Carbon sequestration in cultivated and uncultivated *Vachellia karroo* sites in Tankwa Karoo National Park

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Abstract

The Succulent Karoo Biome (SKB) in South Africa is widely reputed to house Earth's greatest diversity of succulent plants. It is also famous for spectacular displays of annual flowers after good rains. The area experiences winter rainfall which infrequently exceeds 100 mm per annum but certain parts of the SKB can get 250 mm. Irrigated agriculture on a large scale was therefore not a viable option when European farmers began colonizing the land. The land was conquered from the indigenous Khoekhoe herders and San hunter-gatherers, South Africa's first peoples. The biome underwent extreme transformation in the last 200 years following colonisation which resulted in homogenization of the landscape and extinction of many succulents thus reducing biodiversity. The major change to the landscape was the introduction of large herds of livestock which needed feed and therefore farmers started growing lucerne extensively. The study area, Tankwa Karoo National Park, was proclaimed to foster biodiversity conservation thereby allowing landscape recovery in areas that experienced the legacy of farming. Park authorities hence required data on the impact of abandoning farming activities on ecosystem functioning. This study therefore investigated the influence of previously cultivated areas prior to proclamation of the park on carbon storage, microbial community composition and plant physiology. The community-level physiological technique with Biolog EcoPlates was used to estimate microbial community composition and the LI-COR, LI-6400 Portable Photosynthesis System assessed gaseous exchange on the natively occurring *Vachellia karroo (V. karroo).* This plant was the dominant medium to large tree in the landscape and occurs along riparian corridors adjacent to the dry riverbeds.

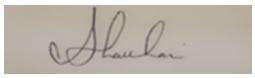
Plants in uncultivated plots were significantly larger than those in which cultivation had taken place ($t_{7.3} = -2.3$, $p = 0.05$ for girth). Soils in the near pristine site had higher bulk density ($t_{18} = -3.4$, $p = 0.003$) and pH ($t_{15} = -3$, $p = 0.01$), and lower moisture content ($t_{10} = 2.1$, $p = 0.06$) and electrical conductivity ($t_{16} = 4.8$, $p = 0.0002$) when compared to

previously cultivated areas. Carbon and nitrogen were significantly different between the two habitats (t_{10} = 2.9, p = 0.02, and t_{11}= 3.3, p = 0.007 respectively). There was no significant difference in soil texture and litterfall rate between the two sites. For net CO_2 assimilation, results show that *V. karroo* displayed a steep rise to an asymptote in carbon utilised as a function of rising light levels in both treatments, in summer and winter. Similarly, in the rainy season, mean C photosynthesised in the cultivated plot was 8.1 ± 3 $\mu mol.m^{-2}.s^{-1}$ while in the pristine site it was 10.3 ± 1.4 $\mu mol.m^{-2}.s^{-1}$. The difference between summer and winter in corresponding plots was not significant either. The Shannon diversity and evenness indices were 4.4 and 2.5, and 4.2 and 2.3 in the cultivated and uncultivated site, respectively. These indicate that there was a slightly greater diversity of microbial activity in the cultivated soil, but not by a significant margin. The study demonstrated that incorporating land that was previously cultivated into protected areas for more than a decade resulted in an improvement of all the measured ecosystem parameters.

Keywords: Soil properties, ecoplates, soil microbes, carbon storage, plant allometry, stomatal conductance, Li-Cor, gaseous exchange, litterfall, plant performance

Declaration

I declare that all of the work in the document is my own work, that it has not been submitted for any degree or examination at any university, and that all the sources which I have used or quoted have been indicated and acknowledged by complete references.



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Paulina Avhavhudzani Phophe Date

Dedication

This study is wholeheartedly dedicated to my beloved mother Mrs Musundwa Shavhani and my late father Mr Muofhe Shavhani whose legacy of love still supports me and my wonderful husband Mr Takalani and my son Thendokhae Phophe who have been my source of inspiration and gave me strength when I thought of giving up, who continually provide their moral, spiritual and emotional support. And lastly, I dedicated this thesis to the Almighty God, thank you for the guidance, strength, power of mind, protection and skills and for giving me a healthy life. All of these, we offer to you.

"Being confident of this, that he who began a good work in you will carry it on to completion until the day of Christ Jesus" Philippians 1:6

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Acronyms and abbreviations list

AWCD:	Average Well Color Development
BD:	Bulk Density
C:	Carbon
CO_2:	Carbon Dioxide
CTGP:	Central Tanqua Grassy Plain
CUL:	Cultivated
DIW:	Distilled Water
E:	Evenness
EC:	Electrical Conductivity
GS:	Stomatal conductance
H:	Shannon-Wiener Index
LU:	Land Use
LUC:	Land-Use Change
N:	Nitrogen
NRDR:	Nieuwoudtville Roggeveld Dolerite Renosterveld
PAR:	Photosynthetically Active Radiation
RK:	Roggeveld Karoo
RSR:	Roggeveld Shale Renosterveld
SANParks:	South African National Parks
SK:	Succulent Karoo
SKB:	Succulent Karoo Biome
TES:	Tanqua Escarpment
TKNP:	Tankwa Karoo National Park
TKR:	Tanqua Karoo Region
TWR:	Tanqua Wash River
UNC:	Uncultivated
V. Karroo:	Vachellia Karroo
WC:	Water Content
WUE:	Water Use Efficiency

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Chapter 1: Introduction

Climate change is the most serious existential threat to the world since a meteor obliterated dinosaurs 64 million years ago (Charig, 1993). The 20th century meteor is unseen but equally sinister is carbon dioxide (CO_2). This gas is considered one of the greenhouse culprits responsible for rising temperatures across the globe (Avola et al., 2008). Rising atmospheric concentrations of CO_2 and other greenhouse gases (GHG's) are key contributing factors which result in rising atmospheric temperatures, and increased frequency of extreme weather events (Drake et al., 1997; IPCC, 2000; IPCC, 2014; Ritchie & Roser, 2017). South Africa has already fallen victim to climate change, one prevalent issue is extreme and prolonged weather calamities like drought, heat waves and flooding (van Wilgen & Herbst, 2016). Considerable evidence showed that the highest portion of climate change is mainly caused by the emission of GHG due to anthropogenic activities and their production and could accelerate the temperature increase in the future (Montzka et al., 2011; Dijkstra et al., 2012; IPCC, 2014).As a result, carbon (C) storage in biogeochemical reservoirs has been a leading topic addressed in global research for the last few decades (Fang et al., 2001; Zhuang et al., 2015). It is therefore essential to evaluate and lower the rate of GHGs accumulation in the atmosphere through enhancing C sequestration in other biogeochemical reservoirs (Conforti et al., 2017). Many studies suggest that the natural environment can potentially act as the ultimate sink for atmospheric CO_2. These natural sinks include the ocean, terrestrial vegetation and soils (IPCC, 2000; Guo & Gifford, 2002; Kirschbaum, 2003; Banasiak et al., 2015).

Soil is the largest terrestrial reservoir of C, it can store about three times more C (around 1500 Pg C) than the atmosphere and vegetation combined (Post et al., 1982; Lal, 2002). According to Fischlin and Gyalistras (1997), soil has acted as a major sink of anthropogenically added atmospheric CO_2 since the industrial revolution over 200 years ago. It can store about 60% in the form of soil organic matter, and the remaining 40% in

the form of inorganic C (Jobbágy & Jackson, 2000). Not only is soil the largest terrestrial reservoir of C, it also plays a role in C cycle (Jobbágy & Jackson, 2000; Lal, 2004; Muñoz-Rojas et al., 2015) and in providing plant nutrients, supporting plant growth and is home to a wide range of organisms other than plants (Buckman & Brady, 1960). Hence, soil is not just an important component of C mitigation but provides many other ecosystem services (Torn et al., 1997).

The C mitigation function of soil is probably the most important. It entails the exchange of C between terrestrial ecosystems and the atmosphere. The flux of C in and out of terrestrial ecosystems can have a significant impact on atmospheric CO_2 concentration (Norman et al., 1997; Houghton, 2003). Measuring the flux of CO_2 from soils to the atmosphere is therefore fundamental in understanding the terrestrial C cycle (Schimel, 1995). This is because that flux is the principal pathway that C takes from ecosystems back to the atmosphere (Kaplan et al., 2012). It is one of the largest fluxes in the global C cycle, at about 50 to 80 Pg C per year from soil to air (Raich & Schlesinger, 1992; Potter et al., 1993), but varies with local conditions.

In South Africa, Barnard (2000) conducted a study on the status of soil organic matter using data from the land type survey, which started in 1970. Approximately 2380 soil profiles were analysed physically and chemically and used to produce a generalized map for organic carbon in virgin topsoils in South Africa. The study showed that the organic C of topsoils ranged from less than 0.5% to more than four percent. Only 4% of the topsoils contained more than 2% organic C, whilst 58% of the topsoils contained less than 0.5% organic C. The variation in soil C in the study was explained mostly by land use (LU). Protected areas appeared to store more C than commercial farmland and communal rangelands. It may thus be possible to mitigate climate change by just switching LU to conservation. This strategy has been attempted elsewhere in the world. In the last decade, soil C management by land use change (LUC) was proposed as an effective means for managing soil fertility, plant productivity and raise soil C sequestration to mitigate climate

change (Lal, 2006; Powlson et al., 2011). According to Detwiler (1986), improvements in LU management potentially increase the sequestration of C in the soil. Therefore, scientific understanding of soil C storage and efflux in relation to LU and conservation practices is essential for managing global climate change and soil fertility (Lal, 2010).

1.1 Problem statement

The change from one LU type to another occurs naturally or through human agency. It has long been recognized that LUC can alter the amount of organic C stored in soil (Laganière et al., 2010; Deng et al., 2016), and this in turn affects both soil fertility and atmospheric CO_2 concentrations (Powers et al., 2011). Land use change is therefore important to grasping the dynamics of soil C, terrestrial ecosystem C balance and the global C cycle. Thus, the change in land use from cultivated land to protected area may potentially increase soil and biomass C sequestration (Mendham et al., 2003), which could mitigate the problem of a rising atmospheric CO_2 concentration (Lal, 2002).

The present study was conducted in Tankwa Karoo National Park (TKNP), the park was initially declared on 19 September 1986. Many land pockets have subsequently been added, including tracts that were previously cultivated fields. The park has been recognised as important in the face of climate change as it provides a refugium for succulent plants adapted to arid and semi-arid areas (SANParks, 2014). It is found in the Tanqua Karoo Region which is a subset of the Succulent Karoo Biome (SKB) in South Africa. This biome has high levels of biodiversity, endemism and it is one of the 36 world biodiversity hotspots (Mittermeier et al., 1999). The SKB (including the TKNP) is truly unique in that it is one of only two arid global biodiversity hotspots and is an epicentre of succulent plant biodiversity.

However, the arid biomes such as the SKB and Nama-Karoo Biomes have experienced dramatic LUC and are among the most threatened ecosystems in South

Africa (Hoffman, 2014). The SKB has undergone major transformations in the last 200 years following European colonisation. The legacy of this land use change is seen in homogenization of the land-scape and extinction of many succulents thus reducing biodiversity (Haarmeyer et al., 2010). The main vehicle for the change was the introduction of great herds of livestock. These animals needed feed and in response farmers began growing lucerne extensively because of its rapid growth high and protein levels, even under relatively dry conditions. Thus large parts of the TKNP used to be grazed by farm animals and cultivated with lucerne. This land use is opposed to the park's current land use as a conservation entity.

The livestock farming and cultivation of lucerne activities are considered to be the main threats to the natural resources in the area especially floristic diversity (Rouget et al., 2003; Hoffman, 2014). Although there is a shift from agriculture to protected areas not a lot is known on how that affects soil C. The main thrust of this study was thus to investigate how land pockets added to a protected area may mitigate C emissions, in the SKB specifically (Fig. 1.1).

When the land was converted from agriculture into a national park, most of the land pockets added were used for agricultural activities, whereby virgin land was converted into agricultural land to suit human needs such as food security. Rising CO_2 which is mainly caused by overpopulation, burning of fossil fuels and LUC leads to global warming which threatens the capacity of Earth to sustain life (Drake et al., 1997; IPCC, 2000; IPCC, 2014; Ritchie & Roser, 2017) (Fig. 1.1). Natural and managed soils however play a significant role as an important source and sink for atmospheric CO_2 and that, primarily as a result of the activities of soil microorganisms (Schlesinger & Andrews, 2000; Noponen et al., 2013). Since, atmospheric CO_2 is controlled by the balance between photosynthesis and respiration, C is transferred from the atmosphere to the soil via C fixing, mainly by photosynthesising plants. Fixed C is then returned to the atmosphere through respiration.

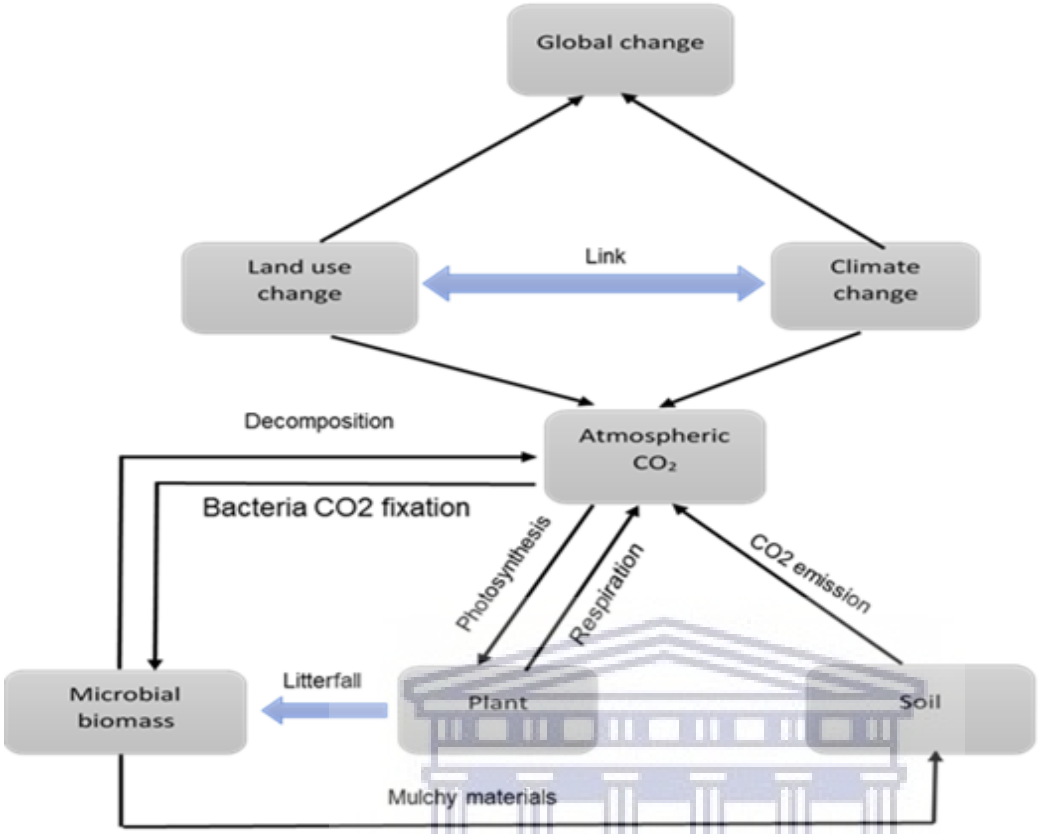

Figure 1.1: A conceptual framework to describe the terrestrial C cycle and review the main processes for quantifying C storage in Tankwa Karoo National Park.

Figure 1.1 shows the link between climate change, LUC and global change. The terrestrial C cycle is mainly controlled by the balance between photosynthesis and respiration. Carbon is transferred from the atmosphere to soil via carbon-fixing, mainly photosynthesising plants and by microbes that synthesise atmospheric CO_2 into organic material. Fixed C is then transferred back to the atmosphere through respiration. The reverse route includes decomposition of organic material, absorbent some C in their biomass and releasing the rest as CO_2 back to the atmosphere. The C cycle is a particularly important ecosystem service because the dynamic balance between C stored in ecosystems and in the atmosphere plays a key regulatory role in the global climate (de Graaff et al., 2015). As such, soil C dynamics and the processes that influence them have the potential to impact atmospheric CO_2 concentrations and associated climate forcing.

Soil management activities in terrestrial ecosystems that positively influence C capturing could potentially help mitigate the current rise in atmospheric CO_2 and associated climate change by promoting soil C storage (Cramer et al., 2001; Johnson & Curtis, 2001). Soil C management is an important strategy for improving soil quality and reducing soil degradation and soil loss (Keller et al., 2018). Capturing C in the soil helps improve soil health, productivity and stabilize the global C cycle, benefiting terrestrial ecosystems. The legacy of historical LU potentially affects the soil C sink in the different areas.

1.3 Aim

The management of protected areas to conserve existing C stocks and to remove C from the atmosphere by adding it to soil are considered essential to mitigate climate change, hence the study investigated how land pockets added to a protected area may play role in mitigating C emissions. The aim of this study is to quantify the influence of LU practice on C storage potential in soil when previously cultivated land is incorporated into a protected area using most dominant tree in the landscape, *Vachellia karroo*.

1.4 Objectives

1. To evaluate soil C storage above and below *V. karroo* as a function of cultivation history in the TKNP.
2. To assess the effect of land use change from cultivated to pristine land on soil microbial biodiversity.
3. To investigate the effects of land use change from cultivated to pristine land on the ecophysiological behaviour of *V. karroo*.

Chapter 2: Literature review

2.1 Impacts of LUC on soil quality

Land use change in ecosystem integrity may lead to degradation and deterioration of soil quality (Jiao et al., 2020). These changes are due to more people on Earth requiring ever increasing yields of food from the land (Gao et al., 2017; Liu et al., 2019). As a result, most tracts of natural land have been converted to agricultural land (Smith, 2008). This transformation is often associated with a breakdown in the provision of ecosystem services such as biodiversity and carbon sequestration (Islam & Weil, 2000; Ayoubi et al., 2011). It is therefore important to investigate the ecological consequence of LUC in order to conserve ecosystem services (Teixeira et al., 2014; Ma et al., 2019). Moges et al. (2013) showed that unmonitored land use changes may lead to land degradation and soil quality deterioration through loss of plant cover and top soil. Soil quality is a complex concept depending on LU, management practices and environmental interactions (Doran et al., 1996; Ayoubi et al., 2011). Evidence has showed that a severe decline in soil quality occurs as a result of agricultural activities following LUC (Sigstad et al., 2002). Clearing natural vegetation for farming led to more erosion and decreased carbon storage. Garcıa-Orenes et al. (2009) also reported that LUC not only impacts soil quality but also soil C sequestration.

2.2 Effects of LUC on carbon storage

Environmental degradation caused by inappropriate LU is a worldwide problem that has attracted attention in the last decade. The United Nations have proclaimed this decade (2020-2030) the UN Decade for Restoration in order to reserve this problem of land degradation worldwide. Land use change is one of the main drivers for many processes of environmental change, as it influences basic resources within the landscape including soil

C storage (Guo & Gifford, 2002). Land use changes could occur naturally or be the result of anthropogenic activities, and this in turn affects both soil C and atmospheric carbon dioxide (CO_2) concentrations (Powers et al., 2011). It is therefore important to understand the dynamics of soil C as a function of LUC. The management of terrestrial ecosystems to conserve existing C stocks and to remove C from the atmosphere by adding it to the soil C stocks has been an agenda in the context of climate change mitigation. Global efforts have focused on both reducing emissions of GHG and increasing the storage of atmospheric C in soil and vegetation via photosynthesis (Lal et al., 2003; Lambers et al., 2008).

Soils, especially undisturbed soil in protected areas, have a large capacity to store C and thereby contribute to the mitigation of GHG emissions, however, LUC could jeopardise this potential (Lal, 2004). Storing more C has additional spin-offs in the form of ecosystem services, it enhances biological activity by increasing fertility and the nutrient holding capacity of the soil (Vaccari et al., 2012; Leksungnoen, 2017). As such, an increase in soil C storage typically results in a more stable C cycle and enhances overall soil productivity in the ecosystem. Schlesinger (1982) suggested that a change in land use from more pristine land to crop farming often goes hand in hand with a loss in soil C storage.

The expansion of agriculture is probably the main mode of LUC globally that returns CO_2 to the atmosphere (Li et al., 2013). This is because the ploughing during cultivation breaks down soil aggregates and exposes previously protected organic matter, thus reducing C in the soil. In addition, the removal of above ground plant biomass during harvest and soil erosion also deplete soil C (Matsuura et al., 2018; Toru & Kibret, 2019). Therefore, the loss of natural ecosystems to anthropogenic activities, especially agriculture, generally reduce C in soil (Chen et al., 2018).

Arid and semi-arid regions are especially vulnerable to LUC, which often leads to land degradation and nutrient loss in these habitats (Xu et al., 2019). These regions are considered severely degraded ecosystems around the world and have the great capacity

for increasing soil C content if they are restored (Yang et al., 2018). Wang et al. (1999) demonstrated that LUC has an effect on soil C storage, and therefore plays a vital role in global C dynamics. According to Lal (2004), small fluctuations of the soil C have large impacts on the atmospheric CO_2 concentration.

2.3 Soil C during LUC from natural to agricultural land

Converting natural land to farming leads to imbalances in the input and output equilibrium of soil C thereby disrupting the C cycle and C storage (Eaton et al., 2008; Muñoz-Rojas et al., 2015; Deng et al., 2016). Cultivating virgin soil results in a net C loss because irrigation and ploughing stimulate decomposition (Mills et al., 2005). Therefore, it can be expected that LUC in the SKB has resulted in less C storage. It is thus important to understand the ramifications of this reduced capacity on ecosystem services.

According to Detwiler (1986), soil C sequestration varies according to the type of change and the ecosystems involved. Their study shows that intensive cultivation changes the natural ecosystems and disturbs the soil, leading to a significant loss of C (van Antwerpen, 2005). Post and Kwon (2000) indicated that when natural ecosystems are farmed, the rapid decline in soil C is due partly to a lower fraction of insoluble material in the more readily decomposed crop residue. Guo & Gifford (2002) suggested that the decline can be reversed with an appropriated soil C sequestration management regime.

Early in the century, studies focused on the importance of increasing soil C storage by eliminating ploughing and maintaining cover crops, this strategy restored up to 70% of lost C in a grassland (Lal, 2002). Recently, Thapa et al. (2018) evaluated soil health in semi-arid areas and recorded 37% more C in grasslands than in cultivated land. Li et al. (2013) estimated soil C in arid areas as a function of LUC, the average soil C content decreased by 14% from 1982 to 2005 as a result of cultivation. Freibauer et al. (2004) and

Zhang et al. (2013) showed that the soil C concentrations in cultivated areas are significantly lower than the corresponding soils under uncultivated land.

Locally, Prinsloo et al. (1990) investigated the impact of cultivation on soil C in the Free State. There was at least 8% soil C lost in surface horizons, the average C loss in the first 1 m of soil was 36%. du Preez & du Toit (1995), also in the central parts of South Africa, examined the top 20 cm of soil at 50 sites with 5 to 90 years of cultivation. The legacy of cultivation led to 10-75% loss on soil organic C. According to Olson (1963) and Minderman (1968), the surface layer of 0-30 cm often loses an average of 20-50% of the C content when soil is cultivated. The rate of loss is greater in the first few years of disturbance and slows thereafter (Olson, 1963; Minderman, 1968). It is reasonable to assume that the observed historical change in land-use has led to a net decrease in the size of terrestrial C stock (Keenan & Williams, 2018).

All these studies highlighted that the C content in soil strongly depends on the type of land cover as well as the LU practice (Arrouays et al., 2001). By contrast with most studies, Yuan et al. (2015) argued that some agricultural activities may enhance soil C and improve soil productivity. For example, cultivation of lucerne can raise soil C and N. This is because lucerne's spreading growth form protects soil from erosion and the plant fixes N. Cultivation of lucerne has been used as a strategy to rehabilitate degraded land and has been very successful in the semi-arid agroecosystem, hence recorded as the most efficient strategy to accumulate soil C (Luo et al., 2011). Freibauer et al. (2004) suggested that management practices leading to increased C storage in soil or vegetation must be continued indefinitely as a climate change mitigation strategy.

2.4 Soil microbial activity and C sequestration

As mentioned above, LUC affects important ecosystem processes including C and N cycles, both these services are mediated by soil microbes (Torsvik & Ovreås, 2002;

Lombard et al., 2011; Spurgeon et al., 2013). The soil microbial community plays a vital role in maintaining soil fertility by liberating nutrients from organic matter via decomposition (Smith et al., 1990), and also help maintain soil structure (Meena et al., 2015). Microbial diversity in soil is a key indicator of microbial function hence can easily be affected by anthropogenic disturbances such as agricultural activities and LUC (Fox & MacDonald, 2003; Wagg et al., 2011). Since agriculture promotes uniformity in plants, it can lead to the loss of microbial diversity (Bossio et al., 2005). In addition, LUC may put a strong selective pressure on the soil microbial community causing short-term adaptation and even a shift in community composition (Szoboszlay et al., 2017).

Comparing microbial composition and diversity in previously cultivated and uncultivated land could reveal the lasting impact of cultivation on the microbial community (Spurgeon et al., 2013). Rodrigues et al. (2013) highlighted that changes in LU possibly shift both microbial community and diversity in terrestrial ecosystems. Therefore, it was expected that there will be lower microbial activity in previously cultivated land. According to Logah et al. (2010), excessive use of fertilizers can jeopardise the health of the microbial community. Katayama et al. (1998) and Wagg et al. (2011) also showed that application of fertilizers and tillage practices can lead to shifts in the microbial community compositions with adverse consequences for the plant community. In contrast to the physical and chemical properties of soil which change very slowly, biological properties are sensitive even to small environmental fluctuations (Jezierska-Tys & Frac, 2008; Gryta et al., 2014). Hence the rationale for the use of microbial characteristics as soil quality indicators is their central role in cycling of C and their sensitivity to change (Nannipieri et al., 2017; Sahu et al., 2017). Freibauer et al. (2004) indicated that any management practices that directly or indirectly impact the microbial activity and decomposition rate affect the amounts of organic C stored in the soils (Jenkinson, 1981; Hariohay, 2013). However, poor understanding of the mechanisms responsible hinders the development of effective land management strategies to enhance soil C storage (Rajendhran &

Gunasekaran, 2008). One way of examining this would be by conducting a study on soil microbial dynamics as affected by specific amendments in various cultivated systems (Lin et al., 2004).

Carbon availability, on the other hand, often controls soil microbial growth and there is evidence that at regional scales soil microbial biomass is positively correlated with aboveground litter inputs (Ruan et al., 2004). Therefore, more plant residue inputs could potentially enhance soil C storage and accelerate decomposition of existing soil C (Gougoulias et al., 2014; Xu et al., 2019). Mazzetto et al. (2016) supported that microbial diversity is sensitive to changes in the soil C that result from LUC, they emphasized that low microbial diversity resulted in loss of ecosystem resilience.

2.5 Gas exchange and C capture by photosynthesis

Soil organic C ultimately comes from atmospheric CO_2 captured by plants through photosynthesis (Cabrera et al., 1998). The rate of change in soil C storage is determined by the difference between C turnover, inputs and outputs (Burke et al., 1989; Tao & Zhang, 2010). A rise in atmospheric CO_2 and global temperatures may have a variety of different consequences for soil C inputs via controls on photosynthetic rates and C losses through respiration (Tao & Zhang, 2010).

It has recently been found that protected areas are the principal C sink in many countries, hence the increased protection has a positive spin-off in more C credits for the country (Conforti et al., 2017). Nogia et al. (2016) highlighted that C fixing through photosynthesis is considered an important strategy for mitigating climate change in the long term (). The exchange of C absorbed by photosynthesis, and released through respiration has resulted in a large and determined net removal of C from the atmosphere by global terrestrial ecosystems (Nogia et al., 2016). It is therefore important to link both

soil properties (i.e. C, nutrients and moisture retention) and gas exchange process with plant physiological performance to assess C storage in ecosystems.

2.6 Effects of LUC on plant physiology

Global terrestrial vegetation plays a critical role in biogeochemical cycles and provides important ecosystem services. However, terrestrial vegetation has been altered by anthropogenic global change drivers including LUC, altered disturbance regimes and climate change (Franklin et al., 2016). de Graaff et al. (2015) supported that these drivers could negatively affect plant physiology. Li et al. (2019) conducted a study looking at peanut (*Arachis hypogaea*) grown in the soil with different management histories, their results suggested that croplands reduced traits related to plant performance such as nutrients metabolism compared to pristine land. In addition, their study showed that cultivated soil displayed a significant reduction in growth coinciding with a decrease of plant hormones related to production such as auxin and cytokinin. This is in agreement with many studies that show that plant performance depends on agricultural management practices (de la Pena et al., 2016). Mattingly and Orrock (2013), showed that historic agricultural land use can alter plant physiological performances.

According to Kgope et al. (2010), plant physiology is controlled by several environmental factors and the interactions of several ecosystem processes, of which photosynthesis, respiration, and decomposition are key. Therefore, information about photosynthetic characteristics and stomatal behaviour of plant species is required to predict C and water fluxes at the leaf, plant, ecosystem and biome levels (Kgope et al., 2010; Schulze et al., 1994). Photosynthesis and stomatal conductance have been intensively studied for a wide range of species belonging to various ecosystems worldwide. Particular attention has been paid to the light saturated photosynthetic rate (A_{max}) and stomatal conductance (gs) (Schulze et al., 1994; Woodward & Smith 1995). In

an analysis of more than 2700 soil profiles from three global databases, Jobbagy & Jackson (2000) found that plant physiology was closely correlated with the amount of soil C and its distribution.

Li et al. (2019) suggested that LUC may potentially affect stomatal conductance, and thus causing a reduction in carbon assimilation by vegetation. In semi-arid environments, the amount of atmospheric gases entering the leaves might be compromised by the exclusion of the influence of drought on stomatal conductance (Mills et al., 2011; Anav et al., 2016). Soil moisture, water stress and CO_2 concentration play a pivotal role in regulating stomatal behaviour of plants. Although the response of stomata to environmental and physiological factors is complex, Buckley and Mott (2002) showed that stomatal conductance varies with leaf irradiance, leaf temperature, atmospheric water vapour pressure deficit and CO_2 concentration.

Gas exchange process at the stomatal scale is a trade-off between the diffusion of CO_2 into the leaf for photosynthesis and water vapour out of the leaf by transpiration (Chapin et al., 1998). The ratio of C assimilation to transpiration is known as the water use efficiency (WUE) (Farquhar & Richards, 1984). Plants with higher WUE tend to have greater growth rate than plants with lower WUE (Marshall et al., 2007). In this study, the leaf photosynthesis and stomatal conductance were measured for *V. karroo* in previously cultivated and uncultivated areas. In particular, we tested whether differences in the photosynthetic rate, stomatal responses and water use efficiency were explained by LU history, or whether the significant differences could be observed between *V. karroo* from cultivated and uncultivated areas.

Chapter 3: Materials and methods

3.1 Study area

The Tankwa Karoo National Park is located in the south central section of the Succulent Karoo Biome (SKB) (SANParks, 2014). It straddles the border between the Western and Northern Cape provinces of South Africa (Fig. 3.1) and lies approximately 140 km north of Ceres, 100 km south of Calvinia, 38 km west of Sutherland and 25 km southwest of Middelpos (SANParks, 2014). The park is bounded by the Cederberg Mountains to the west, the Roggeveld Mountains and Plateau to the east and northeast, and the Klein Roggeveld Mountains to the southeast (SANParks, 2014). Two non-perennial rivers run through the park, the Renoster, which flows across the south-eastern part and the Tanqua River in the south. These rivers experience occasional flooding during heavy storms but are dry for most of the year (Rubin, 1998).

The TKNP was formally proclaimed in 1986 and consisted of a patchwork of commercial farms totalling 270 km^2 (Rubin, 1998). It is currently about 1 486 km^2 and ranges from 316 m above sea level in the west to 1 640 m in the eastern highlands. The TKNP is therefore heterogeneous in landform and vegetation as a result of a wide altitudinal span (SANParks, 2014). It comprises many diverse habitats from the low-lying areas on the plains to the higher-lying areas on the Roggeveld Mountains and Plateau (Fig. 3.2). The dominant landforms in the park consist of open plains, a large flat dolerite plateau, an extensive floodplain, the Roggeveld Escarpment and the Renoster River catchment (SANParks, 2014). The wide range of landforms has a strong influence on microclimate (Van der Merwe et al., 2015), which is reflected as differences in the vegetation characteristics and soil properties (Steyn et al., 2013).

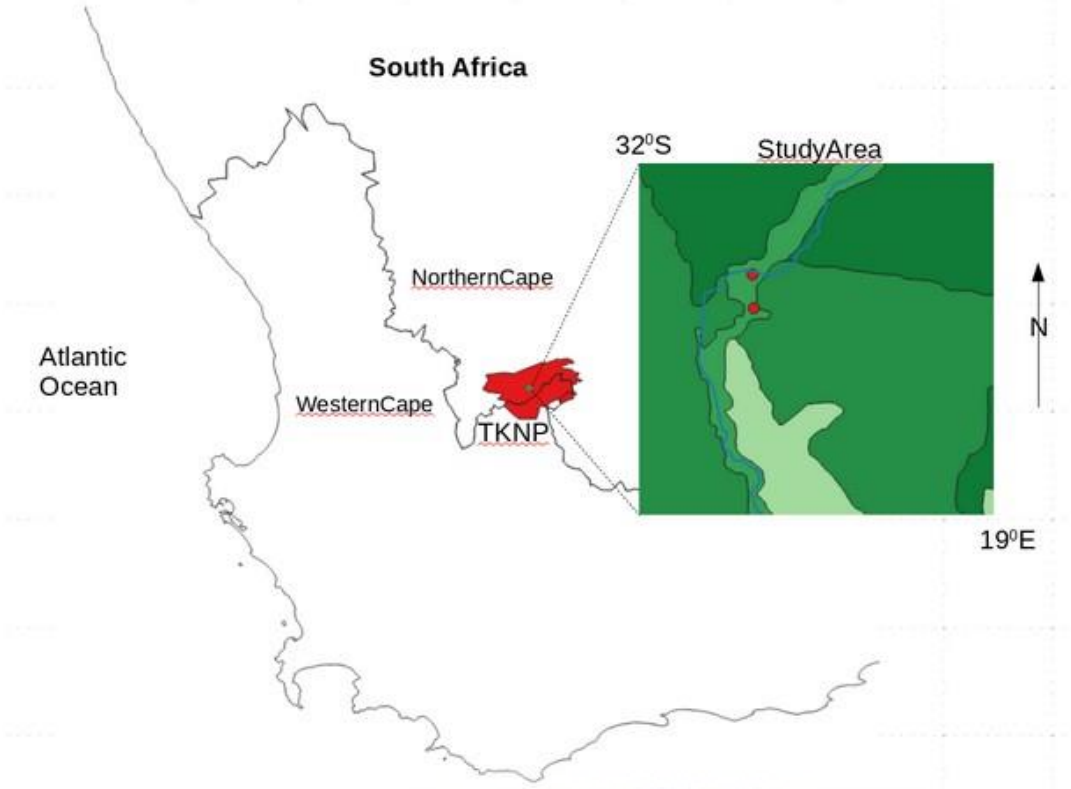

Figure 3.1: Study site in the Tankwa Karoo National Park, South Africa. The red dots represent the cultivated and uncultivated habitats sampled here.

3.1.1 Climate

The Tankwa is characterised by extreme summer aridity with unreliable rainfall occurrence and distribution. Climate in the park is primarily controlled by topography (Van der Merwe et al., 2015). The higher-lying eastern and north-eastern parts have relatively lower temperature and receive significantly more rain compared to the lower-lying western plains. Rainfall is primarily in winter, the west and most of the plains receive 75-155 mm per annum. The eastern higherland on the Roggeveld has a range between 155 and 270 mm per annum. Over two thirds of the rain comes down between March and August. Tropical thunderstorms that originate in the grasslands and savannas of South Africa can penetrate into the TKNP in summer (Desmet, 2007). Mean July (winter) temperature is 6.8°C on the plains and -2.4°C in the mountains, and mean January (summer) maximum temperature is 36.6°C on the plains and 29°C higher up. The average annual relative

humidity on the plains is 42% and in the mountains, 65%. The average evapotranspiration rate on the plains is 1900 mm per annum and on the mountains, 1850 mm (SANParks, 2014).

3.1.2 Geology and soils

According to the Geological Survey of South Africa (1973), the study area is part of the Karoo Supergroup, with three main rock types, old sedimentary rocks, recent deposits and igneous rocks. The sedimentary rocks are the Dwyka Group (tillites, sandstone, mudstone, and shale) on the western edge of the park, the Ecca Group (sandstone and shale) that occupy most of the park, and the Beaufort Group (mudstone) capping the Roggeveld Mountains. The second group consists of alluvium and colluvium from the Quaternary and even more recent deposits, found throughout the park. The igneous rocks are represented by dolerite intrusions that occur sporadically around the park.

Soils of the Tankwa are typical of arid ecosystems, they are shallow, skeletal and young due to intense erosion in a sparsely vegetated environment (Beukes & Ellis, 2003). Soils in the flat plains are shallow lithosols and often form a desert pavement on the surface. The plains also contain deep unconsolidated deposits of alluvium along drainage lines. Thus plains soils are often very sandy with a high proportion of rock fragments, up to 40% in some instances. On the escarpment, soils are mainly shallow, stony lithosols (Francis et al., 2007).

3.1.3 Flora

The SKB is an over 100 000 km^2 semi-arid to arid plant biodiversity hotspot stretching just north of Cape Town to southern Namibia. It is one of the 36 richest and most threatened reservoirs of plant and animal life on Earth. It is one of only two plant biodiversity centres that are entirely arid, the other is in the horn of Africa. The SKB is commonly divided into

Namaqualand on the west coast and the more inland Karoo (Myers et al., 2000). The biome is home to 6 356 plant species, 40% of which are endemic and 936 (17%) of which are listed in the Red Data Book. Not only does the SKB boast the world's richest succulent flora, it also has high reptile and invertebrate diversity. Grazing, agriculture and mining, especially for diamonds and heavy metals threaten this fragile biome. Fortunately, the human population in the SKB is very low compared to other hotspots allowing the vegetation to remain relatively intact. Unfortunately, only 30,000 km^2 of the original land remains in a relatively pristine state while protected areas like the TKNP cover a mere 4457 km^2.

Broadly, the TKNP comprises lowland and upland karoo vegetation types. The lowland succulent karoo on the plains is characterised by very sparse shrubland and dwarf shrubland (<0.3 m). The upland succulent karoo in the mountains is made up of small to medium sized shrubs and succulents (SANParks, 2014). At finer scales, the park has six vegetation types (Mucina & Rutherford, 2006), the Roggeveld Shale Renosterveld (RSR), Tanqua Escarpment (TES), Succulent Karoo (SK), Roggeveld Karoo (RK), Tanqua Wash Rivers (TWR) and Nieuwoudtville Roggeveld Dolerite Renosterveld (NRDR) (Fig. 3.2). This study took place in the TWR wherein a cultivated and an uncultivated site were selected.

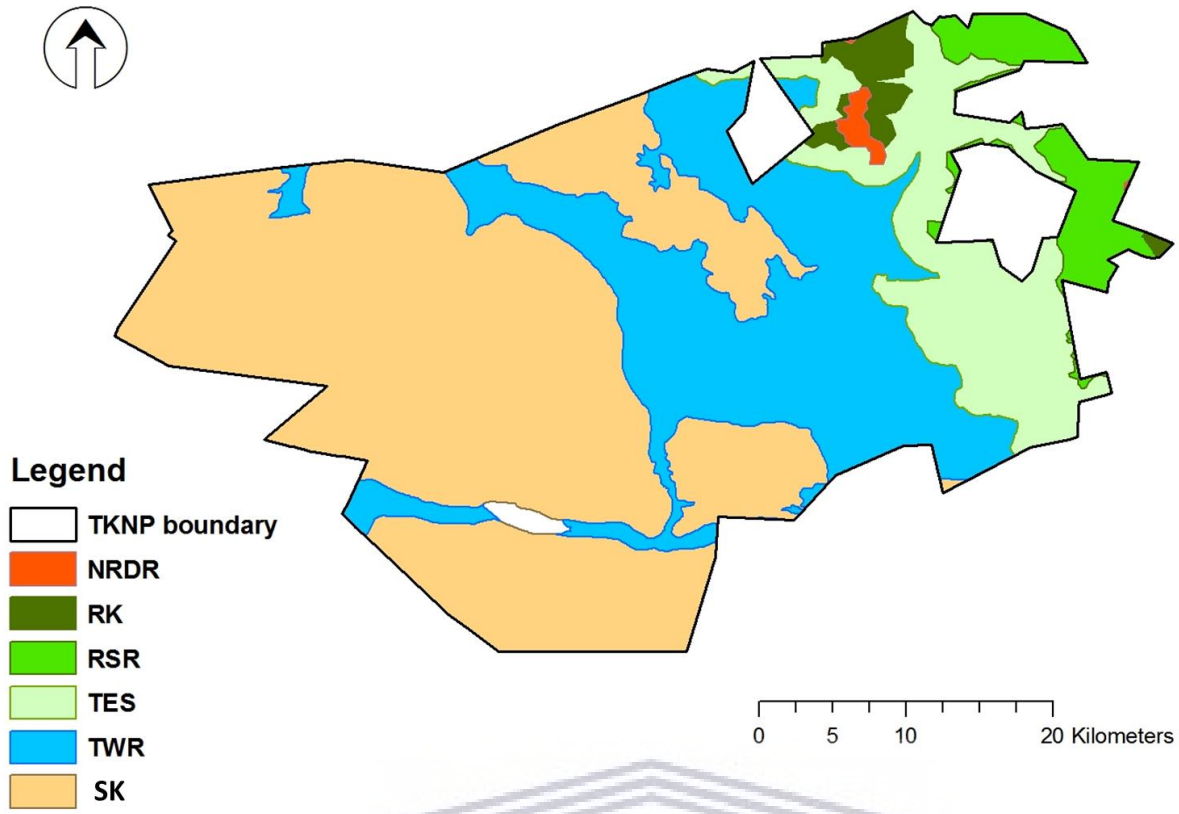

Figure 3.2: Vegetation types in the Tankwa Karoo National Park (TKNP) (Saaed et al., 2018). Where NRDR is the Nieuwoudtville Roggeveld Dolerite Renosterveld, RK is the Roggeveld Karoo, RSR is the Roggeveld Shale Renosterveld, TES is the Tanqua Escarpment, TWR is the Tanqua Wash Rivers and SK is the Succulent Karoo.

3.2 Study species

Until 2005, *V. karroo* was famously known as *Acacia karroo*, but according to current taxonomic research and molecular evidence, the *Acacia* genus was shown to be polyphyletic (Dingaan & du Preez, 2017). So right now, only Australian members retain the genus name. *Vachellia karroo* belongs to Fabaceae (legumes) and is commonly called sweet thorn, soetdoring or mooka (Palgrave, 1977). *Vachellia karroo* grows naturally along the rivers in the SKB and Nama-Karoo Biome and across most of southern Africa except in the Namib and Kalahari Deserts. It grows to peak size under 800-900 mm rain but can survive and even thrive in very dry conditions. Members of *Vachellia* are pod-bearing

woody plants that range from shrubs to large trees. They can be sprawling or climbing depending on the habitat. This genus is readily recognised by its thorns, which are typically paired and straight. These thorns are modified stipules, which become hard and spiny and are important for identification. *Vachellia* trees can further be distinguished by their characteristic growth form, by bark and also by pods (Fig. 3.3). This is however a taxonomically difficult genus containing a number of closely related species whose recognition and identification are not always simple. Sweet thorn has many uses and is seldom cleared by farmers because it provides fodder primarily and has secondary uses like in dyes, ropes, needles, food, medicine, cosmetics and firewood (Dingaan & du Preez, 2017).

Figure 3.3: *Vachellia karroo* trunk (A), branches (B), leaves (C), flowers (D), pods (E) and spines (F)

3.3 Plant stature measurements

Sampling was conducted in two sites in the park, a previously cultivated and an uncultivated site. Sampling was done in two seasons over two years: August 2017 and January 2018. To estimate *V. karroo* plant stature in the two sites, measurements were taken on five fully matured plants randomly selected in each site. Plant total height were measured from the ground to the tip of each tree using tapes. While other studies measure the circumference of the tree at a height of 1.3m above the ground to determine the tree diameter (Diameter at Breast Height Over Bark), this study used height to diameter model to examine the relationship between height and diameter. The following formula were used to estimate basal circumference and canopy volume: (where π=3.14, r= radius and h= height)

Basal circumference = $2\ \pi.r$

Canopy volume = $\pi.r^2.h$

3.4 Soil properties

Ten soil samples per site were collected beneath *V. karroo* with a 113 cm^3 steel cylinder, two soil cores were collected for each sample. Bulk density was determined from the ratio of weight of the soil in the cylinder and its volume (McKenzie et al., 2002). The soil from the two cylinders was then combined, homogenised, and air dried in the laboratory at room temperature. To determine the moisture content, a 5 g sub-sample was dried to constant weight in an ecotherm labotec oven for 24 hours at 105°C (Cresswell & Hamilton, 2002). Soil particle size was measured with a hydrometer in a 1 L plastic cylinder (Gee & Bauder, 1986) on a 40 g sample. The soil was disaggregated by suspending it in 100 ml calgon, mixed end-to-end for 5 minutes, the cylinder was then filled with distilled water (DIW) to the final volume. The hydrometer was immersed gently into the cylinder for measurements (Ashworth et al., 2001). The sand, clay and silt fraction of all samples were recorded as a

percentage. Electrical conductivity (EC) and pH were measured in a 10 g subsample suspended in 20 ml DIW and agitated for 30 min. The soil-water mixture was then subjected to five cycles of 10 min stirring and 5 min settling. The EC and pH were then measured with a standard benchtop meter.

3.5 Leaf litter

Litterfall was estimated in rectangular 325 cm^2 (25×13 cm) traps placed beneath each tree to catch free-falling leaves (Fig. 3.4). These traps were laid out in August 2017 and the leaf litter was collected after 3 days. The leaves were oven-dried to constant weight at 70°C for ~48 hours, then weighed. The rate was then calculated as the ratio of the leaf weight and area over the three days.

Figure 3.4: Litterfall traps set beneath *V. karroo trees.*

3.6 Soil and foliar CN

Carbon and nitrogen were measured by combustion in a Leco Trumac CN analyser (LECO Corporation, Michigan, USA) at 720 mm Hg ballast pressure and 1350°C temperature. The

carbon in the sample is burned into CO_2 while nitrogen is oxidised to NO_2 with oxygen, which also acts as the carrier gas whose flow is carefully regulated. Soil C and N concentrations were determined in a 200 mg oven dried subsample ground to a fine powder. Foliar C and N, on the other hand, were measured in a 300 mg leaf sample, oven dried at 70°C for 48 hours, and finely ground with a mortar and pestle, Vice versa. Sample weights were accurately measured on a Sartorius analytical lab scale 120S MC1 to a < 0.001 g precision.

3.7 Microbial community metabolic profiling

To quantify the impact of cultivation on the potential functioning of the rhizosphere microbial community, the community-level physiological technique with Biolog EcoPlates was used (Biolog Inc., Haywood, CA, USA) to analyse the functional diversity of microbial communities and the physiological activity of microorganisms in soil as a function of historical land use. This scheme uses 3 g of soil suspended in 27 ml sterile 0.85% sodium chloride solution and vortexed for five minutes at maximum speed. After 10 minutes of settling, 180 μl of this suspension was siphoned into the 31×3 ecoplate wells and then incubated at 25°C in the dark for the duration of the experiment. The 31 wells (including control) contain a specific C source that can potentially be metabolised by soil dwelling microbes. Each well also contains some tetrazolium dye which develops upon utilization of the C substrate in the well, unoxidized wells remain colourless while a well in which microbes thrive turns a deep purple (Adams et al., 2017).

The colour was quantified with optical density measured as an absorbance at 590 nm wavelength at least every 24 hours using a Vmax Microplate Reader (Varioskan Flash, Therma Scientific). The actual absorbance was observed at zero, 25, 51, 72, 95, 126 and 165 hours. Data were compared using groupings of C sources. To simplify direct comparison, five categories were chosen, amino acids, amines, carboxylic acids, carbohydrates and polypeptides. Data derived from the wells for evaluation included the

average well color development (AWCD), the Shannon-Wiener Index (H) and Shannon Evenness (E) (Stefanowicz, 2006; Frac et al., 2012). The functional diversity indices were calculated as described by Zhong et al., (2010).

$$H' = \sum_{i=1}^{s} (p_i)(\ln p_i)$$

Where p_i is the proportional colour development of a well over the development of all colour in all wells of the plate. Evenness was calculated as the ratio of H and the natural logarithm of substrate richness, which itself is the number of substrates around which a reaction occurs. Average well color development is an indicator of the general potential metabolic activities of the microbial community, thus it is an index of the total bioactivity for the biological plates. The H and E were used to calculate the physiological diversity of bacterial communities (Frac et al., 2012; Kenarova et al., 2014). This was supplemented with a PCA analysis showing correlation of metabolic functional groups in previously cultivated and uncultivated areas.

3.8 Gas exchange measurements

Gas exchange is commonly used to estimate photosynthetic activity of a plant because it provides a direct estimate of the net rate of photosynthetic carbon assimilation. Photosynthetic gas exchange was measured in the wet (August 2017) and dry season (January 2018) on 10 plants each in the previously cultivated and uncultivated site using an LI-6400 Portable Photosynthesis System (LI-COR, Lincoln, Nebraska, USA). Measurements were performed on a fully expanded mature leaf randomly selected from the tree canopy and chiefly entailed photosynthesis as a function of light intensity, the light response curve (A_N). The by-product of collecting these types of data using a Licor

instrument is that water use efficiency (WUE) and stomatal conductance (g_s) are also simultaneously estimated. The WUE is calculated per mole of CO_2 assimilated basis for every water mole expended. We recorded these metrics after 5-10 minutes following adequate stomatal adjustment at each Photosynthetically Active Radiation (Par) level. To calculate leaf surface area, we harvested the leaves post gas exchange measurements and traced them onto a 2 mm grid paper.

3.9 Analytical framework

To answer the research questions and achieve the study aims, the analytical framework was designed to be structured around the different methods and tools at a species level (Fig. 3.5). The structure of the study considered the above, intermediate and below ground processes and investigated the seasonal and long term state. Thus, it would come up with a broader view and reliable assessment of soil C sequestration potentials in the TKNP. The above ground components include plant productivity and plant allometry, the intermediate components include plant and soil C:N ratio and litterfall, and the below ground components include soil properties and soil microbes.

The analytical framework of this study considers the impact of LUC on soil C sequestration and soil productivity, particularly when the land was converted from agricultural land to a national park which largely has substantial influence on the species ecophysiological behaviour. The different parameters were measured to evaluate the soil C storage above and below *V. karroo* as a function of cultivation history, the effect of LUC on microbial biodiversity and on ecophysiological behaviour of *V. karroo*. The framework suggests different variables and various methods and techniques implemented at species level.

3.10 Statistical analysis

Welch's 2-sample t-tests were used to determine the difference between the girth, canopy volume and basal circumference of plants in cultivated and uncultivated sites. The relationship between height and basal circumference was probed with a simple linear regression model while the height-diameter models were used to estimate the height-diameter relationships. Likewise, bulk density, moisture content, particle size, electrical conductivity, pH, carbon, nitrogen and litterfall rate were compared with t-tests. The microbial metabolic profiles were analysed by calculating the average well colour development (AWCD) after blank and initial absorbance corrections, and setting negative values to zero. A principal components analysis (PCA) was used to further examine the relationship between AWCD and the metabolites. Finally, the AWCD's were used to calculate diversity indices for the metabolites, the Shannon-Wiener Index (H) and Shannon Evenness (E). The gas exchange parameters (C assimilation, stomatal conductance and water use efficiency) as a function of photosynthetically active radiation (PAR) were investigated with simple scattergrams. A summary of all methods employed and framework for analysis is provided in Fig. 3.5

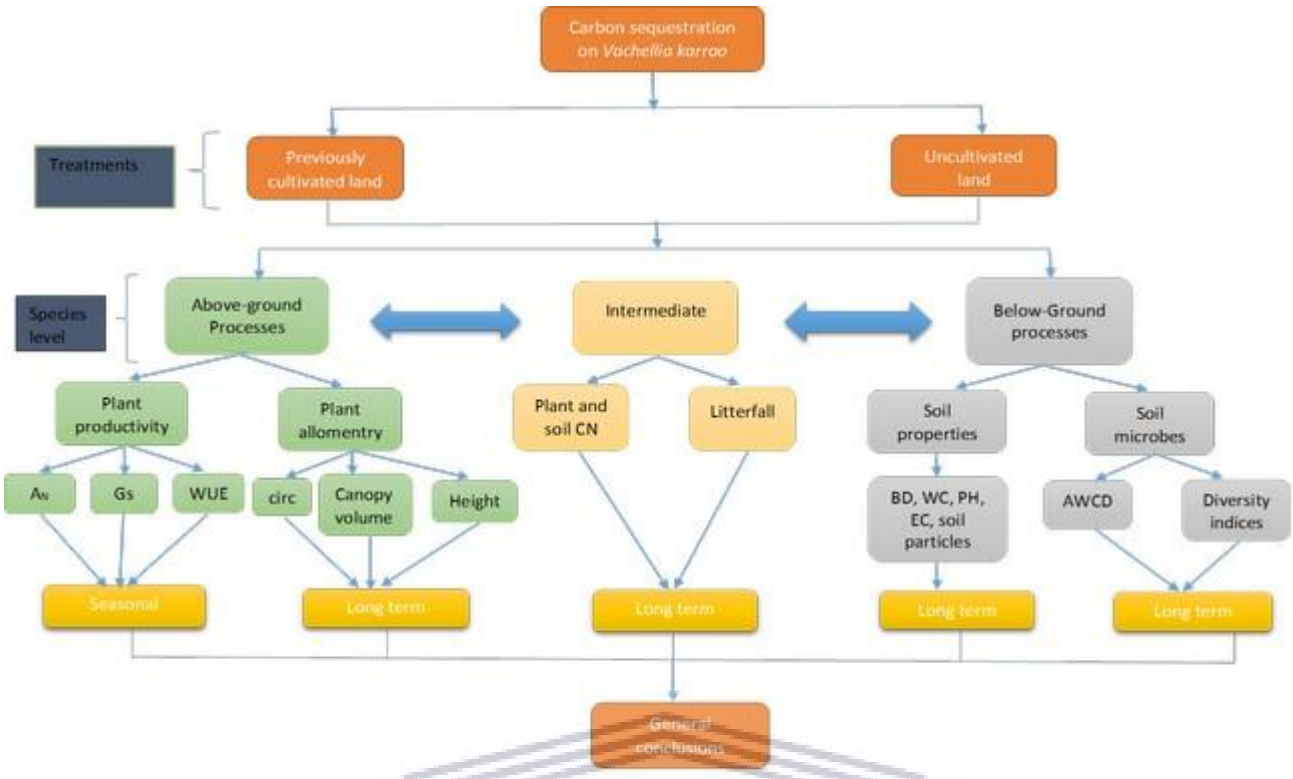

Figure 3.5: Analytical framework of the various approaches and methods applied in this study. To better understand the changes in plant productivity and soil carbon storage under previously cultivated and uncultivated *V. karroo* habitats, a two-year field campaign was undertaken. Soil properties, we analysed microbial diversity and examined stomatal conductance and photosynthetic rate. A_N is net assimilation of carbon, G_s = stomatal conductance, WUE = water use efficiency, circ. = basal circumference, BD = bulk density, WC = water content, EC = electrical conductivity and AWDC = average well color development.

Chapter 4: Results

4.1 Plant stature between sites

Plant stature measured in previously cultivated sites gave a basal circumference ranging from 12 to 24 m with a mean of 18.9 ± 2.1 m while plants in uncultivated sites were between 19 and 35 m in girth with a mean of 26.9 ± 2.84 m. The difference in basal circumference between the two sites was significantly different according to Welch's 2-sample t-test ($t_{7.3} = -2.3$, $p = 0.05$). Canopy volume in the previously cultivated site ranged from 20 to 105 m^3 with a mean of 53.7 ± 14.5 m^3 while in the uncultivated site the canopy volume ranged from 21 to 312 m^3 with a mean of 157.9 ± 49.3 m^3. The difference in canopy volume between the two treatments was not significant ($t_{4.3} = 1.8$, $p = 0.1$). A global model of height and basal diameter explained about 30% of the differences in tree physiognomy ($R^2 = 0.3$), however, different height-diameter relationships between cultivated and uncultivated sites was not significant ($F_{1.8} = 4$, $p = 0.1$). When localized models for cultivated and uncultivated sites were constructed, they also yielded little correspondence in height and diameter, although the uncultivated plants were marginally stunted (Fig 4.1) (*personal observation*).

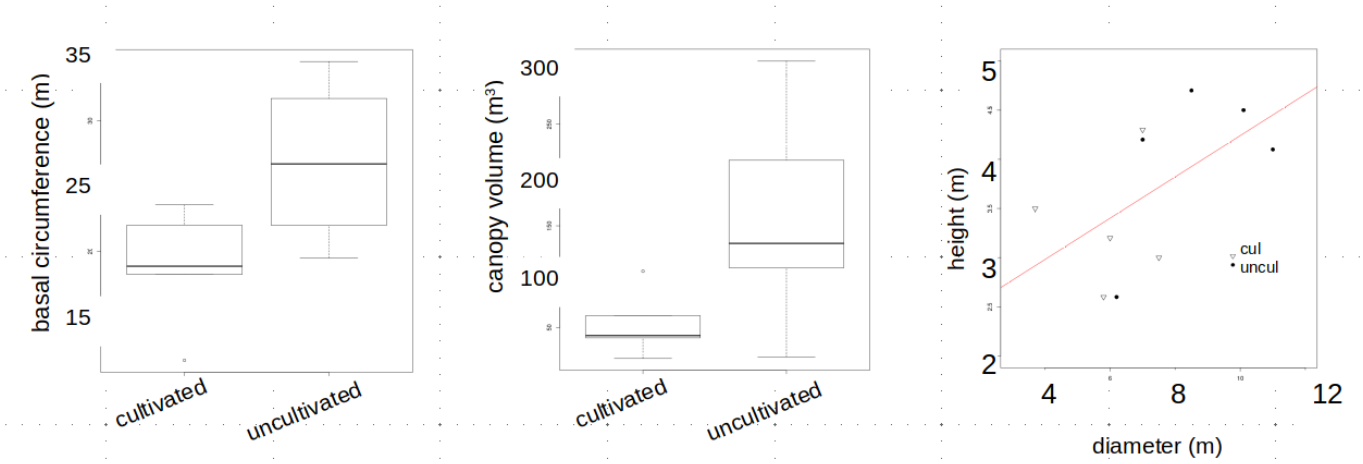

Figure 4.1: Box, whisker plots and scatter plot of plant physiognomy for studied *V. karroo* in previously cultivated (cul) and uncultivated (uncul) sites in the TKNP. The left panel presents the basal circumference, the middle is the canopy volume. Error bars are standard errors of the means. The scatter plot on the right represents the height-diameter relationships between previously cultivated and uncultivated sites. The results from previously cultivated are indicated by the triangle and results from uncultivated site are indicated by solid circle.

4.2 Soil property and litterfall differences

There was a significant differences in bulk density between the two sites according to Welch's 2-sample t-test (t_{18} = -3.4, p = 0.003) (Fig 4.2). Soil bulk density in the previously cultivated site ranged from 0.50 to 1.10 g.cm^{-3} with a mean of 0.84 ± 0.05 g.cm^{-3}, while in uncultivated site, bulk density ranged from 0.90 to 1.40 g.cm^{-3} with a mean of 1.08 ± 0.04 g.cm^{-3}. Between the two sites, significantly lower bulk density values were recorded in soils under the previously cultivated site. Water content in soil, on the other hand, was not significantly affected by cultivation (t_{10} = 2.1, p = 0.06). The water content in previously cultivated sites ranged from 4.3 to 5.4% with a mean of 4.7 ± 0.1%, while in uncultivated sites the range was 2.5 to 6.2% with a mean of 3.9 ± 0.4%. Similarly, there was no significant difference in soil texture between the two sites (t_{15} = 1.3, p = 0.2). Mean percent

fine soil particles (sand +clay) were 58 ± 5% and 45 ± 9% in cultivated and uncultivated sites, respectively (Fig 4.2). The electrical conductivity (EC) ranged from 605 to 1652 $mS.cm^{-1}$ in previously cultivated and 224 to 37000 $mS.cm^{-1}$ in the uncultivated site with respective means of 1082 ± 99 $mS.cm^{-1}$ and 5035 ± 3669 $mS.cm^{-1}$. The large span in EC for the uncultivated site was due to two outliers, which were removed from the statistical test. Without the outliers the mean EC was 456 ± 84 $mS.cm^{-1}$ in the uncultivated site, and the difference between the two treatments was highly significant ($t_{16} = 4.8$, $p = 0.0002$). With outliers retained, the p value was 0.3. The pH ranged from 7.35 to 8.27 in the previously cultivated site, and 7.61 to 8.93 in uncultivated site with respective means of 7.75 ± 0.08 and 8.24 ± 0.14. The difference in pH between the two sites was highly significant ($t_{15} = -3$, $p = 0.01$) (Fig 4.2).

The measured soil C values ranged from 0.4 to 6.3% in the previously cultivated site while in the uncultivated habitat, the values ranged from 0.05 to 1.6% with respective means of 2.3 ± 0.2% and 0.54 ± 0.6%, there was a significant difference in soil C between the two sites ($t_{10} = 2.9$, $p = 0.02$). Between the two sites, significantly higher soil C values were recorded in soils under the previously cultivated site. Measured soil N ranged between 0.09 and 0.56% in the cultivated site while in the uncultivated site, the values ranged from 0.006 to 0.18% with respective means of 0.24 ± 0.05% and 0.07 ± 0.02%, these indicated significant differences in soil N between the two sites ($t_{11} = 3.3$, $p = 0.007$).

Plant C ranged from 44.9 to 47.7% in the previously cultivated site and 42.6 to 48.7% in the uncultivated site with respective means of 46.45 ± 0.3% and 47.08 ± 0.7%, there was no significant difference in foliar C between the two sites ($t_{13} = -0.85$, $p = 0.4$) (Fig 4.2). By contrast, foliar N was highly and significantly different between the two sites ($t_{14} = 4.7$, $p = 0.0004$). The concentration of N in leaves was 1.8 to 2.2% in the previously cultivated site while in the uncultivated habitat, leaf N ranged from 1.3 to 2.0%, with respective means 1.99 ± 0.05% and 1.53 ± 0.09%. Finally, the rate of litterfall ranged from 0.01 to 0.43 $g.m^{-2}.day^{-1}$ in the cultivated site and from 0.02 to 0.19 $g.m^{-2}.day^{-1}$ in the

uncultivated site with respective means of 0.11 ± 0.04 $g.m^{-2}.day^{-1}$ and 0.07 ± 0.02 $g.m^{-2}.day^{-1}$, these were not significantly different ($t_{12} = 0.98$, $p = 0.3$) (Fig 4.2).

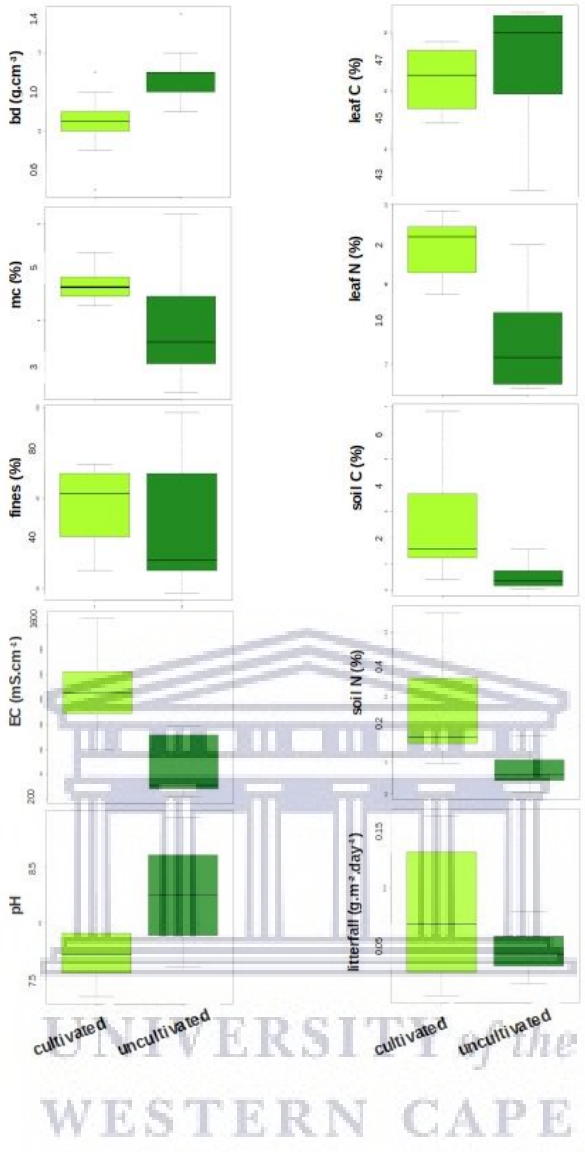

Figure 4.2: Box and whisker plots showing span in bulk density (bd), soil moisture content (mc), soil texture (% fine = % silt + % clay), electrical conductivity (EC), pH, foliar C and N percentage, soil C and N concentrations and litterfall rate in the previously cultivated and uncultivated sites in the Tankwa Karoo National Park.

4.3 Physiological profile of the microbial community

Average well color development (AWCD) of Biolog EcoPlates is an important index for estimating the diversity of the soil microbial community. The values represent the changes of soil microbial community ability to metabolize different substrates. After blank C source

and initial absorbance corrections, and setting negative numbers to zero, AWCD in the uncultivated site ranged between zero and 2.4, with a mean of 0.8 ± 0.6. By contrast, the cultivated site had a mean AWCD of 1.1 ± 0.5 in the range between zero and 2.3. There was no significant difference in AWCD between the two sites according to a t-test (Fig 4.3).

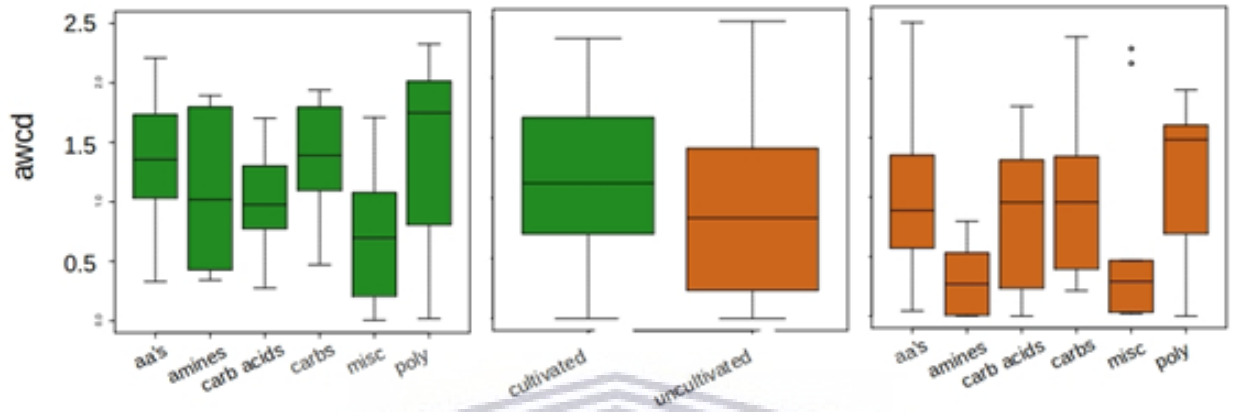

Figure 4.3: Box and whisker plot showing the average well colour development (AWCD) and C source (aa's are amino acids, amines, carb acids are carboxylic acids, carbs are carbohydrates, mics = miscellaneous and poly are polypeptides) in soil samples collected from a previously cultivated and an uncultivated site in the Tankwa Karoo National Park. The central plot represent an aggregate of all the C sources while the adjacent panels show utilization per C source analysed wherein the cultivated is represented by the green whisker plots and the uncultivated represented by the orange whisker plot.

The oxidation of amino acids by the microbial community as indexed by the AWCD had a mean intensity of 1.3 ± 0.5 in cultivated soil and 0.99 ± 0.6 in uncultivated soil, the difference was not significant ($t_{34} = 1.8$, $p = 0.08$). For carboxylic acids as well, there was no effect of cultivation on microbial activity ($t_{40} = 1.2$, $p = 0.2$), higher mean metabolic activity was recorded in soils under the previously cultivated site. Similarly for carbohydrates and polypeptides, these were not utilised to significantly different degrees between cultivated and uncultivated soil (Fig.4.3). In amines, the difference between the

mean metabolic activity in the cultivated (1 ± 0.7) and uncultivated (0.3 ± 0.3) soils was significant ($t_{7.2} = 2.4$, $p = 0.05$). Therefore, this study shows that cultivation have long lasting impact on the composition, diversity and activity of amines, however, there were no any impact on other carbon sources. This is supported by the PCA (Fig 4.4) in which the first axis explains 80% of the clustering, of which there is very little impact on microbial activity and diversity. The first axis corresponds to variation mainly in the uncultivated site from which it can be concluded that the cultivated soil had less metabolism of polymers and minimal activity in amino acids. Indices of diversity were also invariant between the two sites, H and E were 4.4 and 2.5, and 4.2 and 2.3 in the cultivated and uncultivated site, respectively. These numbers indicate that there is a slightly greater diversity of microbial activity in the cultivated soil, but not by a significant margin (Fig. 4.4).

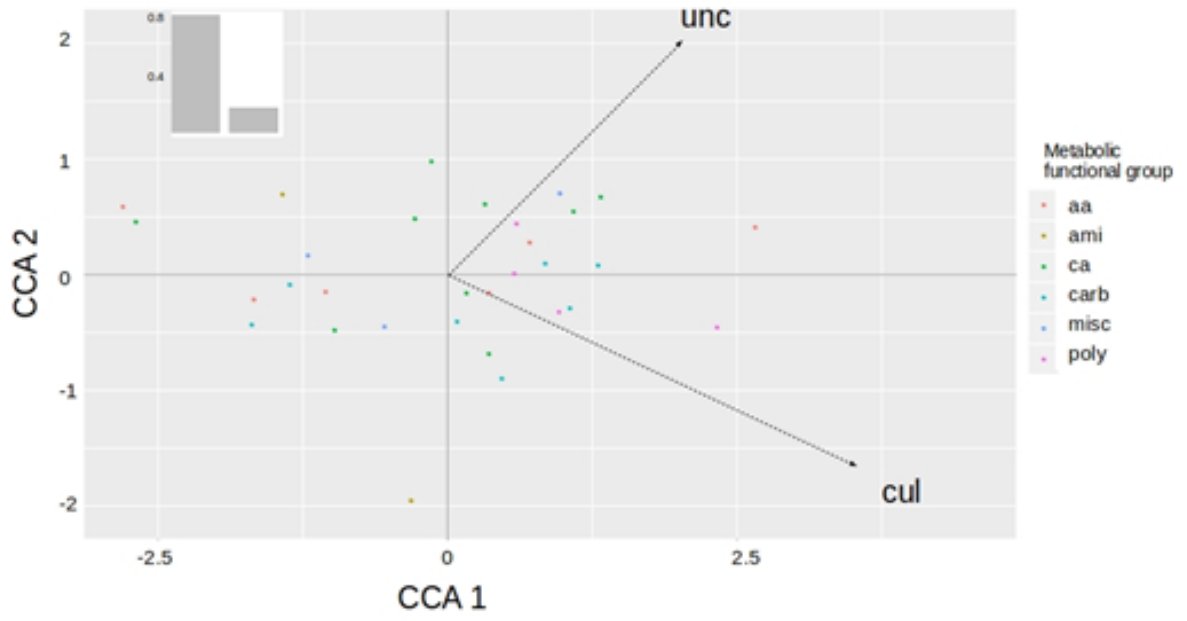

Figure 4.4: Principal Components Analysis (PCA) showing correlation of metabolic functional groups (where aa's are amino acids, ami are amines, ca are carboxylic acids, carbs are carbohydrates, mics = miscellaneous and poly are polypeptides) in previously cultivated (cul) and uncultivated (unc) sites.

4.4 Gas exchange at different light intensities

The light intensity levels investigated in the photosynthetically active radiation (PAR) ranged between a first quartile of 112 to a maximum of 2235 μmol.m^{-2}.s^{-1}. Between these two extremities, carbon assimilation in both habitats and sampling campaigns followed the typical light response curve of photosynthesis. In all four situations, *V. karroo* displayed a steep rise to an asymptote in C utilised as a function of rising light levels. Overall, the carbon assimilation via photosynthesis (A_N) ranged from 3.3 in the first quartile to a peak of nearly 25 μmol.m^{-2}.s^{-1} (Fig 4.5). For stomatal conductance (gs), the range in both sites and the two seasonal measurements was 0.04 in the first quartile to a peak of 0.5 μmol of water m^{-2}.s^{-1}, patterns were erratic for individual plants (Fig 4.5). Likewise, for the water use efficiency (WUE), the response to rising light intensity was very hysterical, but a trend of asymptotic efficiency in water use per unit C could be discerned. The WUE ranged from

43 in the first quartile to a maximum peak of nearly 1500 units of CO_2 per unit water, this wide variability can be garnered from the 150% coefficient of variability (Fig 4.5). Given the inconsistency in individual patterns of gas exchange, some of the more erratic plants were removed from further analysis, and the more consistent plants were used to pool data and decipher general trends.

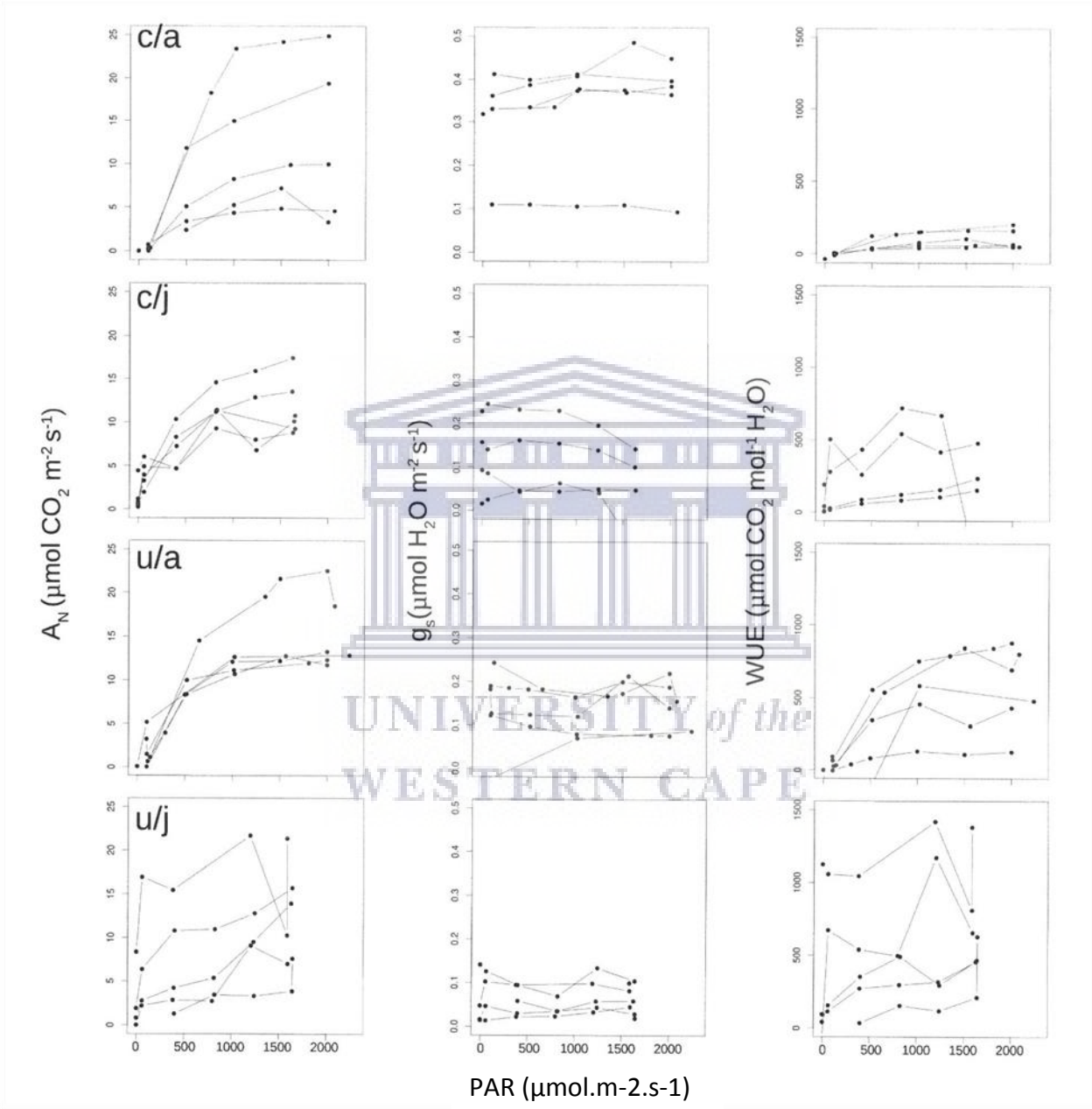

Figure 4.5: The physiological response in carbon assimilation (A_N), stomatal conductance (g_s) and water use efficiency (WUE) as a function of increasing light intensities for *V. Karroo* trees sampled in August 2017 (a) and January 2018 (j) on previously cultivated (c) and uncultivated (u) areas in the TKNP.

4.5 Effect of cultivation on carbon and water relations

The mean C assimilation in the cultivated site in January was 6.9 ± 1.4 μmol.m^{-2}.s^{-1} with a range from 4.6 to 10.33 μmol.m^{-2}.s^{-1}. This was strangely the same as the C accumulating in the uncultivated site in January where the mean C assimilation was 6.9 ± 2.6 μmol.m^{-2}.s^{-1}. In the August, mean C photosynthesised in the cultivated site was 8.1 ± 3 μmol.m^{-2}.s^{-1} while in the uncultivated site it was 10.3 ± 1.4 μmol.m^{-2}.s^{-1} (Fig. 4.6). The difference between summer and winter in corresponding sites was not different either. Therefore, there was no significant seasonal (relatively August vs. January) or spatial (cultivated vs. uncultivated) shift in C assimilation for *V. karroo* in the TKNP.

In contrast, August gs compared between plants in the uncultivated (0.15 ± 0.02 μmol.m^{-2}.s^{-1}) and cultivated (0.3 ± 0.05 μmol.m^{-2}.s^{-1}) sites were significantly different according to the Welch two sample t-test ($t_{5.3}$ = 2.9, p = 0.03). However, in January, there was no significant difference in gs measured in cultivated versus uncultivated sites. In the temporal dimension, the differences in gs in the same habitat compared in winter and summer were both significant. The mean gs in the cultivated site for January was 0.12 ± 0.04 μmol.m^{-2}.s^{-1} which was significantly less than the 0.3 ± 0.05 μmol.m^{-2}.s^{-1} in August ($t_{5.7}$ = 3.3, p = 0.02). Likewise, uncultivated site differences for the two sampling periods were significantly different (t_7 = 2.7, p = 0.03), the winter mean was 0.14 ± 0.02 μmol.m^{-2}.s^{-1} while the summer equivalent was 0.05 ± 0.01 μmol.m^{-2}.s^{-1}. Finally, January WUE in the cultivated site ranged between 57 and 431 units of water per unit of C accumulated. This was not significantly different to the pattern in the uncultivated site for the same season, where the minimum was 34 and the peak 1044 μmol.mol^{-1} ($t_{5.8}$ = -1.3, p = 0.3) (Fig 4.6). Respective means for the two treatments were 208 ± 87 μmol.mol^{-1} and 447 ± 170 μmol.mol^{-1} in the cultivated and uncultivated sites. In the winter when it was wet, the range in WUE for the cultivated site was 32 to 134 μmol.mol^{-1} while for plants growing in uncultivated site it was 79 to 553 μmol.mol^{-1}, with respective means of 73 ± 23 μmol.mol^{-1} and 378 ± 110 μmol.mol^{-1}, which were also not significantly different, but nearly so ($t_{3.3}$ = -

2.7, p = 0.07). As with the other gas exchange parameters measured, there was no significant difference in either the cultivated or uncultivated sites in terms of seasonal water use for both winter and summer sampling periods (Fig 4.6).

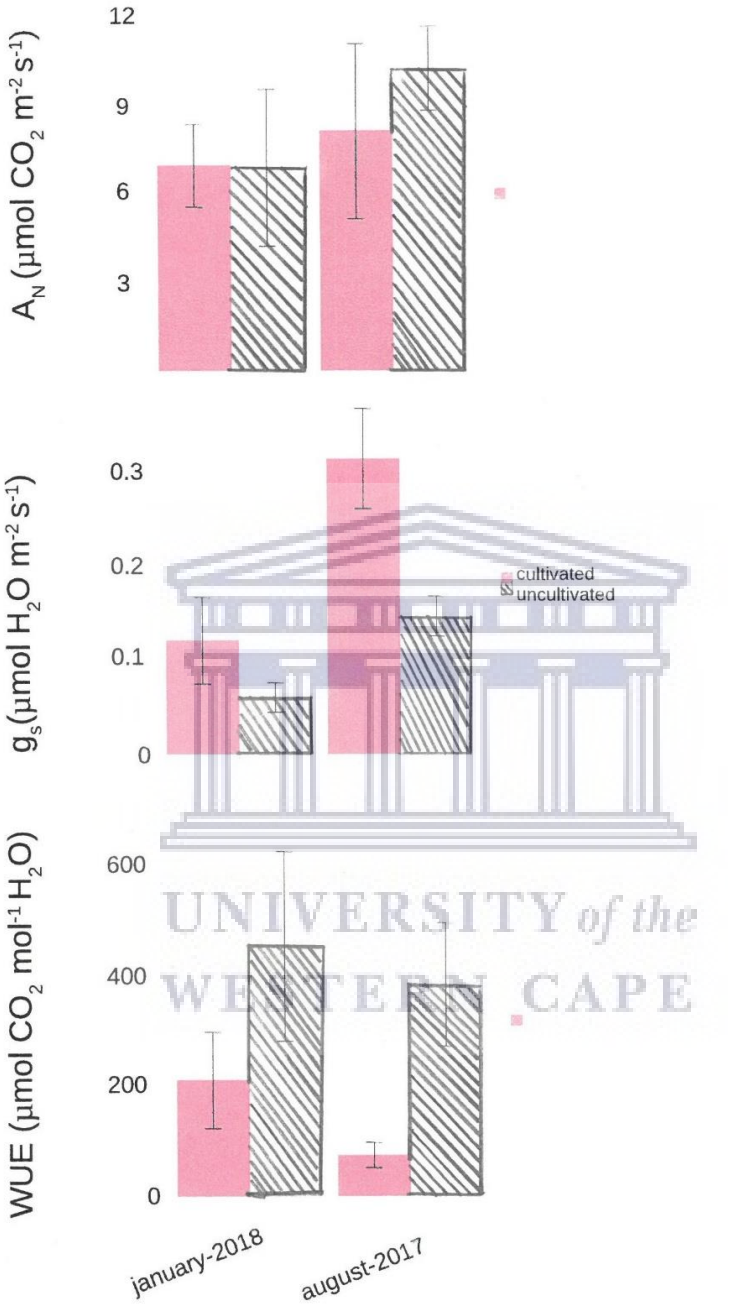

Figure 4.6: The mean seasonal response and standard error of above ground physiological processes for *V. karroo* plants growing in previously cultivated and uncultivated areas. A_N is the assimilation of C, gs is the stomatal conductance and WUE is the water use efficiency.

Chapter 5: General discussion and conclusions

5.1 General discussion

The need to stabilize the atmospheric CO_2 concentration is the greatest environmental challenge of this century (Amundson & Biardeau, 2018; Köne & Büke, 2019). These challenges may be reduced through the sequestration of C in vegetation or soil (Leksungnoen, 2017). According to the IPCC report on land, various forms of land degradation including cultivation are responsible for reducing the ability of ecosystems to sequester C. While the focus is often on degradation in forest and grassland ecosystems since they store the most C, arid ecosystems also have a role to play in reducing CO_2. Very few studies exist in the arid desert ecosystems that investigates the impact of existing or abandoned croplands on C storage potential of pristine ecosystems. This study added to our understanding of soil C storage potential on converted versus pristine land within a protected area. It is important to understand the ecological impact of degraded areas that are incorporated into protected areas by the acquisition of farmland. By examining the responses of soil C sequestration using gas exchange in *V. karroo* and soil microbial functional diversity across cultivated and uncultivated sites in the TKNP, this study will help guide authorities and researchers in the future.

The impact of cultivation of soil C storage is contextual, it depends on the crop being produced. This is because different crops will affect the soil differently, for example, some might promote C sequestration while others may not (Yuan et al., 2015). It is thus important to investigate the effect of abandoned lucerne croplands in the park. Specifically, this study examined (a) plant allometry, soil properties, soil and foliar C:N ratios, (b) identified soil microbes composition, diversity and activity and (c) measured C assimilation, stomatal conductance and water use efficiency. All these components of the thesis help

understand the impact of degraded or abandoned croplands on C sequestration related processes.

5.1.1 Vachellia karroo stature

According to the results, *V. karroo* assumes different growth forms depending on current and historical LU. The differences can be seen in the bimodal distribution of the basal circumference, height and diameter between cultivated and uncultivated sites. The study showed that the uncultivated site had trees with larger girths, more canopy volume and were taller than trees in cultivated sites. Similar findings have been observed by Vanninen and Mäkelä (2005), where tree growth of Scots pine *(Pinus sylvestris L.)* was shown to be more vigorous in sites that were more fertile, like the previously cultivated site, than pristine. Through the observation, and as a results of animal droppings found under the trees in the historically cultivated site, these trees seemed to be under more browsing pressure from mammalian herbivores especially due to proximity of watering points. These trees were seen to have a thornier and cagier architecture as a structural defence against herbivores like springbok and kudu compared to their counterparts (Charles-Dominique et al., 2017). There was also more evidence of animal droppings and footprints. Similar phenomenon has been documented elsewhere in the nearby savannas, but not in the succulent karoo where mammalian biomass is much lower (Archibald & Bond, 2003).

A previous study demonstrated that the selective pressure imposed by heavy browsing is likely to result in an architecture that favours structural defences (Archibald & Bond, 2003; Charles-Dominique et al., 2017). By contrast, sweet thorn trees in uncultivated sites were much more elongated and out of reach from browsers compared to those in cultivated areas. This architecture is reminiscent of the strategy employed by some *Acacias* in defence against giraffe browsing (Midgley et al., 2001). Further studies could be conducted to understand the impacts of LUC on plant architecture. The size and architectural disparities in *V. karroo* may reflect soil nutrient dynamics as conditioned by

historical LU. Indeed, soil and foliar C and N concentration suggest that this was the case in the present study as it was in Vanninen and Mäkelä (2005) as well as Mugunga and Mugumo (2013).

5.1.2 C & N as a function of LU

Land use change shapes soil C content because it controls soil aggregate stability, and could impinge negatively on ecosystem services such as C sequestration. However, land conversion from natural to agricultural land continues to grow due to the huge demand for food and fibre (Spurgeon et al., 2013; Deng et al., 2016; Mazzetto et al., 2016). Due to the high demand for food and increasing population globally, more and more land is being converted to agricultural use like livestock farming and cultivation (Saeed et al., 2018). Hence, when land pockets were added to national parks in the karoo, much of the old lands were previously used for cultivation of lucerne.

In this study, the results show that even though land pockets added into the TKNP were under cultivation, there was more C in cultivated soils than soils with no history of farming. The results comply with Yuan et al. (2015) who reported more soil C in land where lucerne cultivation had occurred. More C was attributed to lucerne which might have improved soil C storage capacity (Yuan et al., 2015). Additionally, these results were supported by Post and Kwon (2000) who reported that soil C accumulated when agricultural land was no longer used for cultivation and allowed to return to natural vegetation. Yuan et al. (2015) demonstrated that cultivation of lucerne had been widely used to rehabilitate degraded ecosystems, to improve vegetation cover and soil productive capacity. However, this improvement was not observed in this study. This may be due to climatic and environmental differences between the two sites. In addition, the overuse of fertilizers in the past, could have negatively affected the plant diversity since there was almost nothing growing in the old cultivated fields (*Personal observation*).

Houghton et al. (2012) emphasise that LUC strongly influences C content in soils. In contrast, Brady and Weil, (1996) argued that tilled soil usually experiences a net loss of C as yields are removed for human and animal consumption. Relatively little C is returned to the soil under agriculture. Niklaus et al. (2001) found that in undisturbed soils where there was no tillage, most of the organic matter produced by the vegetation would be returned to the soil, therefore was expected to have soil C content higher than tilled soil. This study showed that soil C was not affected by LUC, this means that the old lucerne croplands did not reduce soil C. The proximity of watering points and prevalence of animal dung in the old cultivated land might also have influenced soil C results in this study. The trees are also the only form of shade for animals as most plants in the landscape were well below a metre in height. Subsequent studies should sample soil well away from trees in order to control for these effects.

According to Paterson et al. (2009), the balance of C in soils is a function of inputs and outputs. Inputs were approximated in terms of photosynthesis and C inventory in soils, outputs were only estimated by litter fall. There was no account for C exit by soil respiration, which is an important flux (Niklaus et al., 2001). This is a major shortcoming of this study, and further investigations are required to properly account for all the C in the system including the understanding of inputs from animals. However, Osman et al. (2019) did measure estimates of C exhaled from Tankwa soils (5.2 mg C day^{-1}) and these were exceedingly low by global standards (76.5 Pg C yr^{-1}), on account of soils remaining dry for most of the year (Raich & Potter, 1995; Thomas et al., 2014). Therefore, it can also be assumed that allowing the land to rest with implementation of the rehabilitation interventions for more than 10 years since proclamation might have reversed the C losses during cultivation.

As expected, nitrogen concentration in soil was highly correlated with soil C (Cambardella & Elliott, 1993). The results showed a significant difference in soil C & N between the two study areas and thus suggest another mechanism outside mere organic matter was operating (Fig 4.2). To a large extent, the cultivated site had lower soil C & N

than the uncultivated site. More nitrogen may be explained by greater inputs of plant material on cultivated land which may have fertilised the soils with N. Other studies have shown that cultivation has a significant negative effect on the N fertility of soils in South Africa (du Preez & du Toit, 1995), the opposite of what was found in this study. However, this might be an influence of the animal dung inputs below the trees in cultivated area.

It was observed that the cultivated site had greater herbivore activity which may increase N inputs into the soils. Thus these soils may be richer in N as a result of animal micturition and defecation. Since the C:N ratio determines the decomposability of soil organic matter (Ostrowska & Porębska, 2015), it might also have an impact on plant nitrogen availability. It then follows that microbial composition and function will also be affected. The challenge in this study was that nitrogen was not determined in bare patches far away from the trees.

5.1.3 Response of soil microbial diversity to land-use change

Land use changes have major consequences for above-ground biodiversity, but their impact on soil microbial communities is poorly understood (Szoboszlay et al., 2017). A diverse microbial community often leads to greater C storage in the soil (Szoboszlay et al., 2017). In this study, microbial diversity in soils from the previously cultivated site were compared to those more pristine sites. The results indicated that there were no differences in microbial diversity and activity between the two habitats. The variety in C substrates in both sites was a reflection of healthy soil, plant and microbial interactions (Tilak et al., 2005; Yu-Hong et al., 2011). Brady and Weil (1996) argue that cultivation for mass production usually eliminates microbial diversity by optimising soil conditions for monoculture. It was expected that the cultivated site would have less variety of microbial life, however, this was not the case. Others have also found a similar result (Buckley & Schmidt, 2001). The uniformity in microbes showing a slightly greater diversity of soil microbial activity in the cultivated site complies with the uniformity in soil C showing slightly

higher in the cultivated site. The reasons mentioned above such as the ten year rest of the land and animal activity might be responsible for rehabilitating the microbial community.

When considering individual C source responses as a function of historic cultivation practices, results showed that the cultivated land was favourable to the composition, diversity and activity of the amines, carboxylic acid and carbohydrates. However, is was unfavourable to the amino acids and polymers carbon sources. It was therefore assumed that soil from previously cultivated areas contained fertilizer resistant microbes that could use carbon from amines, carboxylic acid and carbohydrates while the amino acid and polymers community couldn't tolerate fertilizer inputs (Marshall et al., 2007). Clearly, agricultural activities and the input of fertilizers in the area caused a significant impact on the amino acid and polymer consuming microbes. This is in agreement with results for catabolic responses of microbial activity in different LU reported by Mazzetto et al. (2016). Their study's findings indicated that few substrates had disappeared as a function of cultivation history, only some bacterial species were able to remain metabolically active. This means microbes were somehow suppressed in sites that were planted with crops, which may imply that the monoculture in plants led to an equivalent monoculture in bacteria.

5.1.4 Gas exchange response to land use change

The sequestration of atmospheric CO_2 as organic C in the biosphere attracts attention as a way to reduce GHG concentrations and curb climate change (Amundson & Biardeau, 2018; Murphy, 2020). Carbon sequestration by plants and soil is now regarded as an essential process to mitigate rising atmospheric CO_2 and ameliorate climate change (Lal,

2001; Leksungnoen, 2017). In this study, the ability of a national park to serve as a sizable C sink was tested with a soil survey and the gas exchange of *V. karroo* growing in two sites, cultivated and uncultivated, during the wet and dry seasons. Measured light saturated C assimilation for *V. karroo* were ±11 μmol.m-2.s-1 higher than the 14.5 μmol.$m^{-2}.s^{-1}$ previously reported for *Acacia* species (Eamus et al., 1999).

Kirschbaum and McMillan (2018) showed that increased CO_2 generally leads to some stomatal closure in most plants. According to Gao et al. (2004), stomatal conductance is an important process to quantify leaf function, since photosynthesis requires CO_2 via open stomata to fix C. The study tested the hypothesis that plant stomatal conductance varies as a function of LU and season. The results indicated that stomatal conductance in the winter rainy season was higher, in both sites, than in the drier summer. These results are consistent with Anav et al. (2018) where moisture availability in response to rainfall was the main driver behind stomatal conductance. Mészáros et al. (2009) and Anav et al. (2016) support that the variability of the stomatal opening was more strongly regulated by the availability of soil moisture than by land use history.

Clavijo-Herrera et al. (2018) suggested that high stomatal conductance could show the capacity of the stomata to allow CO_2 into the leaf, this implies that *V.karroo* in TKNP stored more C in winter as compared to summer. In a combined dataset of both seasons, *V. karroo* in historically cultivated sites maintained stomatal aperture during all seasons while *V. karroo* in uncultivated land opened more during winter, when water availability was higher. As a result*, V. karroo* in uncultivated land maintained a low stomatal conductance in summer seasons in order to conserve water within their tissue as compared to those in previously cultivated land. These results can imply that *V. karroo* in both uncultivated and previously cultivated land open more stomata in the wet season than when the air is drier. However, Urban et al. (2017) showed that stomatal conductance in poplar *(Populus deltoides)* and loblolly pine *(Pinus taeda)* increased with temperature. The difference in growth and size of the *V. karroo* as a function of cultivation histories could

have resulted in different physiological behaviour with the stomata responding to seasonal changes.

5.2 Conclusions

This study was able to show the effects of LU history on C storage and explaining the contribution of old cultivated land to C storage potential when added into protected areas. The results indicate that the variation found in plant stature could explain soil nutrients dynamics as conditioned by historical land use. This study demonstrated that even though the land pockets added to the TKNP were previously used for cultivation of lucerne, those contained more soil C than pristine land. This suggests that cultivation of lucerne in some instances may improve C storage in soil. In terms of microbial diversity and activity, the study showed that there were no differences in microbial responses to land use change, the diversity in C substrates in both sites revealed healthy soil, plant and microbial interactions. Similarly, there was no difference in stomatal conductance, the results showed that *V. karroo* in both uncultivated and previously cultivated land open their stomata more in the wet season.

The results from this study provided the evidence that previously cultivated land with lucerne when incorporated into the park and rested for more than a decade affected soil C storage in the area positively. Therefore park management could consider purchasing land that was previously used for cultivation of lucerne but rested for years if carbon storage is prioritized. However, this should take into context the fact that we did not sample away from the plant in which clearly no biodiversity value besides the improved microbial communities under the tree was accrued by having barren land for so many years despite considerable effort to restore the previously cultivated site. Further research should investigate landscape wide implications of succulent species in both cultivated versus uncultivated versus these approach of only focussing on a specific species in the riverine vegetation type. This would allow for extrapolation across the wider Succulent

Karoo Biome wherein cultivated land of Lucerne are still incorporated in protected areas. A comparison with areas that are still being cultivated might highlight the value of allowing the landscape to rehabilitate itself within a protected area setting.

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APPENDIX A

Vachellia karroo stature on previously cultivated and uncultivated land in the Tankwa Karoo National Park.

hab	plant	diam	ht	x	y	cir	volume
cul	8	6	3.2	3.2	4.10	18.85	41.98
	9	5.8	2.6	2.6	2.90	18.22	19.60
	7	7.5	3	3.9	3.40	23.56	39.78
	6	7	4.3	4.3	5.70	21.99	105.39
	10	3.7	3.5	4.4	4.00	11.62	61.60
unc	6	7	4.2	7.0	7.30	21.99	214.62
	5	8.5	4.7	8.3	8.00	26.70	312.08
	4	6.2	2.6	2.8	2.90	19.48	21.11
	3	10.1	4.5	5.9	5.00	31.73	132.75
	1	11	4.1	5.1	5.20	34.56	108.73

APPENDIX B

Percentage (%) of Soil and foliar C and N content on plant leaf and soil samples collected in previously cultivated and uncultivated land within Tankwa Karoo National Park. Geography

	Leaf			Soil		
Sample ID	Mass Weighed	N% reading	C% reading	Mass Weighed	N% reading	C% reading
cult 01	0.218	1.84	47.3	0.300	0.138	1.25
cult 01	0.217	1.88	47.5	0.303	0.087	0.96
cult 02	0.206	2.09	45.2	0.302	0.210	1.91
cult 02	0.207	2.09	45.3	0.302	0.181	1.71
cult 03	0.206	1.79	47.7	0.301	0.162	1.64
cult 03	0.206	1.77	47.6	0.303	0.165	1.41
cult 04	0.204	2.06	44.8	0.301	0.160	1.26
cult 04	0.204	2.27	44.9	0.301	0.151	1.19
cult 05	0.204	2.02	46.3	0.301	0.141	1.37
cult 05	0.204	2.01	46.4	0.300	0.200	1.66
cult 06	0.204	1.71	46.0	0.304	0.512	6.36
cult 06	0.202	1.79	45.8	0.302	0.604	7.28
cult 07	0.203	2.10	47.5	0.301	0.327	3.39
cult 07	0.204	2.06	47.3	0.301	0.386	3.95
cult 08	0.204	1.92	47.8	0.302	0.402	4.03
cult 08	0.203	2.08	47.7	0.303	0.339	3.39
cult 09	0.202	2.28	46.8	0.302	0.171	1.56
cult 09	0.202	2.00	46.8	0.303	0.193	1.64
cult 10	0.203	2.05	44.8	0.302	0.091	0.39
cult 10	0.202	2.06	44.9	0.301	0.095	0.42
unc 01	0.202	2.00	45.9	0.3017	0.04069	0.29627
unc 01	0.201	1.98	46.0	0.3006	0.06844	0.38885
unc 02	0.201	1.42	47.7	0.3008	0.05217	0.31191
unc 02	0.202	1.64	47.8	0.3004	0.03966	0.2711
unc 03	0.202	1.32	48.6	0.3001	0.01054	0.27328
unc 03	0.201	1.27	48.8	0.3006	0.07416	0.4833
unc 04	0.202	1.32	48.0	0.3011	0.03623	0.16327
unc 04	0.202	1.36	48.0	0.3022	0.03076	0.16052
unc 05	0.200	1.40	48.6	0.3048	0.0013	0.06252
unc 05	0.200	1.16	48.6	0.3044	0.01067	0.04107
unc 06	0.202	1.51	48.6	0.3014	0.07398	0.62972
unc 06	0.202	1.61	48.6	0.3022	0.05409	0.82228
unc 07	0.201	1.31	42.5	0.3003	0.0325	0.05361
unc 07	0.201	1.28	42.7	0.3027	0.03578	0.09216
unc 08	0.201	2.23	44.4	0.3025	0.09312	1.0029
unc 08	0.202	2.46	44.5	0.3038	0.11935	1.1372
unc 09	0.203	1.64	48.0	0.3002	0.13097	1.537
unc 09	0.201	1.69	48.1	0.3016	0.14042	1.5848
unc 10	0.202	1.95	48.1	0.3029	0.07509	0.63253
unc 10	0.203	2.04	48.3	0.3027	0.0757	0.83964

APPENDIX C

Values for the different soil and plant variables in previously cultivated and uncultivated land in Tankwa Karoo National Park.

hab	plant	bd ($g.cm^{-3}$)	wc (%)	sand (%)	clay (%)	silt (%)	EC ($mS.cm^{-1}$)	pH	acC (%)	acN (%)	solC (%)	solN (%)	clip (g)	fall (g)	rate ($g.m^{-2}.day^{-1}$)
cul	1	1.1	4.5	30	23	48	604	8.3	47.1	1.86	1.11	0.11	52.7	12	0.13
	2	0.9	5.4	25	30	45	997	7.9	45.3	2.09	1.81	0.2	57.2	18.8	0.03
	3	0.8	4.7	28	33	40	780	7.8	47.7	1.78	1.53	0.16	55.7	23.2	0.43
	4	1	4.7	33	25	43	890	8	44.9	2.17	1.23	0.16	61.2	27.4	0.16
	5	0.9	4.9	30	30	40	1120	7.7	46.3	2.02	1.51	0.17	59.3	46.2	0.07
	6	0.5	5.2	60	20	20	1652	7.6	45.9	1.75	6.82	0.56	56.8	23.9	0.08
	7	0.8	4.7	45	28	28	1225	7.7	47.4	2.08	3.67	0.36	55.2	31.3	0.15
	8	0.7	4.8	48	38	15	1165	7.4	47.7	2	3.71	0.37	54.5	4.5	0.01
	9	0.8	4.3	73	10	18	941	7.9	46.8	2.14	1.6	0.18	55.8	32.3	0.02
	10	0.9	4.3	58	20	23	1443	7.5	45.4	2.06	0.4	0.09	54.9	26	0.03
unc	1	1.1	2.7	73	8	20	275	8.3	45.9	1.99	0.34	0.06	56	58.8	0.03
	2	1.1	2.5	70	15	15	327	8.6	47.8	1.53	0.29	0.05	52.9	16.1	0.19
	3	0.9	3.4	65	5	30	682	8.9	48.7	1.3	0.38	0.04	52.2	55.3	0.06
	4	1.4	3.1	73	8	20	224	8.8	48	1.34	0.16	0.03	52.6	35.6	0.06
	5	0.9	3.9	83	5	13	299	8.3	48.6	1.28	0.05	0.01	47.9	23.4	0.12
	6	1	3.7	63	5	33	9700	7.7	48.6	1.56	0.73	0.06	51.8	9.5	0.08
	7	1.2	4.5	20	25	55	37000	7.6	42.6	1.3	0.07	0.18	55.5	38.5	0.04
	8	1.1	5.7	30	18	53	754	8	44.4	1.34	1.07	0.11	56.1	11.3	0.02
	9	1	6.2	3	28	70	785	7.9	48	1.66	1.56	0.14	52.4	37.6	0.04
	10	1.1	3.2	80	5	15	305	8.2	48.2	2	0.74	0.08	51.4	46.1	0.05
cul	1	1.1	4.5	30	23	48	604	8.3	47.1	1.86	1.11	0.11	52.7	12	0.13
	2	0.9	5.4	25	30	45	997	7.9	45.3	2.09	1.81	0.2	57.2	18.8	0.03
	3	0.8	4.7	28	33	40	780	7.8	47.7	1.78	1.53	0.16	55.7	23.2	0.43
	4	1	4.7	33	25	43	890	8	44.9	2.17	1.23	0.16	61.2	27.4	0.16
	5	0.9	4.9	30	30	40	1120	7.7	46.3	2.02	1.51	0.17	59.3	46.2	0.07
	6	0.5	5.2	60	20	20	1652	7.6	45.9	1.75	6.82	0.56	56.8	23.9	0.08
	7	0.8	4.7	45	28	28	1225	7.7	47.4	2.08	3.67	0.36	55.2	31.3	0.15
	8	0.7	4.8	48	38	15	1165	7.4	47.7	2	3.71	0.37	54.5	4.5	0.01
	9	0.8	4.3	73	10	18	941	7.9	46.8	2.14	1.6	0.18	55.8	32.3	0.02
	10	0.9	4.3	58	20	23	1443	7.5	45.4	2.06	0.4	0.09	54.9	26	0.03
unc	1	1.1	2.7	73	8	20	275	8.3	45.9	1.99	0.34	0.06	56	58.8	0.03
	2	1.1	2.5	70	15	15	327	8.6	47.8	1.53	0.29	0.05	52.9	16.1	0.19
	3	0.9	3.4	65	5	30	682	8.9	48.7	1.3	0.38	0.04	52.2	55.3	0.06
	4	1.4	3.1	73	8	20	224	8.8	48	1.34	0.16	0.03	52.6	35.6	0.06
	5	0.9	3.9	83	5	13	299	8.3	48.6	1.28	0.05	0.01	47.9	23.4	0.12
	6	1	3.7	63	5	33	9700	7.7	48.6	1.56	0.73	0.06	51.8	9.5	0.08
	7	1.2	4.5	20	25	55	37000	7.6	42.6	1.3	0.07	0.18	55.5	38.5	0.04
	8	1.1	5.7	30	18	53	754	8	44.4	1.34	1.07	0.11	56.1	11.3	0.02
	9	1	6.2	3	28	70	785	7.9	48	1.66	1.56	0.14	52.4	37.6	0.04
	10	1.1	3.2	80	5	15	305	8.2	48.2	2	0.74	0.08	51.4	46.1	0.05

APPENDIX D

Measured light saturated net CO_2 assimilation rates (A_N), stomatal conductance (g_s), transpiration rate (tra), leaf-to-air vapour pressure deficit (Vpd) and water use efficiency (WUE) of *Vk* at different light intensity (par) during winter and summer in previously cultivated and uncultivated land within Tankwa Karoo National Park.

Mon	Hab	plant	Obs	A_N ($\mu mol.\ CO_2.m^{-2}.s^{-1}$)	Gs ($mmol.H_2O\ m^{-2}.s^{-1}$)	Tra ($mol.H_2O\ m^{-2}.s^{-1}$)	Vpd (kPa)	Par ($\mu mol.m^{-2}.s^{-1}$)	Wue ($\mu mol.\ CO_2.m^{-1}.H_20$)
aug	cul	1	1	9.98	0.45	4.29	2.82	1999	65
		1	2	9.9	0.49	4.49	2.74	1599	59
		1	3	8.25	0.41	4.58	3.3	1000	59
		1	4	5.06	0.39	4.45	3.37	499	38
		1	5	0	0.36	3.33	2.7	101	-8
		2	1	24.85	0.38	4.35	2.81	2000	159
		2	2	24.16	0.37	4.1	2.75	1522	161
		2	3	23.39	0.38	4.01	2.63	1023	152
		2	4	18.23	0.33	3.49	2.57	758	134
		2	5	0.27	0.33	3.15	2.35	99	2
		2	6	0	0.32	2.9	2.25	1	-34
		8	1	19.36	0.4	0	5.1	2000	202
		8	2	14.95	0.41	4.96	4.93	1001	150
		8	3	11.81	0.4	4.73	4.87	499	122
		8	4	0.33	0.41	4.85	4.83	120	3
		9	1	4.58	0.09	1.07	1.17	2065	51
		9	2	4.84	0.11	1.27	1.19	1502	46
		9	3	4.34	0.11	1.26	1.22	1001	42
		9	4	3.35	0.11	1.31	1.23	500	32
		9	5	0.7	0.11	1.28	1.2	98	7
		10	1	3.3	0.36	3.16	4.67	1999	49
		10	2	7.18	0.37	3.53	5.06	1501	105
		10	3	5.24	0.37	3.76	5.41	1001	77
		10	4	2.34	0.33	3.37	5.4	499	38
aug	unc	2	1	13.25	0.19	2.25	2.08	2000	123
		2	2	12.19	0.2	2.57	2.23	1502	106
		2	3	12.11	0.16	1.93	2.02	1000	128
		2	4	8.3	0.18	2.04	1.94	500	80
		2	5	3.92	0.18	2.12	1.98	295	37
		2	6	0	0.19	2.11	1.92	100	-6
		3	1	11.75	0.14	1.98	6.99	1999	429
		3	2	12.76	0.21	2.76	6.44	1564	305
		3	3	10.71	0.12	1.74	7.15	1024	455
		3	4	8.38	0.12	1.73	6.89	518	344
		3	5	0.61	0.13	1.72	6.7	109	24
		3	6	3.21	0.18	2.23	6.07	97	90
		7	1	18.45	0.16	1.83	7.68	2079	800
		7	2	22.49	0.22	2.68	8.1	1999	692
		7	3	21.58	0.17	2.28	8.69	1502	841
		7	4	19.52	0.17	2.07	8.15	1344	788
		7	5	14.51	0.18	1.98	7.16	650	536
		7	6	1.08	0.24	2.28	6.26	135	30
		8	1	12.33	0.07	0.79	5.39	2000	876

Mon	Hab	plant	Obs	A_N ($\mu mol.\ CO_2.m^{-2}.s^{-1}$)	Gs ($mmol.H_2O\ m^{-2}.s^{-1}$)	Tra ($mol.H_2O\ m^{-2}.s^{-1}$)	Vpd (kPa)	Par ($\mu mol.m^{-2}.s^{-1}$)	Wue ($\mu mol.\ CO_2.m^{-1}.H_20$)
		8	3	11.96	0.08	0.76	5.12	1806	839
		8	3	11.13	0.08	0.76	4.93	1016	752
		8	4	9.98	0.1	0.91	4.88	520	553
		8	5	1.45	0.12	1.19	5.04	100	63
		9	1	12.86	0.09	0.88	3.15	2235	478
		9	2	12.65	0.07	0.67	2.97	1022	582
		9	3	5.17	-0.02	-0.25	3.02	100	-661
		9	4	0.05	-0.07	-0.72	3.12	0	-2
jan	cul	1	1	10.11	-0.09	-0.86	3.46	1643	-432
		1	2	6.79	0.04	0.37	3.43	1244	666
		1	3	11.41	0.06	0.57	3.4	826	717
		1	4	4.66	0.04	0.38	3.38	400	431
		1	5	6	0.09	0.75	3.3	62	278
		1	6	4.42	0.09	0.81	3.3	0	189
		3	1	8.8	0.05	0.46	2.34	1632	475
		3	2	8.01	0.05	0.56	2.8	1236	415
		3	3	9.28	0.04	0.49	2.75	820	540
		3	4	4.65	0.05	0.49	2.59	398	258
		3	5	4.88	0.02	0.25	2.45	62	502
		3	6	0.24	0.02	0.16	2.46	0	40
		5	1	10.77	0.01	0.05	1.25	1652	2933
		5	2	9.25	0.01	0.09	1.23	1654	1366
		5	3	11.34	-0.03	-0.22	1.12	828	-630
		5	4	7.22	-0.02	-0.13	0.99	403	-568
		5	5	3.92	-0.02	-0.11	0.75	63	-297
		5	6	1.17	0.33	1.19	0.63	0	6
		9	1	13.54	0.1	1.01	1.09	1623	151
		9	2	12.87	0.14	1.32	1.04	1231	104
		9	3	11.19	0.15	1.39	0.99	818	81
		9	4	8.28	0.16	1.38	0.94	397	58
		9	5	1.94	0.14	1.14	0.9	61	15
		9	6	0.5	0.16	1.25	0.88	0	4
		10	1	17.43	0.14	1.6	2.07	1629	233
		10	2	15.91	0.2	2.05	1.94	1232	154
		10	3	14.6	0.23	2.24	1.81	818	121
		10	4	10.33	0.23	2.15	1.72	396	85
		10	5	3.25	0.24	2.13	1.62	61	25
		10	6	0.78	0.23	1.82	1.49	0	6
jan	unc	1	1	15.7	0.1	0.88	2.48	1641	465
		1	2	12.8	0.13	1.05	2.3	1244	293
		1	3	10.96	0.07	0.55	2.35	825	489
		1	4	10.78	0.09	0.73	2.25	401	351
		1	5	6.37	0.13	0.9	2.1	63	155
		1	6	1.9	0.14	1.01	2.1	0	41
		5	1	7	0.04	1.28	11.17	1594	654
		5	2	9.08	0.03	1.06	12.75	1205	1170
		5	3	2.73	0.02	0.72	12.17	799	496
		5	4	2.83	0.02	0.65	11.5	386	539
		5	5	2.19	0.01	0.37	10.67	59	671
		5	6	0	0.01	0.4	10.75	0	-76
		6	1	21.38	0.1	3.29	19.98	1585	1380

Mon	Hab	plant	Obs	A_N ($\mu mol.\ CO_2.m^{-2}.s^{-1}$)	Gs ($mmol.H_2O\ m^{-2}.s^{-1}$)	Tra ($mol.H_2O\ m^{-2}.s^{-1}$)	Vpd (kPa)	Par ($\mu mol.m^{-2}.s^{-1}$)	Wue ($\mu mol.\ CO_2.m^{-1}.H_20$)
	6	2		10.28	0.08	2.88	21.13	1587	809
	6	3		21.7	0.1	3.61	22.09	1194	1418
	6	4		15.45	0.09	2.72	17.3	382	1044
	6	5		16.91	0.1	2.83	16.66	58	1057
	6	6		8.36	0.05	1.45	18.13	0	1124
	8	1		13.94	0.06	0.66	2.03	1629	455
	8	2		9.51	0.06	0.63	1.99	1232	315
	8	3		5.34	0.03	0.37	1.92	818	295
	8	4		4.22	0.03	0.3	1.82	397	270
	8	5		2.75	0.05	0.44	1.73	61	113
	8	6		0.8	0.02	0.15	1.66	0	93
	9	1		7.59	0.02	0.18	1.41	1646	627
	9	2		3.86	0.03	0.28	1.43	1643	210
	9	3		3.31	0.04	0.42	1.42	1242	117
	9	4		3.43	0.03	0.33	1.4	825	152
	9	5		1.28	0.06	0.54	1.35	400	34

APPENDIX E

R Code snippets

```
### budzi vhudzi tankwa cultivation ###

cult <- read.csv("aca.csv",header=T,sep=",")
attach(cult)
se <- function(x) sqrt(var(x)/length(x))
summary(subset(cult[3:7],hab=="cul"))
summary(subset(cult[8:12],hab=="cul"))
summary(subset(cult[3:7],hab=="unc"))
summary(subset(cult[8:12],hab=="unc"))

apply(cult[1:10,3:12],2,se)
apply(cult[11:20,3:12],2,se) ### se"s for everything

se(c(con[11:15],con[18:20])) ### EC outliers removed
se(c(rate[1:2],rate[3:10]))
se(c(rate[11],rate[13:14],rate[16:20])) ### outliers removed from both cul and uncul

t.test(subset(bd,hab=="cul"),subset(bd,hab=="unc"))  ### testing
t.test(subset(wc,hab=="cul"),subset(wc,hab=="unc"))
t.test(subset(fines,hab=="cul"),subset(fines,hab=="unc"))
t.test(subset(con,hab=="cul"),subset(con,hab=="unc"))
t.test(subset(pH,hab=="cul"),subset(pH,hab=="unc"))
t.test(subset(acc,hab=="cul"),subset(acc,hab=="unc"))
t.test(subset(acn,hab=="cul"),subset(acn,hab=="unc"))
t.test(subset(solc,hab=="cul"),subset(solc,hab=="unc"))
t.test(subset(soln,hab=="cul"),subset(soln,hab=="unc"))
t.test(subset(rate,hab=="cul"),subset(rate,hab=="unc"))
t.test(subset(con,hab=="cul"),c(con[11:15],con[18:20])) ### EC outliers removed


boxplot(subset(bd,hab=="cul"),subset(bd,hab=="unc"),col=c(colors()[259],colors()[139]))

boxplot(subset(wc,hab=="cul"),subset(wc,hab=="unc"),col=c(colors()[259],colors()[139]))

boxplot(subset(fines,hab=="cul"),subset(fines,hab=="unc"),col=c(colors()[259],colors()[139]))

boxplot(subset(con,hab=="cul"),subset(con,hab=="unc"),col=c(colors()[259],colors()[139]))   ### outliers not removed

boxplot(subset(con,hab=="cul"),c(con[11:15],con[18:20]),col=c(colors()[259],colors()[139])) ### two outliers removed, both in uncul

boxplot(subset(pH,hab=="cul"),subset(pH,hab=="unc"),col=c(colors()[259],colors()[139]))

boxplot(subset(acc,hab=="cul"),subset(acc,hab=="unc"),col=c(colors()[259],colors()[139]))

boxplot(subset(acn,hab=="cul"),subset(acn,hab=="unc"),col=c(colors()[259],colors()[139]))

boxplot(subset(solc,hab=="cul"),subset(solc,hab=="unc"),col=c(colors()[259],colors()[139]))
```

```
boxplot(subset(soln,hab=="cul"),subset(soln,hab=="unc"),col=c(colors()[259],colo
rs()[139]))
### acacia stature ###

aca  <- read.csv("aca.stat.csv",header=T,sep=",")
se <- function(x) sqrt(var(x)/length(x))

boxplot(subset(cir,hab=="cul"),subset(cir,hab=="unc"))
boxplot(subset(vol,hab=="cul"),subset(vol,hab=="unc"))


summary(cir[1:5])
se(cir[1:5])

summary(cir[6:10])
se(cir[6:10])

summary(vol[1:5])
se(vol[1:5])

summary(vol[6:10])
se(vol[6:10])

t.test(cir[1:5],cir[6:10])
t.test(vol[1:5],cir[6:10])

plot(ht[1:5]~diam[1:5],pch=25,cex=1.5,xlim=c(3,12),ylim=c(2,5))
points(ht[6:10]~diam[6:10],pch=16,cex=1.5)
abline(lm(ht~diam),col="red")
lmaca <- lm(ht~diam)
lmcul <- lm(ht[1:5]~diam[1:5])
lmunc <- lm(ht[6:10]~diam[6:10])


### ecophysiology of acacia karoo ###

aca  <- read.csv("licor.csv",header=T,sep=",")
attach(aca)
summary(aca[5:9])
se(wue)
cv <- function(mean, sd){
               (sd/mean)*100
                }
### cultivates ###
plot(subset(photo, month=="aug"&hab=="cul"&plant=="1")~subset(par,
month=="aug"&hab=="cul"&plant=="1"),
      type="b",ylim=c(0,25),xlim=c(0,2300),pch=16,
     xlab="",ylab="")
lines(subset(photo, month=="aug"&hab=="cul"&plant=="2")~subset(par,
month=="aug"&hab=="cul"&plant=="2"),
      type="b",ylim=c(0,25),xlim=c(0,2300),pch=16,
     xlab="",ylab="")
lines(subset(photo, month=="aug"&hab=="cul"&plant=="8")~subset(par,
month=="aug"&hab=="cul"&plant=="8"),
      type="b",ylim=c(0,25),xlim=c(0,2300),pch=16,
     xlab="",ylab="")
lines(subset(photo, month=="aug"&hab=="cul"&plant=="9")~subset(par,
month=="aug"&hab=="cul"&plant=="9"),
      type="b",ylim=c(0,25),xlim=c(0,2300),pch=16,
     xlab="",ylab="")
```

ecoplates

```
eco <- read.csv("~/Dropbox/R/ecotknp.csv",header=T,sep=",")
attach(eco)
se <- function(x) sqrt(var(x)/length(x))

c(min(cul),max(cul),mean(cul),sd(cul))
c(min(unc),max(unc),mean(unc),sd(unc))

# max of the y -axis
max(eco[,3:4]) # 2.6

svg("tk-ecoplate.svg",height=20,width=20,pointsize=30)
boxplot(cul,unc, col=c(colors()[139],colors()[55], ylim=c(0,2.5)))

boxplot(subset(cul, group=="aa"),subset(cul,group=="ami"),
subset(cul,group=="ca"),subset(cul,group=="carb"),subset(cul,group=="misc"),subs
et(cul,group=="poly"),col=colors()[139], ylim=c     (0,2.5))
boxplot(subset(unc, group=="aa"),subset(unc,group=="ami"),
subset(unc,group=="ca"),subset(unc,group=="carb"),subset(unc,group=="misc"),subs
et(unc,group=="poly"),col=colors()[55], ylim=c(     0,2.5))

c(min(subset(cul, group=="aa")),
max(subset(cul, group=="aa")),
mean(subset(cul, group=="aa")),
sd(subset(cul, group=="aa")))

c(min(subset(cul, group=="ami")),
max(subset(cul, group=="ami")),
mean(subset(cul, group=="ami")),
sd(subset(cul, group=="ami")))

c(min(subset(cul, group=="ca")),
max(subset(cul, group=="ca")),
mean(subset(cul, group=="ca")),
sd(subset(cul, group=="ca")))

c(min(subset(cul, group=="carb")),
max(subset(cul, group=="carb")),
mean(subset(cul, group=="carb")),
sd(subset(cul, group=="carb")))

c(min(subset(cul, group=="misc")),
max(subset(cul, group=="misc")),
mean(subset(cul, group=="misc")),
sd(subset(cul, group=="misc")))

c(min(subset(cul, group=="poly")),
max(subset(cul, group=="poly")),
mean(subset(cul, group=="poly")),
sd(subset(cul, group=="poly")))

c(min(subset(unc, group=="aa")),
max(subset(unc, group=="aa")),
mean(subset(unc, group=="aa")),
```

PCA

```
ecopca <- read.csv("tk-pca.csv",header=T,sep=",")
attach(ecopca)
```

```
373 library(ade4)
374 pca1  <- dudi.pca(ecopca[1:2],scan = FALSE)
375 biplot(pca1) # this is ugly plot
376
377 # set up a better plot we"ll use later
378
379
svg("~/Dropbox/postgrads/pauline/plots/pca.svg",height=20,width=20,pointsize=30)
380 ppp <- ggplot() + coord_fixed() +
381   labs(x="Comp1, Axis1", y="Comp2, Axis2") +
382   geom_hline(yintercept=0, col="darkgrey") +
383   geom_vline(xintercept=0, col="darkgrey")
384
385 pca1.dfs <- data.frame(pca1$li,gro)
386 site <- names(ecopca[1:2])
387 pca1.dfl <- data.frame(5*pca1$co[,1:2], site)
388 ppp + geom_point(data=pca1.dfs, aes(x=Axis1, y=Axis2, col=gro)) +
389   geom_text(data=pca1.dfl, aes(x=Comp1, y=Comp2, label=site))
390
391 barplot(pca1$eig/sum(pca1$eig))
```

APPENDIX F

Microbial response during experiment on soil samples collected from previously cultivated and uncultivated land within Tankwa Karoo National Park.

functional group	carbon source	c0	c25	c51	c72	c95	c126	c165	c189	un0	un25	un51	un72	un95	un126	un165	un189
Amino acids	L-Arginne	0.9	1.5	1.1	1.5	2.1	2.5	2.6	2.6	1.0	1.1	0.8	1.5	1.7	2.0	2.3	2.4
	L-Arginne	0.9	1.7	0.9	1.9	2.2	2.6	2.7	2.6	0.8	1.0	0.8	1.5	1.7	1.9	2.1	2.1
	L-Arginne	1.0	1.4	1.2	1.8	2.1	2.5	2.3	2.3	0.8	1.2	0.9	1.6	1.8	2.0	2.1	2.1
	L-Asparagine	0.8	2.2	1.1	2.1	2.8	3.0	2.7	2.2	1.0	1.7	1.1	2.5	2.9	3.3	3.5	3.5
	L-Asparagine	1.1	2.2	1.7	2.6	3.2	3.1	2.9	2.9	0.9	1.3	0.9	2.0	2.4	2.9	3.0	3.1
	L-Asparagine	0.9	2.1	1.2	2.8	3.0	2.8	3.2	3.0	0.9	1.5	1.0	2.4	2.6	2.8	2.8	2.7
	L-Phenylalanine	0.9	1.6	1.1	1.7	1.8	2.2	3.0	2.5	0.9	1.1	1.1	1.4	1.7	1.7	2.0	2.2
	L-Phenylalanine	1.0	1.0	1.1	1.1	1.3	1.8	2.5	2.7	0.9	0.9	0.9	1.2	1.3	1.3	1.5	1.8
	L-Phenylalanine	1.1	1.3	1.3	1.4	1.6	2.1	2.2	2.2	1.0	1.1	1.0	1.3	1.4	1.5	1.8	1.9
	L-Serine	1.1	1.6	1.0	2.1	2.0	2.1	2.5	2.4	1.0	1.3	1.0	1.6	1.8	2.1	2.6	2.5
	L-Serine	0.8	1.5	1.1	1.9	2.0	2.1	2.0	2.0	1.0	1.3	1.0	1.8	2.2	2.6	2.6	2.5
	L-Serine	1.0	1.7	1.2	2.5	2.4	2.5	2.4	2.4	0.9	1.3	1.0	1.9	2.0	2.0	1.9	1.8
	L-Threonine	1.1	1.4	1.4	1.9	1.8	2.0	2.1	2.2	0.8	0.7	0.8	0.8	0.9	0.9	1.1	1.1
	L-Threonine	0.9	1.1	1.1	1.2	1.4	1.5	1.6	1.8	0.9	0.9	0.9	1.0	1.1	1.1	1.2	1.2
	L-Threonine	1.0	1.1	1.2	1.2	1.2	1.2	1.3	1.5	1.0	1.1	1.1	1.3	1.2	1.2	1.2	1.2
	Glycl-L-Glutamic	0.8	0.9	0.8	1.0	1.3	1.7	2.1	2.4	0.8	0.8	0.8	1.0	1.0	1.2	1.5	1.7
	Glycl-L-Glutamic	0.8	1.5	1.2	1.7	1.7	1.6	2.3	1.8	0.8	0.8	0.8	1.1	1.2	1.3	1.6	1.8
	Glycl-L-Glutamic	1.1	1.2	1.2	1.4	1.5	1.7	1.8	1.7	1.0	0.9	0.9	1.2	1.2	1.2	1.3	1.5
Carbohydrates	b-Methyl-D-Gluc	0.9	1.9	1.3	1.9	1.9	1.9	1.9	1.9	1.0	1.4	1.1	1.4	1.5	1.5	1.4	1.4
	b-Methyl-D-Gluc	1.0	2.4	1.3	2.7	2.6	2.7	2.6	2.6	0.8	1.0	0.7	1.3	1.3	1.3	1.3	1.3
	b-Methyl-D-Gluc	1.2	1.3	1.0	1.6	1.7	1.7	1.7	1.6	0.8	1.2	0.8	1.3	1.2	1.2	1.1	1.1

functional group	carbon source	c0	c25	c51	c72	c95	c126	c165	c189	un0	un25	un51	un72	un95	un126	un165	un189
	D-Xylose	1.0	2.2	1.2	2.5	2.6	2.7	2.6	2.5	1.1	1.3	1.0	1.5	1.5	1.5	1.4	1.4
	D-Xylose	1.0	1.4	1.3	2.1	2.2	2.6	2.5	2.5	0.8	0.9	0.8	1.7	1.8	1.7	1.9	1.7
	D-Xylose	1.0	2.3	1.4	2.9	3.0	3.0	3.0	2.9	0.8	0.9	0.8	1.4	1.7	1.7	1.8	2.0
	i-Erythritol	0.8	1.0	0.9	1.4	2.1	2.5	2.6	2.7	0.9	1.0	0.8	1.3	1.7	1.8	2.2	2.4
	i-Erythritol	1.3	1.3	1.2	1.4	1.4	1.7	2.5	2.4	0.8	0.9	0.7	1.2	1.5	1.5	2.2	2.4
	i-Erythritol	1.1	1.2	1.3	1.4	1.5	2.5	3.0	3.1	0.7	0.9	0.8	1.0	1.2	1.5	1.7	1.9
	D-Mannitol	0.9	2.0	1.1	2.3	2.5	2.5	2.6	2.6	1.0	1.7	1.0	1.8	1.9	2.0	1.9	1.9
	D-Mannitol	0.7	1.7	1.5	2.2	2.6	2.1	2.2	2.3	0.8	1.2	1.2	2.0	2.0	2.3	2.8	2.9
	D-Mannitol	1.1	2.0	1.2	2.2	2.2	2.2	2.2	2.3	0.7	2.5	0.8	3.1	3.2	3.2	3.2	3.1
	N-Acetyl-D-Gluc	1.0	2.0	1.6	1.9	1.9	2.0	1.9	1.9	1.0	1.4	1.0	1.3	1.3	1.3	1.3	1.3
	N-Acetyl-D-Gluc	1.3	2.3	1.5	3.0	2.6	2.7	2.7	2.7	0.8	1.3	0.8	1.5	1.7	1.7	1.7	1.6
	N-Acetyl-D-Gluc	1.0	2.5	1.5	2.7	2.8	2.9	2.8	2.8	0.7	1.5	0.8	1.8	2.0	2.0	2.1	2.0
	D-Cellobiose	0.8	1.7	1.2	1.8	1.7	1.5	1.4	1.3	0.8	0.9	0.8	1.2	1.5	1.7	1.7	1.7
	D-Cellobiose	0.7	2.6	1.1	2.7	2.8	2.8	2.7	2.5	0.7	1.1	0.9	1.6	1.8	1.9	1.9	1.9
	D-Cellobiose	0.7	2.3	0.9	2.5	2.6	2.6	2.5	2.5	0.8	1.1	0.8	2.1	2.4	2.5	2.8	2.7
	a-D-Lactose	0.9	0.8	1.0	1.2	1.4	1.5	1.5	1.5	0.9	0.9	0.8	1.0	1.1	1.2	1.2	1.2
	a-D-Lactose	0.8	0.9	0.9	1.2	1.7	2.4	2.8	2.8	0.8	0.8	0.7	0.8	1.2	2.0	2.7	2.8
	a-D-Lactose	0.8	1.2	1.0	1.8	1.9	2.0	2.0	2.0	0.8	0.8	0.8	0.8	1.1	2.0	2.7	2.9
Polymers	Tween 40	0.8	1.8	1.1	1.8	2.1	2.5	3.0	3.1	0.9	1.4	0.9	2.0	2.2	2.4	2.4	2.5
	Tween 40	0.8	1.7	0.9	2.0	2.3	2.7	3.2	3.4	0.7	0.8	0.8	1.2	1.7	2.0	2.4	2.5
	Tween 40	1.0	1.6	1.2	2.4	2.6	2.8	2.9	3.0	0.7	1.0	0.8	1.5	1.9	2.3	2.7	2.9
	Tween 80	0.8	1.6	1.1	1.8	1.6	1.5	0.9	0.6	1.0	1.3	1.0	1.3	1.3	1.3	1.2	1.0
	Tween 80	0.6	1.5	0.8	2.0	2.3	2.5	2.7	2.7	0.8	1.5	0.7	1.1	1.7	2.3	2.6	2.9
	Tween 80	1.1	2.0	1.1	2.6	3.0	3.1	3.1	3.3	0.7	0.9	0.9	1.6	1.7	2.0	2.4	2.5
	a-Cyclodextrin	0.8	1.1	1.5	0.9	0.8	0.9	0.9	0.9	1.9	1.7	1.6	1.5	1.6	1.5	1.5	1.5
	a-Cyclodextrin	0.8	1.3	1.0	1.8	2.1	2.2	2.0	1.8	0.7	0.8	0.7	1.1	1.3	1.7	2.1	2.3
	a-Cyclodextrin	0.9	1.8	0.9	2.3	2.7	2.7	2.6	2.5	0.7	0.8	0.8	1.2	1.4	1.8	2.4	2.7
	Glycogen	0.8	1.4	1.1	1.6	1.7	1.6	1.4	1.3	0.8	1.2	0.9	1.1	1.1	1.1	1.0	1.0

functional group	carbon source	c0	c25	c51	c72	c95	c126	c165	c189	un0	un25	un51	un72	un95	un126	un165	un189
	Glycogen	1.3	1.7	1.3	2.3	2.6	2.9	3.0	3.0	0.8	1.0	0.8	1.2	1.5	1.9	2.2	2.2
	Glycogen	0.9	1.4	1.2	2.0	2.3	2.7	2.8	2.6	0.8	1.0	0.9	1.3	1.5	1.9	2.4	2.6
Carboxylic acid	D-Galactonic Acid Lac	0.9	2.2	1.3	2.3	2.3	2.2	2.2	2.1	1.6	2.1	1.5	2.3	2.2	2.3	2.3	2.2
	D-Galactonic Acid Lact	0.9	2.2	1.2	2.5	2.4	2.4	2.4	2.4	0.8	1.2	0.7	1.9	2.0	2.0	2.0	2.0
	D-Galactonic Acid Lact	0.9	2.1	1.2	2.1	2.1	2.1	2.0	2.0	0.9	1.8	1.0	2.1	2.0	1.9	2.0	2.0
	D-Galacturonic acid	1.1	2.2	1.3	2.6	2.6	2.4	2.8	2.3	1.1	1.7	1.2	2.3	2.4	2.4	2.5	2.4
	D-Galacturonic acid	1.2	2.5	1.7	2.7	2.8	2.8	2.7	2.8	0.8	1.3	0.8	2.0	2.2	2.2	2.4	2.5
	D-Galacturonic acid	0.8	2.3	1.2	2.4	2.5	2.5	2.4	2.4	1.0	1.5	1.1	2.2	2.5	2.5	2.6	2.5
	2 HydroxyBenzoicAcid	0.9	1.6	1.5	1.2	1.2	1.2	1.2	1.3	1.1	1.1	1.1	1.1	1.1	1.1	1.2	1.2
	2 HydroxyBenzoicAcid	0.9	1.4	1.3	1.7	1.9	1.7	2.0	1.9	0.8	1.0	0.8	1.1	1.2	1.2	1.1	1.0
	2 HydroxyBenzoicAcid	0.8	1.3	1.4	1.3	1.3	1.4	1.4	1.5	0.9	0.9	0.9	0.9	0.9	1.0	1.0	1.0
	4-HydroxyBenzoicAcid	1.0	2.0	1.2	2.2	2.3	2.3	2.4	2.5	1.2	1.4	1.1	2.2	2.5	2.7	3.0	3.1
	4-HydroxyBenzoicAcid	0.9	1.7	1.0	1.3	1.9	1.6	1.9	1.8	0.7	0.8	0.8	1.1	1.4	1.7	2.0	2.0
	4-HydroxyBenzoicAcid	0.8	1.8	0.8	2.6	2.6	2.5	2.5	2.5	0.9	1.0	0.9	1.5	1.9	2.4	2.8	2.8
	r-Hydroxybutyric Acid	0.7	1.3	1.0	1.5	1.4	1.6	1.5	1.5	2.0	2.6	1.2	2.6	2.9	3.1	2.8	2.7
	r-Hydroxybutyric Acid	1.0	1.5	1.1	1.8	1.5	1.4	1.6	1.4	1.5	2.1	1.7	2.9	2.8	3.0	3.3	3.3
	r-Hydroxybutyric Acid	1.0	1.4	1.1	2.0	2.0	2.1	2.1	2.1	0.9	1.3	0.9	2.1	2.2	2.2	2.2	2.2
	Itaconic Acid	0.8	1.3	1.1	1.6	1.6	1.6	1.6	1.7	0.9	1.1	0.9	1.5	1.7	1.8	1.9	1.9
	Itaconic Acid	1.1	1.4	1.2	1.7	1.8	1.9	1.9	1.9	2.4	1.6	1.5	2.2	2.2	2.2	2.3	2.3
	Itaconic Acid	0.8	1.1	0.9	1.6	1.6	1.8	1.8	1.8	2.1	2.0	1.8	2.2	2.4	2.4	2.5	2.5
	a-KetobutyricAcid	1.0	1.0	1.0	1.0	1.1	1.3	1.7	1.9	0.8	0.8	0.8	0.8	0.8	0.8	1.1	1.7
	a-KetobutyricAcid	0.7	0.8	0.9	0.8	1.0	1.1	1.5	1.7	0.8	0.7	0.8	0.7	0.7	0.7	0.8	0.8
	a-KetobutyricAcid	0.8	0.9	1.0	0.8	0.9	1.2	1.6	1.7	0.8	0.8	0.8	0.8	0.9	0.8	0.9	1.4
	D-Malic-Acid	0.9	1.3	1.1	1.7	1.7	1.7	1.7	1.7	0.9	1.1	0.9	1.5	1.5	1.5	1.4	1.4
	D-Malic-Acid	1.0	1.5	1.2	1.5	1.5	1.5	1.5	1.5	0.8	0.7	0.7	1.0	1.0	1.3	1.3	1.3
	D-Malic-Acid	0.9	1.4	1.1	1.7	1.8	1.8	1.7	1.7	0.7	0.7	0.7	0.9	1.0	1.1	1.3	1.4
	D-GlucosaminicAcid	1.1	1.2	1.2	1.7	1.9	2.0	2.0	2.0	0.9	1.1	0.9	1.4	1.8	2.2	2.2	2.1
	D-GlucosaminicAcid	1.1	1.3	1.3	1.8	2.1	2.3	2.2	2.2	0.8	0.8	0.8	1.2	1.4	1.7	2.2	2.4

functional group	carbon source	c0	c25	c51	c72	c95	c126	c165	c189	un0	un25	un51	un72	un95	un126	un165	un189
	D-GlucosaminicAcid	1.0	1.2	1.3	1.5	1.8	2.2	2.3	2.3	0.8	0.9	0.9	1.3	1.5	1.7	2.1	2.2
Miscellaneous	PyruvicAcidMethylEster	0.8	1.4	1.1	1.4	1.6	1.7	1.8	1.7	1.6	2.0	1.5	1.9	1.9	2.0	2.0	2.0
	PyruvicAcidMethylEster	0.7	1.4	0.9	1.5	1.6	1.8	1.9	1.8	0.8	2.1	0.7	2.5	2.8	2.7	3.1	3.0
	PyruvicAcidMethylEster	0.8	1.9	1.1	2.2	2.3	2.5	2.6	2.6	0.7	1.9	0.8	2.3	2.6	2.7	3.0	2.9
	Glucose-1-Phosphate	1.0	1.6	1.2	1.5	1.5	1.5	1.4	1.4	0.9	1.1	0.9	1.2	1.1	1.1	1.1	1.1
	Glucose-1-Phosphate	0.9	1.7	1.1	1.7	1.7	1.7	1.7	1.6	0.8	1.1	0.8	1.3	1.3	1.3	1.3	1.2
	Glucose-1-Phosphate	0.9	2.0	1.2	2.0	2.0	2.0	2.0	2.0	0.7	0.9	0.8	1.1	1.2	1.2	1.3	1.3
	D,L-a-GlycerolPhos	0.7	1.0	1.0	1.0	1.0	0.9	0.9	0.9	0.8	0.9	0.9	0.9	0.9	0.9	1.0	1.0
	D,L-a-GlycerolPhos	0.9	1.0	1.1	1.0	0.9	0.9	0.9	0.9	0.8	0.8	0.8	0.9	0.9	0.9	0.9	0.9
	D,L-a-GlycerolPhos	0.8	1.0	1.1	1.0	1.0	1.0	0.9	0.9	0.8	0.9	0.8	0.9	0.9	0.9	0.9	0.9
Amines	Phenylethyl-amine	1.0	1.9	1.3	2.3	2.7	3.0	2.9	2.8	0.7	0.8	0.8	0.8	0.8	0.8	0.8	0.8
	Phenylethyl-amine	0.9	1.8	1.2	2.7	3.0	3.0	2.9	2.9	0.8	0.8	0.8	0.8	0.9	0.9	0.9	0.8
	Phenylethyl-amine	1.0	1.6	1.1	2.6	2.6	2.6	2.6	2.5	0.9	1.0	1.0	1.0	1.0	1.1	1.1	1.1
	Putrescine	1.2	1.8	1.3	1.7	1.7	1.6	1.6	1.6	0.8	0.9	0.8	1.1	1.3	1.4	1.4	1.4
	Putrescine	1.0	1.6	1.2	1.5	1.4	1.4	1.4	1.4	0.8	0.9	0.8	1.1	1.4	1.5	1.7	1.7
	Putrescine	1.0	1.7	1.2	1.6	1.6	1.6	1.6	1.6	0.9	1.0	0.9	1.2	1.4	1.6	1.5	1.5