BIOCONDITIONING AND NITROGEN FERTILITY EFFECTS OF SELECTED CYANOBACTERIA STRAINS ON TWO DEGRADED SOILS IN THE EASTERN CAPE PROVINCE, SOUTH AFRICA

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DECLARATION

I, Mfundo Phakama Maqubela, declare that the dissertation hereby submitted for the degree of Doctor of Philosophy (PhD) at the University of Fort Hare is my work and has not been previously submitted to another university.

Signature: 

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ABSTRACT

Some cyanobacteria strains have biofertilization and bioconditioning effects in soils. The objective of this study was to identify cyanobacteria with potential to improve the N fertility and structural stability of degraded soils and evaluate their effectiveness in soils of the Eastern Cape, South Africa. Isolation and characterization of the indigenous cyanobacteria strains with desirable properties was first to be undertaken because their effects are known to differ from strain to strain. Cyanobacteria strains 3g, 3v, and 7e were identified from 97 strains isolated from selected soils. *Nostoc* strains 3g and 3v had greater ability to produce exocellular polysaccharides (EPS) but low potential to fix atmospheric N$_2$ (4.7 and 1.3 nmol C$_2$H$_4$ µg chl$^{-1}$ h$^{-1}$, respectively). On the other hand, strain 7e had the highest capability to fix atmospheric N$_2$ (16.1 nmol C$_2$H$_4$ µg chl$^{-1}$ h$^{-1}$) but had the least ability to produce EPS.

Evaluation of the strains was done in glasshouse studies starting with *Nostoc* strain 9v isolated from a Tanzanian soil, followed by the indigenous strains isolated from soils in Hertzog and Qunu, South Africa. Inoculation was done by uniformly applying cyanobacteria on the surface of potted soils at a rate of 6 g m$^{-2}$. First harvest and soil sampling took place after six weeks, and the top 25 mm of the soil was mixed, replanted, and sampled again after a further six weeks (second harvest). Inoculation with *Nostoc* strain 9v increased soil N by 40% and 17% in Guquka and Hertzog soils, respectively, and consequently increased maize dry matter yields by 40 and 49%. Soil C increased by 27% and 8% in Guquka and Hertzog soils, respectively, and this increase was significantly associated with that of soil N ($R^2 = 0.838$). Higher contents of soil C, soil N and mineral N, however, were found in non-cropped soils. Scanning Electron Microscopy (SEM) revealed coatings of EPS on soil particles and fragments of non-cropped inoculated soils, with
other particles enmeshed in networks of filaments, in contrast to cropped and/or non-inoculated soils. The proportion of very stable aggregates was increased by inoculation but cropping with maize reduced the aggregate stability.

Inoculating Hertzog soil with indigenous strains 3g and 7e increased the nitrate N in the first cropping by 49% and 69% respectively, in cropped soils. In the second cropping increases in mineral N were 41% and 43% in 3g and 7e inoculated soils, respectively. Maize dry matter yields were higher on inoculated soils both in the first and second harvest in response to the improved N status of the soil. Increases in aggregate MWD in cropped soil as determined by fast wetting, mechanical breakdown and slow wetting were 85%, 33%, 33%, respectively, for 3g inoculation, 64%, 41%, and 41%, respectively, for 7e inoculation and 60%, 24%, 50% for inoculation with 9v. In non-cropped soil, increases in MWD as determined by fast wetting, mechanical breakdown and slow wetting were 11%, 0%, 7%, respectively for 3g inoculation, 21%, 11%, and 7%, respectively for 7e inoculation, and 25%, 36%, and 19% for strain 9v inoculation. Scanning electron microscopy observations, which were confirmed by chemical results, revealed that inoculated soils had high EPS and filaments that encouraged soil aggregation and improved aggregate stability. Results of this study show that cyanobacteria strains isolated and selected for their ability to fix atmospheric N$_2$ and produce EPS improved the fertility status and aggregate stability of degraded soils from South Africa.
PREFACE

All the work summarised in the Abstract of this dissertation is explained in details in the seven chapters that follow. Chapter 1 is a general introduction and literature review which establishes the justification for the study. The literature review interrogates the need for the study, the causes of the problem that justify the study, the possible solutions to the problem, and the potential of the studied solution in resolving the identified problem. Subsequent chapters report the details of the work written in paper format. Chapter 2 reports on the method employed to isolate and characterise indigenous cyanobacteria strains with biofertilization and bioconditioning effects from the soils. Chapter 3 reports findings on the utilization of cyanobacteria strain 9v (*Nostoc*) with potential to fix high quantities of N and to produce high EPS on improving the nitrogen content of the soil and increasing maize yield. Chapter 4 explains the findings that were obtained when strain 9v was utilized to improve soil aggregate stability in glasshouse studies. The findings of Chapters 3 and 4 will be published in *Plant and Soil: Volume 315*: 79 – 92. The online published version of the manuscript is appended to this thesis as Appendix F. Chapter 5 reports results on the effects of inoculating Hertzog soil with strains 3g and 7e (indigenous to South Africa) and strain 9v on soil N content and maize yield. Chapter 6 explains the effects of the experiment in Chapter 5 on EPS production and aggregate stability. Chapter (7) gives a general discussion, conclusions and recommendations for future studies.
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LIST OF ABBREVIATIONS AND ACRONYMS

SOM = Soil organic matter.
EPS = Exo-cellular polysaccharides.
OM = Organic matter.
ESP = Exchangeable sodium percentage.
EC = Electrical conductivity.
MWD = Mean weight diameter.
cfu = colony forming units.
w = weight.
v = volume.
MPN = Most probable numbers.
DM = Dry matter.
SEM = Scanning electron microscope.
rpm = revolutions per minute.
kPa = kilo Pascal.
FSD = Fragment size distribution.
FW = Fast wetting.
WS = Wet stirring.
SW = Slow wetting.
I = Inoculated.
NI = Non-inoculated.
FSSA = Fertilizer Society of South Africa.
ns = not significant.
nd = not determined.
LSD = Least significant difference.
CHAPTER ONE

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 GENERAL INTRODUCTION

The nitrogen (N) content of cultivated soils in the Eastern Cape and other parts of South Africa is low and is a major factor limiting crop production (Laker, 1976; Bembridge, 1984; du Preez and du Toit, 1995; du Toit and du Preez, 1995; Lobe et al., 2002). The capacity of soil to supply N to plants is inextricably linked to the amount and nature of the soil organic matter (SOM) (Giller et al., 1997) and changes in SOM, caused by cultivation (Barnard and du Preez, 2004). Thus the majority of cultivated soils in the Eastern Cape Province and other parts of South Africa are low in N since they are generally low in organic carbon (C) (Lobe et al., 2002; Laker, 2004; Mandiringana et al., 2005). However, the low levels of N could also be caused by nutrient removals that are not balanced by matching additions.

Low organic C levels of Eastern Cape soils also contribute to poor soil structural stability. It has been shown that below a threshold SOC content of 2%, water–stable aggregates quickly break down and become extremely erodible (Elwell, 1989; Kay and Angers, 2000). A study by van der Merwe et al. (2001) also showed increased erodibility when organic C content of a melanic soil decreased from more than 2.5% to less than 1.5%. The problem is further compounded by the fact that the majority of soils in the Eastern Cape, especially in the former Ciskei region, are medium textured and shallow (Laker, 1978), which make them susceptible to crust formation.
The formation of soil crusts reportedly enhances erodibility of soil and reduces water infiltration and crop yields (Shipitalo and Protz, 1988; Pagliai and Vignozzi, 1998). On an area basis, erosion of soil by water action is the most important form of soil degradation in South Africa (Laker, 2004). Soil crusts also impede seedling emergence by their hardness, and tear seedling roots since they crack upon drying (Hillel, 1980).

Approaches to improve the productivity of Eastern Cape soils must therefore address the constraints of low N status as well as that of physical degradation. The tendency for soil surface crusting can be reduced by improving the resistance of aggregates to withstand physical and physico-chemical mechanisms of breakdown that operate during rainfall and irrigation events. Adding organic matter to soil was reported to increase the stability of aggregates to breakdown (Baldock and Nelson, 2000) by reducing the rate of wetting and increasing the resistance to stresses generated during wetting (Rasiah and Kay, 1995, Caron et al., 1996).

A number of organic amendments are commonly used to improve SOM and N content of soils (Flavel and Murphy, 2006). Similarly, biological nitrogen fixation systems have been used to increase soil C and N (Hubbell and Kidder, 2003). One such system uses cyanobacteria which are essentially free-living prokaryotic bacteria. These photoautotrophic microorganisms have the potential to fix atmospheric di-nitrogen (Buttars et al., 1998) though this capability is known to vary widely among different strains (Steppe et al., 1996). Therefore, in order to be effective, cyanobacteria strains with a high potential to fix atmospheric nitrogen must be isolated and mass produced for soil inoculation. In addition to their ability to increase soil N, cyanobacteria also incorporate C in the soil through their biomass and therefore can minimize soil erosion through
stabilization of aggregates (Metting, 1981). Several studies have also shown that cyanobacteria
have the capability to increase the aggregation and also to improve the aggregate stability of
degraded soils (Metting, 1987; de Caire et al., 2006; Nisha et al., 2007). Improvement of
aggregate stability has been attributed to production of extra-cellular polysaccharides (EPS) and
to accumulation of organic C.

There is little or no information on the effects of cyanobacteria on soil N content, physical
properties and plant growth under South African conditions. The objectives of this study were
therefore to: (i) isolate and characterise cyanobacteria strains from South African soils for their
potential to fix atmospheric N$_2$ and produce EPS, (ii) determine the effects of inoculation of soil
with the cyanobacteria strains on soil N availability, organic matter content and maize yields, and
(iii) evaluate the effectiveness of the selected strains to improve the aggregate stability of
selected degraded soils in the Eastern Cape Province, South Africa.

**Hypotheses of the study**

(i) Eastern Cape soils have cyanobacteria strains with capacity to fix atmospheric N$_2$ and/or
produce EPS.

(ii) Inoculating degraded soils with cyanobacteria strains screened for their ability to fix N$_2$
increases soil N content, N uptake and crop yields.
Inoculating degraded soils with cyanobacteria strains increases soil C and the aggregate stability of the soils.

1.2 GENERAL LITERATURE REVIEW

1.2.1 Introduction

Most soils in South Africa have low productivity as a result of chemical and physical degradation. The chemical degradation is largely a result of soil nutrient depletion while the physical degradation is largely a result of the breakdown in soil structure. This review highlights both problems and examines the possible role of cyanobacteria in the amelioration of both problems.

1.2.2 Nutrients Depletion in Agricultural Soils

Many agricultural soils of the world are deficient in one or more of the essential nutrients needed to support healthy plants (Baligar et al., 2001). An increasing number of African farmers also mention soil fertility decline as a major constraint to farming (Smaling et al., 1997). The decline in fertility is largely attributed to soil nutrient depletion through crop removal, leaching, and soil erosion (Drechsel et al., 2001). One of the nutrients that is found in low quantities in arable soils of the Eastern Cape Province is N (Laker, 1976; van Averbeke and Yoganathan, 1997; Maqubela, 1999). Nitrogen (N), which is a constituent of all proteins and nucleic acids, is
required by plants in greater quantities compared to all other mineral nutrients and is absorbed by plant roots either as a cation or anion (Wilkinson et al., 2000).

When soil is cultivated the N content usually decreases mainly as a consequence of increased rate of oxidation of the SOM (Wild, 1988). Nutrients lost in the soil can be replenished by adding mineral fertilizers, manure, or returning crop residues (Sanchez et al., 1997). The use of mineral fertilizers by many small-holder farmers, however, remains low because of the high cost of the fertilizers (Bekunda et al., 1997; Scherr, 1999). van Averbeke et al. (1998) and Del Rio et al. (2005) also observed that subsistence farmers in some parts of the Eastern Cape Province could not afford mineral fertilizers due to high costs. Thus, most smallholder farmers in the Province use kraal manure as a source of nutrients for crops whilst a very small proportion of them combine it with affordable amounts of mineral fertilizers (Bembridge, 1984; Bembridge et al., 1992; Mandiringana et al., 2005). However, using kraal manure alone or in combination with small amounts of mineral fertilizers does not adequately address the crop nutrients needs in smallholder farming due to a number of factors including limited quantities of manures and variability in their nutrient composition. Since most farmers cannot afford artificial fertilizers, it is important that alternative sources of N be identified / developed and utilized to increase soil productivity.

1.2.3 Soil Aggregation

Aggregation determines the mechanical strength of structured soil with comparable internal parameters (Horn and Baumgartl, 2000). It is important therefore that soil surfaces have stable
aggregates that will reduce soil physical degradation, which, according to Baligar et al. (2001), is mainly caused by anthropogenic processes, the nature of farming, and erosion. Soil aggregation begins when colloidal materials suspended in soil water get connected to each other, by electrostatic forces, to form clusters. As the soil water evaporates the length of each linkage between colloids becomes shorter and stronger, thereby pulling colloidal material closer together. According to Millar et al. (1958), further dehydration of the soil will cause the colloidal materials to stick or cement the formed clusters into aggregates which are permanent until rehydration of the colloids reduces its binding power.

Soil aggregates have been classified as microaggregates, which are clusters of primary soil particles (sand, silt and clay) that are very small to be recognised by the naked eye, and as macroaggregates, which are larger, visible and exist in different shapes and sizes (Millar et al., 1958). The stability of aggregates is determined by the internal binding forces within aggregates that bind the colloidal material (clay or organic materials) to mineral particles of soil (Hillel, 1980). In the presence of excess cementing materials (clay minerals, colloidal oxides of Fe and Al, and colloidal organic matter) internal binding of soil particles into clusters is accompanied by cementing.

Soil aggregate degradation, by wetting in particular, which is common during irrigation and rainfall events, may cause aggregates to collapse as the bonding substances dissolve and clays swell. Soil that has undergone aggregate degradation can be noted by changes in bulk density, total porosity, pore size distribution, aggregate stability, and mechanical strength (Varela et al., 2001). Feddema and Freire (2001), using a model, showed that wet and dry climate regions are
particularly susceptible to impacts caused by soil degradation. Therefore, South Africa, with its nearly 91% arid, semi arid and dry sub-humid climatic conditions (van der Merwe et al., 2001) should be susceptible to impacts caused by soil degradation especially erosion. Soil erosion by water on an area basis is by far the most important form of soil degradation worldwide (Oldeman, 1994). Therefore, soil surface aggregate formation and stability of cultivated soils in South Africa needs to be improved to reduce erosion.

1.2.3.1 Factors Affecting Soil Aggregation

The aggregation and stability of soils is affected by: (i) the organic matter (OM) content of the soil, (ii) free iron (and aluminium) oxides, (iii) exchangeable sodium percentage (ESP), (iv) clay mineralogy, (v) sodium and magnesium contents, (vi) rainfall and land use management, (vii) Mg:Ca ratios and (viii) particle size distribution (Laker, 2004). Multiple interactions between these properties can modify their individual influence (Le Bissonnais, 1996). Other properties known to influence aggregate stability include the composition of the pore fluid, adsorbed or exchangeable solutes, plants and soil organisms and depth in the profile (Kay and Angers, 2000).

(i) The organic matter content of the soil

The stability of soil aggregates to water disruptions in many soils depends on soil SOM, which cements soil particles into microaggregates and macroaggregates that exist in various stages of stabilization (Tisdall and Oades, 1982). The OM content of soils in South Africa is low as a result of intensive cultivation (Lobe et al., 2002; Barnard and du Preez, 2004) and overgrazing.
(Laker, 2004). In the Eastern Cape Province in particular, Mandiringana et al. (2005) found that organic C contents of 62% of cultivated fields, depending on location, were low. van der Merwe et al., (2001) related the SOM content with soil erodibility and reported that in some soils in South Africa the erodibility increased sharply when organic C content decreased from more than 2.5 % to less than 1.5% indicating that low SOM content contributes to low aggregate stability. Some studies elsewhere, showed that the formation of crusts can be reduced or prevented by land spreading of livestock effluents or the application of farmyard manure, and water-stable aggregates can be formed by incorporation of organic residues (Lynch and Bragg, 1985; Pagliai et al., 1987; Pagliai et al., 1998). Working with 26 and later with 120 British soils from agricultural areas, Chaney and Swift (2006) demonstrated highly to very highly significant correlations between aggregate stability and OM, but no correlation with other soil constituent investigated. The findings indicated that OM was mainly responsible for the stabilization of aggregates in the studied soils (Chaney and Swift, 2006). Marshall et al. (1996) reported an indirect relationship between crust formation and the quantity of OM in the soil. In aggregate pore spaces, SOM reduces the rate of water entry and thereby reduces the extent of slaking (Rasiah and Kay, 1995; Caron et al., 1996). Organic matter and its various fractions can therefore contribute to both the formation and the stabilization of soil aggregates. In order to be effective in improving soil aggregate stability the added organic matter must be broken down and modified by soil microorganisms (Lynch and Bragg, 1985).

Microorganisms are also involved in improving aggregate stability by providing mechanical binding between soil particles, producing cementing materials during the decomposition of organic materials, and serving as substrate for further microbial growth. Fungi are believed to be
the most efficient group of soil microorganisms in terms of soil aggregation (Lynch and Bragg, 1985). The hyphal length of fungi and its biomass has been correlated to aggregation as the hyphae bind the soil particles together (Tisdall et al., 1997). Fungi are believed to be involved in both formation and stabilization of soil aggregates (Tisdall et al., 1997). The physical entangling of soil particles by fungi in the process of aggregate formation is coupled with the production of polysaccharides, which promotes aggregate stability (Lynch and Bragg, 1985).

Other microorganisms found in the soil in large quantities are bacteria, but because of their size they are not likely involved in the direct binding of soil particles to form aggregates (Dorioz et al., 1993). However, filamentous bacteria such as blue – green algae (cyanobacteria) and Streptomycetes have the potential to improve aggregate stability of the soil (Metting, 1987). Extracellular polysaccharides that have a binding effect on mineral particles can be produced in large quantities by soil bacteria (Lynch and Bragg, 1985). Unlike other organic materials like kraal manure that are limited to the quantities available in farmyards, and synthetic materials that are expensive to the farmers, microorganisms can be propagated in large quantities and used to improve soil aggregation. One type of bacterium well known to increase soil aggregation and improve other properties of the soil is cyanobacteria (Metting, 1981; Falchini et al., 1996; Malam Issa 1999, 2001, 2007; Nisha et al., 2007).

(ii) **Free iron (and aluminium) oxides**

Iron and aluminium form bridges between clay surfaces and OM and encourage stable aggregates in the soil. The destruction of the linkages between clay surfaces and OM result in the
reduction of aggregate stability (Reid et al., 1982). The sorption of polycations like Fe (III) on clay surfaces causes irreversible flocculation of the clay and results in microaggregation (Shanmuganathan and Oades, 1982).

(iii) Exchangeable sodium percentage

Exchangeable sodium percentage (ESP) is the amount of exchangeable sodium that can be replaced by another ion such as calcium. Sodium is by far the most dispersive cation in soils, and therefore, enhances soils erosion (Laker, 2004). Generally, once the ESP value is equal to 15% or more of the soil cation exchange capacity soils are considered to be sodic (Bresler et al., 1982), and their physical conditions adversely affected (Richards 1954 as cited by Laker, 2004). However, the effect of ESP on aggregation differs from soils to soil (Levy et al., 1988). Soils with excessive amounts of exchangeable sodium experience deflocculation, whereby aggregates breakdown to their constituent individual soil particles.

(iv) Clay mineralogy

Clay mineralogy, in the absence of OM, controls aggregation and soil structure (Baver et al., 1972). Stable and porous structure is found in soils which contain clays with variable charge due to the presence of oxides of aluminium and iron (Taylor and Ashcroft, 1972). The kind of aggregates formed by the various clay types are frequently different (Taylor and Ashcroft, 1972). Sandy soils do not shrink or swell when they are dry or wet because the particles are independent from each other and are only attached to each other in small surface areas. The particles do not
aggregate when disturbed by mechanical activities and the structure is single grained, solely dependent on the size, shape and packaging of the non-porous grains (Taylor and Ashcroft, 1972).

(vi) **Rainfall and land use management**

Rainfall can cause the breakdown of weakly stable soil aggregates resulting in reduced macroporosity and increased runoff, mainly because of rapid wetting. This was demonstrated by the work of Bertrand and Sor (1962) which showed that the aggregate stability of three soil types decreased significantly with rainfall. Land use practices that involve conventional tillage have also been shown to cause intensive erosion if they are not executed properly (Kay and Angers, 2000). This is as a result of decreased organic matter levels. Therefore, O’Connell *et al.* (2007) have suggested that better management practices like adoption of conservation agricultural practices need to be implemented to reduce or mitigate the degradation caused by conventional practices through increasing SOM levels.

(vii) **Mg: Ca ratios**

Magnesium can result in the destruction of soil structure, especially on sodic soils, if the concentration of calcium is low, resulting in surface sealing, decreased infiltration, increased runoff and erosion during rainfall events. Dontsova and Norton (2001), after modifying Mg and Ca in four soils with low organic matter content, clay and mineralogy, demonstrated that Mg has specific effect on clay flocculation and surface sealing due to its hydration behaviour that differs
from that of calcium. Magnesium has a greater hydration radius than calcium and this causes larger separation distance between clay layers and less attraction between them to cause flocculation (Dontsova and Norton, 2001), therefore a soil with high Ca:Mg ratio will have more stable aggregates than a soil with low Ca:Mg ratio.

(viii) **Particle size distribution**

Soils with low clay content (<20%) generally have low aggregation potential, especially on cultivated land, and therefore have low aggregate stability (Hillel, 1980; Horn and Baumgartl, 2000). Soils with high silt and fine sand contents tend to be eroded easily because they have low aggregate stability (Haarhoff *et al*., 1995; Laker, 2004). Soils with high clay content tend to have high percentage of aggregates with more than 0.5 mm in diameter (macroaggregates) (Millar *et al*., 1958).

1.2.3.2 **Mechanisms of Aggregate Breakdown**

Aggregates in agricultural soils are broken down because of stresses experienced, related to tillage, traffic, wetting and mechanical abrasion by flowing water. The breakdown of soil aggregates by water is related to unstable aggregates and can result in intensive soil erosion both on cultivated and uncultivated lands. Four mechanisms of aggregate breakdown have been identified as (i) slaking, the breakdown caused by the compression of entrapped air during wetting (ii) breakdown by differential swelling (iii) breakdown by raindrop impact (iv) physico-
chemical dispersion due to osmotic stress (Le Bissonnais, 1996). The other breakdown of soil aggregates is caused by traffic and active tillage.

(i) Slaking

The compression of air entrapped inside an aggregate during wetting either by rainfall or irrigation causes aggregate breakdown, called ‘slaking’ (Emerson, 1997). The effect of trapped air depends on the volume of air inside the aggregate (Le Bissonnais, 1996), on the rate of wetting (Loch, 1994), and on the sheer strength of wet aggregate (Nearing and Bradford, 1985). Slaking was found to decrease when the initial moisture content was high (Truman et al., 1990) and when the clay content of the soil increased. The resultant fragments caused by slaking are micro-aggregates that increase in size with increases in clay content and type (Le Bissonnais, 1996). The effect of clay on the size of micro-aggregates is associated with the resistance of the bonds it forms between skeleton grains of the soil.

(ii) Breakdown by differential swelling

Unlike in slaking, slow wetting of dry clayey soils causes differential swelling and shrinkage of different minerals in the soil that result in microcracking of aggregates (Barzegar et al., 1995). The breakdown by differential swelling increases as the clay content increases, and is also determined by the type of clays and the wetted portion of the aggregates (Hillel, 1980). As water moves into an aggregate, and the affinity for water of surfaces particles forming an aggregate exceeds the cohesion forces, the aggregates lose their cohesion and the cementing bonds are
destroyed (Baver et al., 1972). The micro-aggregates formed in the process of differential swelling accelerate the formation of structural crusts by reducing the mean aggregate size (Le Bissonnais, 1996).

(iii) Mechanical breakdown by raindrops impacts

Mechanical breakdown, often called ‘splash’, is caused by raindrop impact on aggregates, and it encourages the displacement of detached soil particles or micro-aggregates (Ghadiri and Payne, 1979). According to Morgan and Davidson (1986), it is the raindrop impact, and not only slaking and differential swelling, that disrupts some of the saturated soil aggregates. The breakdown is caused by instantaneous slaking of soil aggregates followed by dispersion and orientation of the finer particles (Baver et al., 1972). Its resultant fragments are generally small, being either individual particles or micro-aggregates (<100 µm) (Le Bissonnias, 1996). The effect of mechanical breakdown is more severe on wet aggregates, because aggregates are weaker when they are wet. In dry areas where mulching material is limited and the rainfall is often accompanied by strong winds, the potential for splash effect will be high.

(iv) Physicochemical dispersion

Aggregate breakdown by this mechanism results in the reduction of the attractive forces between colloidal particles during rainfall or irrigation spells. The dispersion of soil aggregates into elementary particles depends on cations size and valence, decreasing with the polyvalent cations and increasing with monovalent cations (Le Bissonnias, 1996). Dispersion is affected by the
electrolyte concentration (EC) of solution, the EC of applied water, and mechanical disturbance from raindrops’ impact (Agassi et al., 1985). Exchangeable sodium percentage (EPS) also affects dispersion of aggregates (Le Bissonnias, 1996). Since dispersion breaks the aggregate into particles it can be considered as the most effective process of aggregate breakdown, and can also increase the effect of the other mechanisms. Soil aggregates prone to dispersion breakdown can make the soil surface develop a crust very fast, and/or cause slow infiltration of water into the soil whilst increasing the mobility of clay particles in water.

(v) Aggregate Breakdown by Traffic and Active Tillage

Tillage operations and field traffic in particular intensify the reduction in total void length, total void volume and tortuosity, and encourage the development of soil crust with the consequent runoff and erosion (Pagliai and Vignozzi, 1998; Langmaack et al., 2002). The type of tillage operation undertaken in the seedbed preparations results in different sizes of fragments that are the result of aggregate breakdown. Disruption of aggregates leads to exposure of OM to microbial attack (Mills and Fey, 2004), and quick oxidation of OM that will result in a decline in SOM content. The breakdown of soil aggregates by tillage is influenced by soil properties and water content at the time of tillage (Kay and Angers, 2000). Heavy equipment, repeatedly running over the land during soil preparation, breaks the aggregates of the soil. However, tillage also has positive effects on the soil, because implements break up large clods, incorporate the OM in the soil, and make a more favourable seedbed (Brady, 1974).
1.2.3.3 Measurement of Aggregate Stability

Methods for the measurement of aggregate stability try to simulate forces that will occur in the field in order to determine the fraction of the original sample (mass) that withstands destruction and retain its integrity under certain specified conditions. Prolonged dry sieving or crushing is used to measure the resistance of aggregates to mechanical breakdown, and other methods like dropping of aggregates at certain heights can also be employed (Hillel, 1980). The other methods commonly used to measure aggregate stability are based on wet sieving, and were reviewed by Hillel (1980), and they involve testing the stability of aggregates to breakdown in water. This method resembles flooding because aggregates are immersed in water in different graduated sieves and oscillated vertically and rhythmically up and down. Le Bissonnais (1996) proposed a method that involves measurement of aggregates subjected to fast wetting, mechanical breakdown, and slow wetting. The fast wetting resembles a field environment where aggregates are exposed to rainfall of high intensity. Mechanical breakdown resembles the case where aggregates are exposed to physical activities in the field like ploughing, and slow wetting resembles the situation where aggregates are exposed to rainfall of low intensity. Mean weight diameter (MWD) of soil aggregates is then used to determine the aggregate stability of the soil.

1.2.4 Approaches to address Soil Degradation Problems

A number of organic amendments are commonly used to improve SOM and N content of soils (Flavel and Murphy, 2006). Similarly, biological nitrogen fixation systems have been used to
increase soil C and N (Hubbell and Kidder, 2003). One such system uses cyanobacteria, which are free-living prokaryotic bacteria.

1.2.4.1 Overview of Cyanobacteria

Cyanobacteria are blue-green, photoautotrophic, free-living bacteria that are found in many ecosystems (Gibson and Smith, 1982; Fogg, 1987; Harper and Pendleton, 1993). They belong to the kingdom Procaryotae, and they contain chlorophyll and phycobiliprotein pigments such as phycocyanin (Pelczar et al., 1986). Their chlorophyll pigments and photosynthetic process are of the same type as those of algae and higher plants (Nester et al., 1978). Cyanobacteria exist in unicellular, colonial, and filamentous forms, and usually occupy the top 1 cm depth of the soil.

Cyanobacteria Effects on Nutrient Dynamics of Soils

Cyanobacteria increase soil fertility in arid lands through their ability to fix atmospheric N (Arora, 1972; Carr and Whitton, 1982; Buttars et al., 1998). They grow on soil surfaces, form dark coloured surfaces and “fix” N into a biologically available form (Harper and Pendleton, 1993). The N fixed by cyanobacteria is available to higher plants (Harper and Pendleton 1993). Vascular plants that grow in the vicinity of biological soil crusts have been found to have higher levels of N in their leaf tissues, due to uptake of N from N fixing crusts (Belnap et al., 1996). The N fixation rates range from 10 to 25 kg N per ha annually (Harper and Marble, 1988; Buttars et al., 1998), and most vascular plants in cool deserts generally take up 10 to 12 kg N per ha annually (Rychert and Skujinš, 1974; West and Skujinš, 1977 as cited by Buttars et al., 1998).
In addition to N, cyanobacteria have been reported to increase the contents of available phosphorus (P), exchangeable potassium (K), and surface SOM (Kleiner and Harper, 1972). Nitrogen fixation by cyanobacteria is heavily dependent upon environmental conditions including an appropriate combination of light intensity, moisture and temperature (Buttars et al., 1998). High light intensity often inhibits N fixation (Reddy and Giddens, 1975 as cited by Buttars et al., 1998). In the absence of water there is no N fixation, but the rate of fixation increases as soon as the water becomes available (Rychert and Skujinš, 1974). The optimum temperature range in cold desserts for N fixation is 19 to 23°C but it declines above and below this range (Rychert et al., 1978). Some strains could be adapted to higher temperatures such as those of the Eastern Cape, thus the need to isolate and characterise such strains.

**Cyanobacteria Effects on Soil Aggregate Stability**

Cyanobacteria inoculation studies have also shown improvement of soil properties particularly soil C and aggregate stability. A study by Metting and Rayburn (1983) showed that repeated annual inoculation of three soils of loamy fine sand, loamy sand and silt loam texture with *Chlamydomonas mexicana* reduced aggregate breakdown by either wet sieving or rotary dry sieving. Similar results were reported by Nisha et al. (2007) who used multi- cyanobacteria strains on a clay loam soil; Falchini et al. (1996) who used two *Nostoc* strains (chosen for their ability to colonise soil and to produce polysaccharides) on two clay soils; and Malam Issa et al. (2007) who used a *Nostoc* strain in two loam soils.
The formation of aggregates on these soils has been associated with the filaments of cyanobacteria that trap sand particles with finer soil particles sticking on the sticky surface of the filaments (Malam Issa et al., 1999). In addition to the role played by filaments, Belnap and Gardner (1993) showed that the sticky polysaccharide sheath material on the surface of cyanobacteria filaments wind through and across the soil surfaces, attaching and binding soil particles together. A study by Barclay and Lewin (2005) showed that the EPS produced by cyanobacteria was more effective in the top 2 mm of the soil and decreased with the soil depth. They attributed this observation to the fact that more than 99% of cyanobacteria cells and EPS produced remain in the top 2 mm of the soil. The amount and type of EPS produced depends on the species employed and the cultivation conditions (Nicolaus et al., 1999). Phormidium species is the best producer of EPS in non–heterocystous cyanobacteria, while Anabaena belonging to Nostocaceae, and Chlorogloeopsis species are the best producers of EPS among heterocystous cyanobacteria (Nicolaus et al., 1999). Anabaena produces amino sugars such as glucosamine whilst Chlorogloeopsis produces glucose and galactose (Nicolaus et al., 1999). Generally, the EPS contain glucose, galactose, xylose and mannose, but do not characterize a particular cyanobacterial group, because the composition differs quantitatively and qualitatively within a given genus (Nicolaus et al., 1999).

1.2.4.2 Justification for Utilizing Cyanobacteria

The foregoing review shows that cyanobacteria could increase the N content and improve the aggregate stability of degraded soils in South Africa and contribute to increased soil productivity. Cyanobacteria will not only provide the soil with the required form of N, but will also cause no
harm to the environment as commonly caused by long term use of inorganic fertilizers (Conway and Pretty, 1988). Cyanobacteria improve aggregate stability through their ability to bind soil particles together mostly on the surface layer. This results in improved porosity and reduced extent of crust formation, leading to improved movement of water into the soil and reduced soil erosion. There is, however, no work that has been conducted in South Africa to establish the role cyanobacteria, in particular local strains, play in improving soil productivity. This study was, therefore, undertaken to address this need.
1.3 REFERENCES


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Soils from Guquka, Hertzog, and Qunu villages, and Fort Cox College, in the Eastern Cape, were selected for use in the study because of observed surface crusts and had low nutrient contents, especially soil C and N. Indigenous cyanobacteria were isolated by culture and plate techniques using growth media BG 11 for all strains and BG 11\( _0 \) for atmospheric N\( _2 \) fixing strains. The numbers of cyanobacteria strains isolated with capability to produce EPS and fix atmospheric N\( _2 \) were 2, 28, 35, and 32 for Guquka, Hertzog, Fort Cox College, and Qunu soils, respectively. Greater numbers of strains were isolated from virgin soils except for the soil from Fort Cox College. The strains differed with respect to their ability to fix N\( _2 \) and produce EPS. Cyanobacteria strain 7e exhibited high N\( _2 \) fixing capability but had very low EPS production capability, whereas strain 3v produced large quantities of EPS but exhibited low N\( _2 \) fixation capability. Cyanobacteria strain 3g was equally good in producing EPS and fixing atmospheric N\( _2 \). The N\( _2 \) fixation capabilities of cyanobacteria strains 7e, 3g and 3v were 16.1, 4.7, and 1.3 nmol C\(_2\)H\(_4\) µg chl\(^{-1}\) h\(^{-1}\), respectively. Other isolated cyanobacteria strains including 22b, 21a, 1af, 2i, and 8a produced no EPS, and fixed negligible quantities of atmospheric N\( _2 \). The results
suggest that degraded soils in the Eastern Cape Province have cyanobacteria strains with the potential to improve the physical and chemical conditions of soils.

**Key words:** Characterisation; Cyanobacteria strains; Degraded soils; EPS production; Nitrogen fixation.
2.2 INTRODUCTION

Cyanobacteria are known to improve chemical and biological characteristics of soils, increasing the content of N (by N\textsubscript{2}-fixing activity) and C (by photosynthesis) and stimulating activities of microbial communities. The ability of these microorganisms to increase N, a major limiting factor in the productivity of many South African soils, is shared by many species belonging to the genera *Nostoc, Anabaena, Fischerella* and *Scytonema* (Steppe et al., 1996).

The ability of cyanobacteria to fix dinitrogen (N\textsubscript{2}) has been exploited in Asian countries for many years to increase soil organic N content in rice cultivation through soil inoculation with living N\textsubscript{2}-fixing cyanobacteria (algalization) (Ghosh and Saha, 1997). Data obtained in several experiments indicated improvements of total N of around 10 - 30\% in alkaline soils and of up to 120\% in neutral soils. The photosynthetic activity of these organisms involves other beneficial effects such as the increase of soil OM as cyanobacterial biomass under favourable conditions of light, moisture and pH (Roger and Burns, 1994). In the long-term, the large amount of organic C arising from cyanobacteria biomass contributes to the stable C pool in soils. The improvement of biological soil conditions by cyanobacterial inoculation reportedly also results in increases in other microbial populations (Roger and Burns, 1994; Nisha et al., 2007).

In well-aggregated soils, OM and iron oxides bind clay and other particles into water-stable structures which resist the destructive energy of raindrop impact. However, under conditions of arable agriculture, particularly those prevailing in arid and semi-arid areas, soil degradation takes place over time as a result of the progressive loss of OM, leading to a substantial weakening of
aggregates that become very vulnerable to raindrop impact. Cyanobacteria can contribute to the stabilization of these weakened aggregates through the OM they produce (Barclay and Lewin, 1985) and through the production of EPS that have been demonstrated to be among the most effective organic substances for enhancing soil aggregates stability (Rao and Burns, 1990; Rogers and Burns, 1994).

Some cyanobacteria strains exhibit both bio conditioning and bio fertilization effects but others exhibit only one of the effects (Skujinš and Klubek, 1978; Klubek and Skujinš, 1980; Falchini et al., 1996). Therefore, screening of these organisms is necessary for any program that seeks to exploit these organisms in improving degraded soils.

As a first step in exploring the possibilities of exploiting the positive benefits of cyanobacteria in South Africa this study sought to isolate, characterize and select cyanobacteria strains capable of fixing atmospheric N\textsubscript{2} and producing EPS.

2.3 MATERIALS AND METHODS

2.3.1 Site Selection, Soil Sampling and Soil Analysis

Soils used in this study were from Hertzog, Guquka, and Qunu villages in Seymour, Alice and Mthatha, respectively, and from Fort Cox College in Middledrift. The site selection was based on susceptibility of the soils to form crust and low crops yields (a possible indicator of poor soil fertility). Soil samples were collected from cultivated, fallow and/or virgin lands, and the
characteristics of cultivated soils are presented in Table 2.1. Fallow land was considered as land that had not been cultivated for a period of two consecutive years. Five soil samples were collected randomly from the 0 – 5 cm depth of 5m x 5m plot areas. The samples were mixed in a sterilized container to form a composite sample that was subsequently used for soil analysis and the isolation of cyanobacteria strains.

The soils were air – dried, sieved (< 2 mm) and analysed for pH, extractable P, exchangeable K, Ca, Mg, soil C and soil N. Soil pH was measured in a 1 : 2.5 (w:v) soil : water suspension. Extractable phosphorus was determined following the Bray and Kurtz (1945) procedure. Extraction of available K, Ca, and Mg was done with 1 mol dm$^{-3}$ NH$_4$Ac (pH 7) and analysed on an atomic absorption spectrophotometer. The soil C and N were determined by a LECO C & N analyzer. Soil texture was determined using the hydrometer method as described by Bouyoucos (1962). Soil crusts were carefully scooped and their thickness measured using a ruler.

### 2.3.2 Isolation and Purification of Cyanobacteria Strains

The isolation and purification of cyanobacteria was done with the help of the Centro de Investigaciones Científicas Isla de la Cartuja laboratory in Spain. Firstly BG 11 medium was used to isolate all cyanobacteria strains, and secondly BG 11$_0$ medium was used to isolate strains that can fix atmospheric N$_2$. The composition of BG 11 was (g L$^{-1}$): 1.5 NaNO$_3$; 0.04 K$_2$HPO$_4$,3H$_2$O; 0.075 MgSO$_4$,7H$_2$O; 0.036 CaCl$_2$,2H$_2$O; 0.02 Na$_2$CO$_3$; 0.006 Citric acid; 0.006 ferric ammonium citrate; 0.001 EDTA Na$_2$ (disodium – magnesium salt). One litre of BG 11 was mixed with 1 ml of trace metal mix which was composed of (g L$^{-1}$): 2.86 H$_3$BO$_3$; 1.81
MnCl$_2$.4H$_2$O; 0.222 ZnSO$_4$.7H$_2$O; 0.39 NaMoO$_4$.2H$_2$O; 0.079 CuSO$_4$.5H$_2$O; 0.0494 CoCl$_2$.6H$_2$O. The growth medium BG 11$_0$ was the same as BG 11 but did not contain NaNO$_3$ so as to make it N free. Two grams of soil were suspended in 18 ml of physiologic solution (9 g NaCl in 1 L of sterile water) in 100 ml Erlenmeyer flask. After vortexing and disaggregation of soil, six serial dilutions ($10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}$) were made in sterile glass test tubes containing the physiologic solution. Solid growing media were prepared by adding 10 g of purified agar in a liter of BG11 or BG11$_0$ solution, autoclaved at $110^\circ$C for 60 minutes, allowed to cool to approximately $40^\circ$C, and poured into petri-dishes under a sterile laminar flow before cooling to room temperature for about 10 minutes (Rippka et al., 1979). When the agar was cool, 500 µL of the $10^{-2}, 10^{-3}$ and $10^{-4}$ dilutions were poured on the surfaces of the agar growing medium, in triplicate, and distributed (streaked) uniformly using a sterile loop and covered. The plates were incubated at $28^\circ$C under continuous light radiation of about $10 – 30$ µmoles of photons/m$^2$/second. After two weeks of incubation the cyanobacteria colony forming units (cfu) were enumerated.

Isolation of the cyanobacteria strains was done by randomly picking different types of colonies developed on the petri-dishes. The Harrison disc was used to obtain a better random isolation of cyanobacteria colonies based on the diversity in the morphology, colour of the colonies, and the micromorphology of organisms observed under light microscope. The picked colonies were purified by repeatedly streaking to single colonies on new growing media mixed with cyclohexamide (an antieucaryotic compound) so as to get strains free of fungi and other eucaryotic microorganisms. Cyanobacteria strains with only one kind of colony morphology were grown separately and labelled following the guidelines by Stanley and Krieg (1984) to
separate them from other strains. The isolated cyanobacteria strains were sequentially assigned reference numbers using a combination of numerical figures and randomly assigned letters for the purpose of separating strains from one another. Quantitative determination was also done by estimating the number of cyanobacteria per 100 ml of sample using probability tables (Most Probable Numbers, MPN, method) (Petterson, 2006).

2.3.3 Determination of the Nitrogen Fixation and EPS Production Capabilities of Isolated Cyanobacteria Strains

Cyanobacteria in liquid culture containing 25 mL of the BG 110 broth that was inoculated with cyanobacteria strains were set in Erlenmeyer flasks and incubated at 30°C under light with continuous shaking. After 10 days of incubation, cells were harvested by centrifugation, re-suspended in 2 ml of fresh BG110 medium, and incubated in air composed of 13.3 % acetylene in 17mL Erlenmeyer flasks that were tightly sealed with rubber stoppers. The introduction of acetylene and sampling of the gas phase in the flask were done by introducing through the rubber stopper a needle connected to a syringe. The amount of ethylene produced after 90 minutes was determined by gas chromatography. The amount of chlorophyll present in the assays was determined in methanolic extracts of the cell suspensions used in the nitrogenase assay according to Mackinney (1945). Nitrogenase activity was then expressed as nmol of ethylene produced per µg chlorophyll per hour. The strain Anabaena Sp. PCC 7120, a filamentous, heterocyst-forming cyanobacteria commonly used for laboratory work and thoroughly characterized, with a nitrogenase activity of 17.04 nmol C₂H₄ µg chl⁻¹ h⁻¹, was used as a reference strain for nitrogenase activity.
An aqueous solution of India ink was used to detect the presence of EPS. The intensity of EPS was used to quantify the presence of EPS from different cyanobacteria strains as India ink does not penetrate the EPS, which appears as white unstained areas around the cells of cyanobacteria. Equal volumes of India ink and liquid cultures of cyanobacteria (0.5 mL) were gently mixed, and India ink-stained samples were observed under a light microscope. Cyanobacteria with large white zones around its cells were considered as having high EPS content compared to those with smaller white zones around their cells.

2.4 RESULTS

2.4.1 Characteristics of selected soils

The pH, sodium (Na), potassium (K), calcium (Ca), magnesium (Mg) and phosphorus (P) contents of cultivated soils used in the isolation and characterisation of different strains of cyanobacteria are presented in Table 2.1. The pH was low, 5.95 and 5.45 from Guquka and Qunu soils, respectively, and high, 7.46 and 6.79 from Hertzog and Fort Cox soils, respectively. The sodium content was 0.03 and 0.005 g kg\(^{-1}\) from Hertzog and Fort Cox soils respectively whilst Guquka and Qunu soils contained untraceable amounts. The potassium, calcium and phosphorus were low from Guquka and Qunu soils, but higher from Hertog and Fort Cox soils. The magnesium content from Hertzog soil was very high (27.36 g kg\(^{-1}\)) compared to 0.006 g kg\(^{-1}\), 0.319 g kg\(^{-1}\) and 0.08 g k g\(^{-1}\) from Guquka, Fort Cox and Qunu soils, respectively.
The quantities of a combination of silt and fine sand were 83.47%, 77.59%, 79.27% and 84.88% in Guquka, Hertzog, Fort Cox and Qunu soils respectively, whilst the clay content was 15.63%, 21.76%, 19.88%, 7.62% in Guquka, Hertzog, Fort Cox and Qunu soils respectively (Table 2.1). The crusts on the surfaces of Guquka and Hertzog soils were more than 2 mm thick, whilst on Fort Cox and Qunu soils were less than 2 mm thick.

Table 2.1: Selected chemical and physical properties of Guquka, Hertzog, Fort Cox and Qunu cultivated soils

<table>
<thead>
<tr>
<th>Soil property</th>
<th>Guquka</th>
<th>Hertzog</th>
<th>Fort Cox</th>
<th>Qunu</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (H₂O)</td>
<td>5.95</td>
<td>7.46</td>
<td>6.79</td>
<td>5.45</td>
</tr>
<tr>
<td>pH (KCl)</td>
<td>4.38</td>
<td>6.92</td>
<td>6.26</td>
<td>4.86</td>
</tr>
<tr>
<td>Na (g kg⁻¹)</td>
<td>0.00</td>
<td>0.03</td>
<td>0.005</td>
<td>0.00</td>
</tr>
<tr>
<td>K (g kg⁻¹)</td>
<td>0.070</td>
<td>0.34</td>
<td>0.325</td>
<td>0.001</td>
</tr>
<tr>
<td>Ca (g kg⁻¹)</td>
<td>0.102</td>
<td>1.61</td>
<td>1.698</td>
<td>0.112</td>
</tr>
<tr>
<td>Mg (g kg⁻¹)</td>
<td>0.006</td>
<td>27.36</td>
<td>0.319</td>
<td>0.088</td>
</tr>
<tr>
<td>P (g kg⁻¹)</td>
<td>0.0008</td>
<td>0.03</td>
<td>0.03</td>
<td>0.0005</td>
</tr>
<tr>
<td>Mean weight diameter (mm)</td>
<td>0.56</td>
<td>0.51</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Coarse sand (%)</td>
<td>0.20</td>
<td>0.12</td>
<td>0.20</td>
<td>0.15</td>
</tr>
<tr>
<td>Medium sand (%)</td>
<td>0.70</td>
<td>0.53</td>
<td>0.65</td>
<td>7.35</td>
</tr>
<tr>
<td>Fine sand (%)</td>
<td>33.55</td>
<td>32.22</td>
<td>36.85</td>
<td>60.25</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>49.92</td>
<td>45.37</td>
<td>42.42</td>
<td>24.63</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>15.63</td>
<td>21.76</td>
<td>19.88</td>
<td>7.62</td>
</tr>
<tr>
<td>Crust formation</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

++, means a crust more than 5 mm was observed; + means a crust less than 5 mm was observed.
The C and N contents of soils used in the isolation and characterisation of different strains of cyanobacteria are presented in Table 2.2. The soil C increased in the order of virgin > fallow > cultivated soils in Guquka, Hertzog and Fort Cox soils respectively, except in Qunu soil. In cultivated land practices Fort Cox had higher (8.5 g kg$^{-1}$) soil C followed by Hertzog (7.7 g kg$^{-1}$), Guquka (6.1 g kg$^{-1}$) and Qunu had the lowest (1.7 g kg$^{-1}$) quantity. The soil N content also increased in the same order as that of soil C for Hertzog soil, but did not increase in Guquka soil when cultivated and virgin practices were compared.

Table 2.2: Carbon and nitrogen from cultivated, fallow and virgin practices in Guquka, Hertzog, Fort Cox and Qunu soils

<table>
<thead>
<tr>
<th>Soil type</th>
<th>Soil C (g kg$^{-1}$)</th>
<th>Soil N (g kg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cultivated</td>
<td>Fallow</td>
</tr>
<tr>
<td>Guquka</td>
<td>6.1</td>
<td>nd</td>
</tr>
<tr>
<td>Hertzog</td>
<td>7.7</td>
<td>9.0</td>
</tr>
<tr>
<td>Fort Cox</td>
<td>8.5</td>
<td>10.6</td>
</tr>
<tr>
<td>Qunu</td>
<td>1.7</td>
<td>1.5</td>
</tr>
</tbody>
</table>

nd – not determined

2.4.2 Cyanobacteria Enumeration, Isolation, Purification and Growth

The number of cyanobacteria strains isolated from Guquka, Hertzog, Fort Cox College and Qunu soils were 2, 28, 35, and 32, respectively (Table 2.3). There was one cyanobacteria strain isolated from each of the cultivated and virgin soils in Guquka. The Hertzog soil had the highest number
of cyanobacteria strains isolated from the virgin (11 strains), followed by fallow (9 strains) and cultivated (8 strains) soils. In contrast, the highest number of cyanobacteria strains isolated from Fort Cox College was from cultivated (22 strains), followed by fallow (8 strains) and virgin (5 strains) soils. The number of cyanobacteria strains isolated in cultivated, fallow, and virgin soils from Qunu was 0, 10, and 22 strains, respectively (Table 2.3). Considering land use systems, the highest number of cyanobacteria strains were isolated from virgin (39), followed by cultivated (31), and fallow soils (27) (Table 2.3).

Table 2.3: Quantities of cyanobacteria strains isolated and purified from soils sampled from cultivated, fallow, and virgin land use systems at different sites

<table>
<thead>
<tr>
<th>Site</th>
<th>Number of different cyanobacteria strains</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cultivated</td>
<td>Fallow</td>
</tr>
<tr>
<td>Guquka</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Hertzog</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Fort Cox College</td>
<td>22</td>
<td>8</td>
</tr>
<tr>
<td>Qunu</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>31</strong></td>
<td><strong>27</strong></td>
</tr>
</tbody>
</table>

2.4.3 Nitrogen Fixing Capability and EPS Production from Isolated Cyanobacteria

Cyanobacteria strains 3v, 3g and 7e had higher N fixation capability (based on acetylene reduction) than all the other strains, which only showed trace amounts of ethylene. The amount of ethylene produced by strain 7e was higher (16.1 nmol C₂H₄ µg chl⁻¹ h⁻¹) than those of 3v and
3g which were 1.3 and 4.71 nmol C$_2$H$_4$ µg chl$^{-1}$ h$^{-1}$, respectively (Figure 2.2). The amount of ethylene produced by 7e was comparable to that of the reference strain (*Anabaena Sp.* PCC 7120) indicating that cyanobacteria strain 7e could fix high quantities of N.

Figure 2.1: Nitrogen fixation capability in nmol C$_2$H$_4$ µg chl$^{-1}$ h$^{-1}$ by cyanobacteria strains 3v, 3g, 7e, and a reference strain *Anabaena*

Only a few of the isolated cyanobacteria strains could grow in liquid and/or solid growing culture medium (BG11$_0$) and fewer still could produce EPS (Table 2.4). Strains 22b and 1af were isolated from cultivated soils (Guquka and Hertzog, respectively), strains 2i and 8a were isolated from fallow soils (Hertzog and Qunu, respectively) and 21a (Guquka), 3g and 3v (Hertzog) and 7e (Qunu) were isolated from virgin soils. None of the isolated strains from Fort Cox soil could grow well in solid and liquid media (BG 11$_0$). Growth of strains 1af, 2i and 21a was good on solid but weak on liquid medium (BG 11$_0$) whereas that of 3v was very good both on solid and
liquid media. Growth of strains 3g and 7e was weak in liquid media and no growth was observed on solid media. Cyanobacteria strain 8a showed good growth both on solid and in liquid BG11\textsubscript{0}, whereas strain 22b showed weak growth both on solid and in liquid medium (Table 2.4). Only strains 3v and 3g grew well and produced observable EPS (Table 2.4). The EPS was indicated by a white zone around the filament when stained with india ink (Appendix A).

Table 2.4: Growth on solid and liquid medium BG11\textsubscript{0} and EPS production capability of the different strains of cyanobacteria

<table>
<thead>
<tr>
<th>Site</th>
<th>Land use system</th>
<th>Strain number</th>
<th>Growth on BG 11\textsubscript{0}</th>
<th>EPS observed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>---- Solid ------ Liquid -----</td>
<td></td>
</tr>
<tr>
<td>Guquka</td>
<td>Cultivated</td>
<td>22b</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Natural</td>
<td>21a</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Hertzog</td>
<td>Cultivated</td>
<td>1af</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Fallow</td>
<td>2i</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Virgin</td>
<td>3v</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Virgin</td>
<td>3g</td>
<td>nd</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Virgin</td>
<td>7e</td>
<td>nd</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Fallow</td>
<td>8a</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

For growth on BG 11\textsubscript{0}: +++ is very good growth, ++ is good growth, + is weak growth, nd is nothing depicted

For EPS production: ++ means thick EPS zone, + means thin EPS zone, - means no EPS observed
2.5 DISCUSSION

2.5.1 Characteristics of Selected Soils

The pH of all selected cultivated soils was within the suitable range for most crops, however soils from Guquka and Qunu had pH values falling below the range preferred by some strains of cyanobacteria. Several studies reported the abundance of cyanobacteria in soils with neutral to slightly alkaline pH whilst they struggled to survive in soil pH values that are below 5 (Kaushik, 1994; Nayak and Prasanna, 2007).

The soil C contents of the four cultivated, as well as Hertzog and Qunu fallow soils were lower than the lower threshold value of 10 g kg\(^{-1}\) as suggested by Landon (1991). Very low soil C quantities observed in cultivated Guquka (6.1 g kg\(^{-1}\)) and Hertzog (7.7 g kg\(^{-1}\)) soils, and in cultivated (1.7 g kg\(^{-1}\)) and fallow (1.5 g kg\(^{-1}\)) Qunu soils, were possibly caused by low additions of organic matter on the one hand, and continual breakdown of SOM accompanying cultivation. In contrast to cultivated soils, the soil C contents were higher in Guquka and Hertzog virgin soils suggesting accumulation of organic matter under the virgin conditions where the SOM is not disturbed. The lower quantities of soil N (< 1 g kg\(^{-1}\)) in all the soils indicate that these soils were deficient in soil N. In the cultivated soils this status could be a result of continuous cropping with low or no use of organic and/or inorganic fertilizers. The lower soil N content can be related to low soil C quantities because it is well known that soil organic matter is a very important source of soil N. According to Bremmer (1965) and Wolf & Snyder (2003) the highest proportion of soil N is found in organic forms. Guquka, Hertzog and Fort Cox College are in arid to semi arid
climatic conditions. So the limited amounts of OM that build up when climatic conditions permit could quickly be lost through decomposition in summer.

Organic matter also helps in soil aggregate stabilization especially in soils with high silt content and low clay content like the ones used in this study (Table 2.1) (Pagliai et al., 1987; Sheperd et al., 2002; Riley et al., 2008). The observed low organic C content therefore suggests poor aggregate stability which could also partly explain the crusts observed on the surface of the soils studied.

The high silt plus fine sand content (Table 2.1) could make soil structural units unstable upon wetting or due to physical disturbances like ploughing or animal movement. This could account for the observed crusting tendency at some of the sites. The tendency for soils with high silt content and low clay content to form crust during rainfall or irrigation events has been reported by other authors (Le Bissonnais et al., 1989; Pagliai and Vignozzi, 1998). In Hertzog soil, the structure degradation could be exaggerated by high magnesium contents. Studies conducted by Dontsova and Norton (2001) showed that soils with high Mg content had greater aggregate destruction and clay translocation that leads to soil crust formation and erosion even when the sodium content was low. The sodium content in Hertzog and Fort Cox soils was very low and could not have affected flocculation of soil particles.
2.5.2 Cyanobacteria Enumeration, Isolation, Purification and Growth

A greater proportion of the cyanobacteria strains isolated from Qunu soil (22 strains) was from virgin soil, followed by fallow soil (10 strains), with no strains isolated from the cultivated soil. A similar trend for Hertzog soil was observed with higher number (11 strains) of cyanobacteria isolated from virgin soil compared to nine from fallow and eight from cultivated soil. Virgin soils might have higher proportions of cyanobacteria strains because of higher organic matter and better fertility. Tillage exposes organic matter to decomposition, resulting in lower total C. The higher number of cyanobacteria strains isolated from Fort Cox College soil (22 strains) was possibly because of higher use of fertilizers and irrigation. Cyanobacteria are known to flourish on moist or wet conditions with adequate nutrients. Thus, the cultivated soils from Fort Cox College, with their higher fertility due to fertilizer use, could have offered ideal conditions of N and SOM for cyanobacteria growth. However, all the strains isolated from this soil could not fix N and neither did they produce EPS.

The very good growth of the cyanobacteria strain 3v and a good growth of strains 1af, 2i, 8a, and 21a in solid growing medium indicated their preference for solid than for liquid BG110 growing media. Only strains 3g and 7e could not grow on solid medium, but grew weakly on liquid (Table 2.4), while the rest of the strains grew well on solid medium (Table 2.3). Mass production of cyanobacteria can be done more easily in liquid than solid growing medium, hence the need to select for those strains that not only fix high amounts of N and/or produce large quantities of EPS, but also are also able to grow well in liquid culture.
2.5.3 Nitrogen Fixing Capability and EPS Production of Isolated Cyanobacteria

Only strains 3v, 3g and 7e had a potential to fix atmospheric N\(_2\), but only strain 7e exhibited nitrogenase activity comparable to that of the reference strain. The capability of strain 7e to fix higher quantities of nitrogen than other cyanobacteria strains suggests that it probably had a higher percentage of heterocysts which are the cells devoted only to N\(_2\) fixation (Clark, 2002). Strains 3g and 3v and other cyanobacteria strains (1af, 2i, 8a, 22b, and 21a) that grew well (Table 2.4) but had limited potential to fix atmospheric N\(_2\) may not be heterocystous.

As shown in Table 2.4 and Figure 2.1, cyanobacteria strains like 7e, 3v, and 3g displayed an inverse relationship between EPS production and nitrogen fixation. Cyanobacteria strains 3v displayed a high capability to produce EPS but very low potential to fix atmospheric nitrogen. The cyanobacteria strain with the highest nitrogen fixation capability, 7e, displayed untraceable amounts of EPS. This inverse relationship is possibly caused by fewer heterocysts cells and many vegetative cells in strains that fixed lower atmospheric N and produced higher EPS compared to strains that fixed higher atmospheric N and produced lower quantities of EPS. Vegetative cells are responsible for the development and production of EPS in processes that involve oxygen-evolving photosynthesis (Evans and Ehrlinger, 1993; Eldridge and Green, 1994).

2.6 CONCLUSIONS

The numbers of strains isolated from virgin soils were larger than those in cultivated soils, except for Fort Cox College soil where the cultivated soil, as a result of receiving large amounts of

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fertilizer and irrigation, provided conditions requisite for growth of diverse strains of cyanobacteria. Cyanobacteria strain 7e had the highest potential to fix atmospheric N\(\text{2}\). Cyanobacteria strain 3g had the ability to produce EPS and fix atmospheric nitrogen. Cyanobacteria strain 3v exhibited the greatest potential to produce EPS and could thus be utilized in improving the aggregate stability of weakly aggregated soils. The degraded soils studied contained cyanobacteria strains which if successfully mass produced could be used to improve the status of degraded soils through improved N contents and structural stability. The combined soil inoculation of cyanobacteria strains 7e and 3g, if compatible, has the potential to improve both the soil N status and aggregate stability.
2.7 REFERENCES


CHAPTER THREE

NOSTOC CYANOBACTERIA STRAIN 9v INOCULATION OF TWO SOUTH AFRICAN AGRICULTURAL SOILS ENHANCES SOIL FERTILITY AND MAIZE GROWTH

3.1 ABSTRACT

Many soils in South Africa, especially in the smallholder sector, have low nutrient supply because nutrient removal often exceeds replenishment. Two crusting soils from the Eastern Cape Province, South Africa, were used to evaluate the effects of inoculation with a strain of Nostoc (coded as 9v) on soil fertility and maize growth. The Nostoc 9v suspension was uniformly applied over potted soils at the rate of 6 g (dry weight) m\(^{-2}\) soon after maize germination. Inoculation increased soil N by 17 and 40% in Hertzog and Guquka soils, respectively. Soil C was also increased significantly and this increase was strongly associated with that of soil N (R\(^2\) = 0.838). The highest contents of soil C, soil N and mineral N, however, were found in non-cropped Nostoc 9v inoculated soils. Inoculation with Nostoc strain 9v increased maize dry matter yields by 49 and 40% in Hertzog and Guquka soils, respectively. Corresponding increases in maize tissue N were 23 and 14%, respectively. The results suggested that Nostoc strain 9v could improve the fertility of degraded soils.

**Key words:** Cyanobacteria, Inoculation, Nostoc, Nutrients, Soil N, South Africa.
3.2 INTRODUCTION

Most soils used for crop production in South Africa have low fertility due to nutrient removal without adequate replenishment and loss of topsoil through erosion (Scotney and Dijkhuis, 1990; du Toit and du Preez, 1995; Mills and Fey, 2003; Barnard and du Preez, 2004). Similar soil fertility status has also been reported for most Sub-Saharan countries (Sanchez, 2002; Mafongoya et al., 2006). The decline in soil fertility has led to corresponding decreases in crop yields, increased food insecurity and has enhanced environmental degradation. Many farmers practise injudicious management geared towards soil fertility improvement especially in their gardens by applying mineral fertilizers and available farm yard manure (Mandiringana et al., 2005). Most of the time the quantities of mineral fertilizers the farmers use are comparatively small (McIntyre et al., 1992) and not based on sound fertilizer recommendations. Furthermore, the animals they own do not produce enough quantities of manure to meet crop nutrient needs. In other countries like Tanzania, farmers also use leaves of certain trees and local shrubs as green manure (Wickama and Mowo, 2001).

Soil inoculation with N$_2$-fixing cyanobacteria has been shown to result in increases in rice yields in countries like China, Vietnam, Philippines, Japan, Egypt, the Soviet Union and India (Metting, 1981). Total N, SOC, and available nutrients in the topsoil have also been shown to increase with inoculation. Thus, Rogers and Burns (1994) demonstrated that inoculation of a poorly structured silt loam soil with *Nostoc muscorum* led to a pronounced effect on soil chemical properties, with total C increasing by 50–63% and total N increasing by 111–120%. In a laboratory experiment, Acea et al. (2003) also showed that soil inoculation with different cyanobacterial strains induced
great microbial proliferation as well as high increases in SOC and available nutrients, though the efficacy of the treatment was strongly influenced by soil type.

Observed increases in soil total N following cyanobacteria inoculation is attributed to the ability of cyanobacteria to fix atmospheric nitrogen and increase SOM (Harper and Marble, 1988). Soil inoculation with cyanobacteria with these attributes may therefore represent a simple and low-cost method for improving the productivity of degraded lands in developing countries where very little or no inorganic fertilizers are usually used. So far no research has been undertaken with regard to the possibility of using N\textsubscript{2}-fixing cyanobacteria in South African soils. A strain of cyanobacteria (9v) was isolated from a Tanzanian soil showed greater potential to fix atmospheric N\textsubscript{2} and produce EPS and could therefore play an important role in improving degraded soils in the Eastern Cape Province. The purpose of this study was to assess the effectiveness of cyanobacteria strain 9v for the enhancement of the fertility of two degraded arable soils from the Eastern Cape Province, South Africa, under tunnel house conditions.

### 3.3 MATERIALS AND METHODS

Soil samples for this study were collected near the villages of Guquka (32°39’ S, 26°57’ E) and Hertzog (32°35’ S, 26°43’ E) in the Eastern Cape Province of South Africa. The soils were classified according to Soil Survey Staff (1975) as Typic Plinthustalf (Guquka) and Typic Haplustalf (Hertzog) and their chemical and physical properties are given in Table 2.1 (Chapter 2). The profile characteristics are given in Appendices C and D for Guquka and Hertzog soils,
respectively. Guquka soil had been cropped to maize in the previous summer season but was presently on fallow, whereas Hertzog soil was in its second fallow year, at the time of sampling. Bulk soil samples collected from the 0 – 15 cm depth were air-dried and sieved (> 4 mm) before use in the tunnel house study.

The cyanobacterium strain used in this study was isolated from a tropical soil from Tanzania, another site studied during the EU – funded CYANOSOILS project. It was isolated from colonies that developed on solid and selective growth medium BG 11\textsubscript{0} as described in Chapter 2. This \textit{Nostoc} strain (9v) was selected because it showed a high growth rate on culture medium, and had abilities to produce EPS and fix nitrogen at the same time.

Growth of cyanobacteria for pot studies was done by picking clean colonies and growing them in 10 ml BG11\textsubscript{0} liquid growing medium in test tubes. The test tubes were closed with cotton wool to allow diffusion of carbon dioxide and oxygen. The growth of cyanobacteria in the liquid growing medium was observed daily, and when the concentration of cyanobacteria had doubled an equal volume of growing medium was added to the test tubes, until the test tube was full. Cyanobacteria cultures were subsequently transferred into new growing medium in 25 ml conical flasks, and subsequently successively into 50, 100, 250, 1000, and 2000 ml conical flasks when the dry matter concentration reached 1 g L\textsuperscript{-1}. The cyanobacteria from the 2000 ml conical flasks were transferred to 25 L glass bottles to prepare more biomass for pond production (Appendix B). The dry matter was determined weekly when the volume of cyanobacteria was more than 250 ml by taking 25 ml of cyanobacteria and filtering it through pre-weighed filter paper. The filter paper with cyanobacteria was then dried at 60 °C to a constant weight after which it was weighed
to determine its mass with cyanobacteria. The pH of cyanobacteria culture was measured weekly and adjusted by adding 0.1 M HCl to pH 7 whenever pH was greater than 7. Every time cyanobacteria were transferred to the new growing medium the light intensity was reduced by using one light bulb, because cyanobacteria growth is inhibited by high light intensity. As the concentration of cyanobacteria increased the light intensity was also increased from one fluorescence light bulb to four light bulbs when cyanobacteria concentration was 1 g/L. At least 100 L of concentrated (1 g L\(^{-1}\)) cyanobacteria were transferred to outdoor ponds. An additional 100 L of new growing medium was applied to the ponds to reduce the concentration of cyanobacteria to 0.5 g L\(^{-1}\). Ponds were covered with a shade cloth, allowing 50 % light penetration to reduce light intensity until the concentration of cyanobacteria were 0.8 g L\(^{-1}\) and above. The shade cloth was removed on rainy days, clouds overcast days and when the concentration of cyanobacteria was above 0.8 g L\(^{-1}\).

Pots with a diameter of 20 cm were filled with 4 kg of the sieved soils (< 4mm) and used in this study. The treatments were arranged in a split split-plot design with four replications in which soils (Hertzog and Guquka) were the main plots, cropping treatments (cropped and non-cropped with maize) were the subplots and inoculation treatments (inoculated and non–inoculated with the Tanzanian \emph{Nostoc} strain) were the sub sub-plots. A basal rate of 40 mg P kg\(^{-1}\) as potassium phosphate was applied to each pot to provide P and K before five maize seeds were sown in each. In the inoculated pots a suspension of \emph{Nostoc} 9v, at a concentration of 1 g (dry weight) L\(^{-1}\), was uniformly poured on the soils soon after maize germination to provide an equivalent dry biomass of 6 g m\(^{-2}\), whilst non-inoculated soils were irrigated with water equivalent to the liquid introduced via inoculation in the \emph{Nostoc} 9v treated pots. The C and N contents of the inoculated
Nostoc strain were 359 g kg\(^{-1}\) and 35 g kg\(^{-1}\), respectively. The maize plants were thinned to two plants per pot seven days after germination. Pots were watered as necessary to compensate for water loss due to evapo-transpiration.

Six weeks after inoculation the maize plants were harvested by cutting the stems just above the soil surface, and the plants were cleaned with distilled water and dried to constant weight in an oven at 60 °C. The dried plant materials were weighed, ground and analysed for tissue N using a LECO C&N analyzer (LECO Corporation, 2003). The top 5 mm of the soils was sampled by gently scooping from the centre and from four positions along the edges of each pot. The samples were uniformly mixed and a portion was kept in the refrigerator (<4 °C) until it was used for inorganic N determination, whilst another portion was dried, ground and used for soil C and N determination using the LECO C&N analyser. Inorganic N was determined colorimetrically as described by Okalebo et al. (2002).

Statistical analysis was done by analysing the variance using the Genstat statistical software (Genstat Release 4.24DE, 2005) but mean separation was done using LSD.

3.4 RESULTS

3.4.1 Effect of Nostoc Strain 9v Inoculation on Selected Soil Chemical Properties

Nostoc strain 9v significantly increased soil C (p < 0.001), soil N (p < 0.01) and mineral N (p < 0.05) contents in the 5 mm depth of the soil (Table 3.1). The percentage increases when averaged
across the two soils were 50% and 6% for total soil N and mineral N content, respectively. There was no significant interaction (p=0.199) between inoculation with the *Nostoc* strain 9v and soil type on total soil N, but soil N increased following inoculation (Table 3.2) from 0.03 to 0.05 % in Guquka soil (67%) compared from 0.05 to 0.06 % in Hertzog soil (20%).

Table 3.1: Effects of *Nostoc* strain 9v inoculation on the total N and mineral N of soil samples taken to a depth of 5 mm

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Inoculated</th>
<th>Non-inoculated</th>
<th>% increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total soil N (%)</td>
<td>0.06&lt;sup&gt;a&lt;/sup&gt; ±0.005&lt;sup&gt;**&lt;/sup&gt;</td>
<td>0.04&lt;sup&gt;b&lt;/sup&gt; ±0.004</td>
<td>50</td>
</tr>
<tr>
<td>Mineral N (mg kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>50.0&lt;sup&gt;a&lt;/sup&gt; ±0.80</td>
<td>47.0&lt;sup&gt;b&lt;/sup&gt; ±0.90</td>
<td>6</td>
</tr>
</tbody>
</table>

* Different letters following means in a row indicate that means were significantly different at P ≤ 0.05

** Standard error of means
Table 3.2: Interaction between *Nostoc* strain 9v inoculation, soil type, and cropping on soil C, total soil N and mineral N content of soil samples taken to a depth of 5 mm

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Soil C (%)</th>
<th>Total soil N (%)</th>
<th>Mineral N (mg.kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Soil type</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guquka</td>
<td>Inoculated</td>
<td>0.57(^{b#}) ±0.03</td>
<td>0.05 ±0.008(^*)</td>
</tr>
<tr>
<td></td>
<td>Non-inoculated</td>
<td>0.45(^c) ±0.01</td>
<td>0.03 ±0.005</td>
</tr>
<tr>
<td>Hertzog</td>
<td>Inoculated</td>
<td>0.80(^a) ±0.03</td>
<td>0.06 ±0.005</td>
</tr>
<tr>
<td></td>
<td>Non-inoculated</td>
<td>0.74(^a) ±0.03</td>
<td>0.05 ±0.002</td>
</tr>
<tr>
<td><strong>Cropping</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cropped</td>
<td>Inoculated</td>
<td>0.62 ±0.05</td>
<td>0.06 ±0.004</td>
</tr>
<tr>
<td></td>
<td>Non-inoculated</td>
<td>0.56 ±0.05</td>
<td>0.05 ±0.005</td>
</tr>
<tr>
<td>Non –cropped</td>
<td>Inoculated</td>
<td>0.74 ±0.05</td>
<td>0.05 ±0.009</td>
</tr>
<tr>
<td></td>
<td>Non-inoculated</td>
<td>0.64 ±0.06</td>
<td>0.04 ±0.006</td>
</tr>
</tbody>
</table>

* For each treatment (Guquka and Hertzog or Cropped and Non-cropped), means in a column followed by the same letter or none at all are not significantly different at \(P \leq 0.05\)

* Standard error of means

### 3.4.2 Effect of *Nostoc* Strain 9v Application on Maize Growth and Nitrogen Uptake

*Nostoc* strain 9v improved the growth and N uptake of maize (Table 3.3). In Guquka soil, dry matter (DM) yield was increased by 49% (8.1 to 12.1 g pot\(^{-1}\)) while in Hertzog soil it increased by 40% (5.3 to 7.4 g pot\(^{-1}\)). A similar trend was observed for maize tissue N concentration and N
uptake in both soils (Table 3.3). The increases in maize tissue N concentration and N uptake following *Nostoc* strain 9v inoculation mirrored observed improvements in soil N and mineral N content of the soils following inoculation with the *Nostoc* strain 9v (Table 3.1).
Table 3.3: Dry matter yield and nitrogen uptake of maize in response to inoculation with a *Nostoc* strain 9v in Hertzog and Guquka soils

<table>
<thead>
<tr>
<th>Inoculation</th>
<th>Hertzog</th>
<th>Guquka</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry matter</td>
<td>Tissue N</td>
</tr>
<tr>
<td></td>
<td>(g pot⁻¹)</td>
<td>(%)</td>
</tr>
<tr>
<td>Inoculated</td>
<td>12.1ᵃ⁺ ±1.35**⁺⁺</td>
<td>0.53ᵃ⁺ ±0.06</td>
</tr>
<tr>
<td>Non-inoculated</td>
<td>8.1ᵇ⁻ ±0.40</td>
<td>0.43ᵇ⁻ ±0.03</td>
</tr>
</tbody>
</table>

¹⁺ Means in a column followed by the same letter indicate that means are not significantly different at $P \leq 0.05$

⁺⁺ Standard error of means
3.5 DISCUSSION

3.5.1 Effect of Nostoc Strain 9v Inoculation on Selected Soil Chemical Properties

The *Nostoc* strain 9v showed an ability to colonize Guquka and Hertzog soils quickly, irrespective of their physical and chemical characteristics. This good establishment of *Nostoc* in the two soils was responsible for the observed improvement in the soil C and soil N contents determined in the top 5 mm of these C and N poor soils (Table 3.1). This increase was presumably the result of the photosynthetic increment in both soil carbohydrate C (polysaccharide) and biomass C (Lange *et al.*, 1992). Treatment performance, however, depended on soil type with the largest increases in both soil C and N occurring in Guquka soil, possibly because it had much lower levels of these nutrients to start with (Table 3.2). The observed increases in soil N, particularly in Guquka soil, were comparable to those reported by Harper and Pendleton (1993) in a similar study. The observed trends in soil N were also similar to those reported by Skarpe and Henriksson (1987) and Buttars *et al.* (1998) even though their studies used the more accurate acetylene reduction method for assessing N-fixation, as opposed to this study where total N was used.

A strong positive relationship of soil C and soil N ($R^2=0.838$, Figure. 3.1) indicated close association of C and N dynamics in the inoculated and non-inoculated soils, as reported by Nisha *et al.* (2007). Generally, the increases in N due to inoculation were proportionately greater than those of C, which resulted in a narrowing of the C: N ratio. In Guquka soil, for example, the C: N ratio dropped from 15.0 in non-inoculated soils to 11.4 when soil was inoculated with *Nostoc,*
which impacted positively on the mineralization of N (Table 3.2). Nisha et al. (2007) observed greater C loss from cyanobacteria – treated soils than from non – treated ones and attributed it to greater microbial activity and respiration in the former. The greater increase in N relative to C observed in cyanobacteria treated soils in the present study could probably have been caused by the N – fixation by the cyanobacteria strain 9v.

![Graph showing the relationship between soil C and soil N](image)

Figure 3.1 Relationship between soil C and soil N in soil samples collected to a depth of 5 mm and treated with or without a Nostoc strain 9v

### 3.5.2 Effect of *Nostoc* strain 9v Inoculation on Maize Yield and Nitrogen Uptake

Inoculation with *Nostoc* resulted in improved maize growth in both Guquka and Hertzog soils. The improved growth appeared related to the observed increases in soil N and favourable mineralization of the same in inoculated soils. Plants that had higher dry matter had higher tissue
N. These results were in agreement with those of Nisha et al. (2007) who also observed improvements in the growth of a pearl millet-wheat sequence in response to cyanobacteria biofertilization.

The observed increases in soil C could have improved the water holding and infiltration capacities of the soils, and potentially the plant water use efficiency from the soils. However, water was not a limiting factor in the tunnel house experiment, so the observed improvement in maize dry matter yields may not be attributed to improved water use efficiency but rather to improved soil N levels. Such an effect could, however, translate also to improved water retention and use efficiency under the water–scarce field conditions that often prevail in the semi-arid environment of the Eastern Cape, South Africa. Generally, the results indicate that cyanobacteria screened for N-fixing ability could improve the productivity of nitrogen poor soils of the Eastern Cape Province of South Africa.

3.6 CONCLUSIONS

The Nostoc strain 9v improved the soil C and N contents of both Guquka and Hertzog soils. The increases in soil N translated to improved maize growth and N uptake in both soils. Cropping with maize reduced the effectiveness of inoculation due to its negative effect on Nostoc 9v establishment and OM production. Nevertheless, the results suggested that cyanobacteria screened for N$_2$-fixing ability could improve the productivity of N poor soils.
3.7 REFERENCES


Genstat Release 4.24DE (2005). Lawes Agricultural Trust (Rothamsted Experimental Station) UK.


CHAPTER FOUR

BIOCONDITIONING EFFECTS OF NOSTOC CYANOBACTERIA STRAIN 9v INOCULATION IN TWO SOUTH AFRICAN AGRICULTURAL SOILS

4.1 ABSTRACT

The majority of soils in South Africa have poor structural stability and are prone to soil erosion due to susceptibility to surface sealing and crusting. Two crusting soils from the Eastern Cape Province, South Africa, were used to evaluate the effects of inoculation with a strain of Nostoc (coded as 9v) on soil structure. The Nostoc strain 9v suspension was uniformly applied on potted soils at the rate of 6 g (dry weight) m$^{-2}$ soon after maize germination. Inoculation significantly increased soil C by 27 and 8% in Guquka and Hertzog soils, respectively. The highest contents of soil C, however, were found in non-cropped Nostoc 9v inoculated soils. Scanning Electron Microscopy (SEM) revealed that soil particles and fragments of non-cropped inoculated soils had coatings of EPS, with other particles enmeshed in networks of filaments, whilst by contrast little or no EPS and/or filaments were observed on cropped and/or non-inoculated soils. This was consistent with chemical analysis which showed that the Nostoc strain 9v caused significant increases in the EPS and soil C contents of non-cropped soils. The proportion of very stable aggregates was increased by inoculation with Nostoc strain 9v, possibly due to the greater quantities of soil C and EPS observed in inoculated soils. Inoculated soils cropped with maize had a lower proportion of stable aggregates presumably due to their low soil C and EPS contents compared to non-cropped soils. The results suggested that Nostoc strain 9v could improve the structural stability of the studied degraded soils.

Key words: Aggregate stability, Cyanobacteria, Exo-cellular polysaccharides, Inoculation, Nostoc, South Africa.
4.2 INTRODUCTION

Most soils used for crop production in South Africa have high silt and low (SOM) contents (Oldeman, 1994; Barnard and du Preez, 2004). The aggregates of these soils, therefore, quickly collapse under pressure on irrigation or high rainfall, resulting in surface sealing and physical crust formation (Davies et al., 1993). The tendency for soil surface crusting can be reduced by improving the resistance of aggregates to physical and physico-chemical mechanisms of breakdown that operate during rainfall and irrigation events. According to Le Bissonnais (1996) aggregate breakdown is caused by one or more of the following mechanisms: (i) slaking, caused by the compression of entrapped air during wetting; (ii) breakdown by differential swelling related to the behaviour of clay material after wetting; (iii) breakdown by raindrop impact, and (iv) physico-chemical dispersion due to osmotic stress. Methods proposed by Le Bissonnais (1996) used to test the occurrence of these mechanisms are fast wetting, mechanical breakdown, and slow wetting.

Soil organic matter content has been reported to be of utmost importance in improving degraded soils (Domini and Haynes, 2002). Adding OM to soil increases the stability of aggregates to breakdown (Balock and Nelson, 2000) by reducing the rate of wetting and increasing the resistance to stresses generated during wetting (Caron et al., 1996). Below the SOM content threshold of 2%, water–stable aggregates have been shown to break down and became extremely erodible (Elwell, 1989; Kay and Angers, 2000). Filamentous cyanobacteria have been shown to contribute to macro-aggregation and result in improved resistance to soil erosion (Aspiras et al., 1971; Falchini et al., 1996; Kay and Angers, 2000). As primary producers, they contribute to the
enrichment of soil with SOM and to the improvement of biological activity (Acea et al., 2003). Exo-cellular polysaccharides produced by some cyanobacteria strains are dominated by polysaccharides which can bind soil particles (Belnap and Gardner, 1993; Malam Issa et al., 2001), in addition to their role in the protection of the cyanobacteria against environmental conditions (De Winder, 1990) and in assisting cyanobacteria motility (Stal, 1995).

Inoculating soil with cyanobacteria has been reported to improve the aggregation of the top soil (Malam Issa et al., 2007) and to increase water retention, and ecosystem regeneration (Eldridge and Greene, 1994). All these benefits on physical properties are in addition to increased SOC, total N and available nutrients in the topsoil as a result of inoculation with N\textsubscript{2} fixing cyanobacteria. In a laboratory experiment, Acea et al. (2003) also showed that soil inoculation with different cyanobacterial strains induced great microbial proliferation as well as high increases in SOC and available nutrients, the efficacy of the treatment depending on the type of soil. The effects of *Nostoc* strain 9v inoculation on selected soil properties and maize growth was reported in a glasshouse experiment in Chapter 3. The objective of the present study was to assess the effectiveness of *Nostoc* strain 9v for the enhancement of the structure of two degraded arable soils from the Eastern Cape Province, South Africa.

4.3 MATERIALS AND METHODS

The experiment described in Chapter 3, section 3.3, was used for the pursuance of the objectives of the present study. Soon after the maize plants were harvested soil sampling for aggregate
stability determination was done by gently scooping the top 5 mm of the soil and pouring this into rigid containers to avoid further breakage of the aggregates. Microscopic investigations of soil structure and aggregate stability measurements were simultaneously run for inoculated and non-inoculated samples. The micromorphological characteristics of the selected samples were investigated with a scanning electron microscope (model QUANTA 200 Phillips). Exo-cellular polysaccharides/sugars were analysed by extracting 10 g of soil with 30 ml of 0.5 \( M \) NaOH and then hydrolysing overnight in 5 ml of 12 \( M \) \( H_2SO_4 \). Samples were then centrifuged at a relative centrifugal force of 15557 \( x \) \( g \) for 30 minutes using the Eppendorf Centrifuge 5810 centrifuge and the extracted EPS was determined spectrophotometrically using the phenol-sulphuric acid method (Dubois et al., 1956). A blank which did not contain the phenol was also prepared.

Aggregate stability measurements were performed in triplicate using 5g soil samples according to the method of Le Bissonnais (1996). The samples were first sieved between 3 and 5 mm mesh and dried at 40°C for 48 hours. The samples were then subjected to fast wetting, slow wetting, or mechanical breakdown treatments according to Attou et al. (1998). Fast wetting tests forces related to entrapped air within the aggregates and differential swelling of clays (Chenu et al., 2000). Fast wetting was done by gently immersing soil aggregates in 50 ml deionised water for 10 minutes. The water was drawn off by pipette leaving behind the slaked aggregates. Slow wetting tests the stability of aggregates under low moisture conditions such as those subjected to moderate rains. It measures aggregate stability under conditions in which air entrapment and differential swelling are minimized. Slow wetting was performed by placing aggregates on a filter paper maintained at a matric potential of – 0.3 kPa. After 30 minutes the residual aggregates were collected. The mechanical breakdown method tests the cohesion of soil
aggregates independently of slaking by air entrapment and the effect of differential swelling. For mechanical breakdown air was removed from the aggregates before energy was applied, by immersing soil aggregates into 50 ml ethanol. After 10 minutes the ethanol was drawn by pipette and aggregates were transferred to another flask with 50 ml of deionised water. Distilled water was added to the flask to the 200 ml mark, corked and agitated end over end 20 times and left to stand for 30 minutes to allow coarse particles to settle. Suspended material and excess water were removed by pipette and the residual aggregates collected.

Following each test method, the residual aggregates were transferred to a 50 µm sieve immersed in ethanol. The aggregates which were retained on the sieve were transferred to evaporation dishes and dried at 40°C for 24 hours. The fragment size distribution (FSD) was measured by dry sieving the aggregates with a set of six sieves of 2, 1, 0.5, 0.2, 0.1 and 0.05 mm in diameter. The weight of aggregates collected on each sieve was determined and expressed as a percentage of the initial sample dry mass. Aggregate stability was described using the resulting fragment size distribution in the seven granulometric classes and the mean weight diameter (MWD) calculated as follows:

\[
MWD = \frac{\sum \bar{x}_i w_i}{100}
\]

Where \(\bar{x}_i\) is the mean intersieve size and \(w_i\) the percentage particles left on each sieve.

The results of the fragment classes were also grouped into macro (>0.2 mm in diameter) and micro (<0.2 mm in diameter) aggregates. Statistical analysis was done by analysis of variance using the Genstat statistical software (Genstat Release 4.24DE, 2005) but mean separation was done using LSD.
4.4 RESULTS

4.4.1 Observations on cyanobacterial colonization of the soil surface

Three weeks after inoculation about 90% of the entire surfaces of inoculated soils were covered with layers of *Nostoc* strain 9v in combination with other cyanobacteria, whilst only thin patches of cyanobacteria were found on the surface of non-inoculated soils. These observations suggested that the inoculated *Nostoc* strain 9v established well on the surfaces of the two experimental soils. Further observations under SEM showed great differences between inoculated and non-inoculated samples (Figures 4.1a – f). Inoculated soil samples had networks of filaments among which mineral particles were enmeshed (Figure 4.1a and b), and mineral particles and soil fragments were coated by EPS presumed to have been secreted by the *Nostoc* strain (Figure 4.1c and d). By contrast little or no EPS and/or filaments were observed on non-inoculated soils, especially on the cropped ones (Figure 4.1e and f). In non-cropped Guquka soil, fragments and soil particles were enmeshed among cyanobacteria filaments and EPS coating (Figure 4.1a) and other particles were enclosed in the networks of filaments (Figure 4.1b). Mineral particles and soil fragments were trapped in the network and superficial pores resulting from the intertwining of filaments. On the surface of non-cropped inoculated Guquka soil massive accumulation of EPS was observed (Figure 4.1c). On non-cropped inoculated Hertzog soil EPS coating formed organic bridges between soil particles (Figure 4.1d). When cropped and non-inoculated, only mineral particles and fragments were observed on Guquka soil (Figure 4.1e) and Hertzog soil (Figure 4.1f).
Figure 4.1: SEM micrographs of the soil surface: (a) Non-cropped inoculated Guquka soil with filaments and EPS coating, (b) Non-cropped inoculated Guquka soil with networks of filaments, (c) Non-cropped inoculated Guquka soil, (d) Non-cropped inoculated Hertzog soil, (e) Cropped non-inoculated Guquka soil and (f) Cropped non-inoculated Hertzog soil.
4.4.2 Effect of *Nostoc* Strain 9v Inoculation on Soil C and EPS Content

Inoculation with *Nostoc* strain 9v significantly increased soil C (p < 0.001) and EPS (p < 0.001) contents in the 0 – 5 mm soil depth (Table 4.1). The percentage increases when averaged across the two soils were 14% and 37% for soil C and EPS, respectively. There was a significant interaction (p=0.049) between soil type and inoculation with *Nostoc* in regard to soil C content (Table 4.2) in that soil C was only increased in the Guquka soil but not in Hertzog soil as a result of inoculation. The interaction between inoculation and cropping was not significant (p=0.212) but a greater increase in soil C following inoculation was observed in non-cropped soils (16%) than in cropped soils (11%).

Table 4.1: Effects of *Nostoc* strain 9v inoculation on soil C and EPS of soil samples taken to a depth of 5 mm

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Inoculated</th>
<th>Non-inoculated</th>
<th>% increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil C (%)</td>
<td>0.68a ±0.04</td>
<td>0.60b ±0.04</td>
<td>14</td>
</tr>
<tr>
<td>EPS (mg.g⁻¹)</td>
<td>2.6a ±0.2</td>
<td>1.9b ±0.1</td>
<td>37</td>
</tr>
</tbody>
</table>

* Different letters following means in a row indicate that means were significantly different at P ≤ 0.05

** Standard error of means

Production of EPS was significantly (p < 0.001) favoured in *Nostoc* strain 9v inoculated soils as reflected by observed increases in EPS production of 31% and 42% in Guquka and Hertzog soils, respectively (Table 4.2). The interaction between soil type and inoculation was not significant.
(p=0.256) on EPS but the interaction between cropping and inoculation was significant (p=0.013). Inoculation with *Nostoc* strain 9v increased EPS by 58% (2.05 to 3.24 mg g\(^{-1}\)) in non-cropped soils whereas in cropped soils EPS increased by only 13% (1.83 to 2.07 mg g\(^{-1}\)) (Table 4.2).

Table 4.2: Interaction between *Nostoc* inoculation, soil type, and cropping on soil C and EPS of soil samples taken to a depth of 5 mm

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Soil C (%)</th>
<th>Exo-cellular polysaccharides (mg g(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Soil type</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guquka</td>
<td>Inoculated</td>
<td>0.57(^{b#}) ±0.03(^{**})</td>
</tr>
<tr>
<td></td>
<td>Non-inoculated</td>
<td>0.45(^{c}) ±0.01</td>
</tr>
<tr>
<td>Hertzog</td>
<td>Inoculated</td>
<td>0.80(^{a}) ±0.03</td>
</tr>
<tr>
<td></td>
<td>Non-inoculated</td>
<td>0.74(^{a}) ±0.03</td>
</tr>
<tr>
<td><strong>Cropping</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cropped</td>
<td>Inoculated</td>
<td>0.74 ±0.05</td>
</tr>
<tr>
<td></td>
<td>Non-inoculated</td>
<td>0.64 ±0.06</td>
</tr>
<tr>
<td>Non-cropped</td>
<td>Inoculated</td>
<td>0.62 ±0.05</td>
</tr>
<tr>
<td></td>
<td>Non-inoculated</td>
<td>0.56 ±0.05</td>
</tr>
</tbody>
</table>

\(^{#}\) For each treatment (Guquka and Hertzog or Cropped and Non-cropped), means in a column followed by the same letter or none at all are not significantly different at \(P \leq 0.05\)

\(^{**}\) Standard error of means
4.4.3 Effect of *Nostoc* Strain 9v Inoculation and Cropping on Soil Aggregate Stability

**Fragment size distribution (FSD)**

The percentage of fragments >2 mm in non-cropped inoculated soils from Guquka ranged from 22-32 % and were significantly higher than those obtained in corresponding non-inoculated soil which ranged 19-24 % for all three test methods (Figure 4.2a). The FSD of non-cropped soils from Hertzog exhibited lower values of coarse aggregates compared to that of Guquka. The proportion of aggregate fragments >2mm collected after fast wetting (FW), wet stirring (WS) and slow wetting (SW) on non-cropped soils from Hertzog ranged from 13 to 21 % and 13 to 20 % when inoculated and non-inoculated, respectively (Figure 4.2b).

The FSD of cropped and inoculated Guquka soil showed the proportion of fragments >2mm to range from 14 to 18 % which was higher than the 5-15% observed in the corresponding non-inoculated soil (Figure 4.3 a). The proportion of aggregates >2 mm for Hertzog soil were 11 to 14 % when cropped and inoculated and 7 to 14 % when cropped and non-inoculated (Figure 4.3b).
Figure 4.2: Effect of *Nostoc* inoculation on the fragment size distribution (FSD) of non-cropped Guquka (a) and Hertzog (b) soil samples taken to a depth of 5 mm (I and NI stand for inoculated and non-inoculated, respectively). Error bars represent standard deviations.
Figure 4.3: Effect of *Nostoc* strain 9v inoculation on the fragment size distribution (FSD) of maize cropped Guquka (a) and Hertzog (b) soil samples taken to a depth of 5 mm (I and NI stand for inoculated and not-inoculated, respectively). Error bars represent standard deviations.
When grouped into macro aggregates (>0.2 mm) and micro aggregates (<0.2 mm) the results showed that inoculating with the *Nostoc* strain 9v increased the proportion of macro-aggregates with a corresponding decrease in the proportion of micro-aggregates in both Guquka and Hertzog soils (Figure 4.4). This effect was the same in non-cropped and cropped soils. The percentage of macro-aggregates and micro-aggregates for non-cropped and inoculated soils from Guquka ranged between 50 and 71% and between 29 and 50%, respectively while corresponding values for non-cropped and non-inoculated soils ranged between 42 and 65% and between 35 and 58%, respectively. A similar pattern was observed on Hertzog soil but the negative effect of cropping on aggregate stability was less pronounced compared to that in Guquka soil (Figure 4.4).
Figure 4.4: Interaction effects of *Nostoc* inoculation with cropping and soil type on soil macro-aggregates (>0.2mm) and micro-aggregates (<0.2mm) sampled to a depth of 5mm and determined by fast wetting, wet stirring, and slow wetting methods (I and NI stand for inoculated and non-inoculated, respectively). Error bars represent standard deviations.
Mean weight diameter (MWD)

Inoculation with *Nostoc* strain 9v significantly increased the MWD of soil aggregates as determined by fast wetting (p=0.005), wet stirring (p=0.002) and slow wetting (p<0.001) methods. However, the inoculation effect was interactive with soil type (p=0.012) in that for the wet stirring and slow wetting methods it was more pronounced for Guquka than for Hertzog soil (Figure 4.5a). Cropping resulted in significantly lower aggregate MWD as determined by all three test methods (Figure 4.5b). Values measured in samples from cropped soils ranged from 0.5 to 0.9, 0.4 to 0.8, and 0.8 to 0.9 mm when determined by fast wetting, wet stirring, and slow wetting methods, respectively, which were lower than corresponding values obtained in non-cropped soils, which ranged from 0.7 to 1.2, 0.7 to 1.2 and 1.0 to 1.4 mm, respectively. There was, however, a significant interaction between cropping and inoculation for MWD$_{sw}$ (p=0.008) in that inoculation increased MWD$_{sw}$ significantly where the soils were non-cropped but not when cropped (Figure 4.5b). A positive association between MWD and soil C ($R^2 = 0.485$) and EPS ($R^2 = 0.763$) was observed (Figures 4.6a and 4.6b), indicating that aggregate stability increased with increases in the levels of soil C and EPS in the soils.
Figure 4.5: Interaction effects of *Nostoc* inoculation with soil type and cropping with maize on the Mean Weight Diameter (MWD) of soil aggregates sampled to a depth of 5 mm and determined by fast wetting, wet stirring and slow wetting methods. Error bars represent standard deviations.
Figure 4.6: Relationship between the soil C (a) and EPS (b) contents and mean weight diameters (MWD) calculated as means of values determined by fast wetting, slow wetting, and wet stirring methods.
4.5 DISCUSSION

4.5.1 Effect of *Nostoc* Strain 9v Inoculation on Soil C Content

*Nostoc* strain 9v used for inoculation showed the ability to colonize Guquka and Hertzog soils, irrespective of their physical and chemical characteristics. This establishment of *Nostoc* strain 9v in the two soils was responsible for the observed improvement in the soil C content determined from the top 5 mm layer of these C poor soils (Table 4.2). This increase was presumably the result of the photosynthetic increment in both soil carbohydrate C (polysaccharide) and biomass C (Lange et al., 1992). Treatment performance, however, depended on soil type, with the largest increase in soil C occurring in Guquka soil, possibly because it had much lower levels of this nutrient to start with (Table 4.3).

4.5.2 Effect of *Nostoc* Strain 9v Inoculation on Soil Aggregate Stability

The MWD is qualitatively related to aggregate strength and increases with increasing aggregate stability (Horn and Baumgart, 2000). Le Bissonnais (1996) divided MWD into five classes and related them to soil aggregate stability as follows: <0.4 mm is very unstable, 0.4 – 0.8 mm is unstable, 0.8 – 1.3 mm is partly stable, 1.3 – 2 is stable, and >2 mm is very stable. The non-inoculated Guquka soil had aggregates with MWD of 0.84, 0.88, and 0.62 mm as determined by the fast wetting, slow wetting, and wet stirring methods, respectively (Figure 4.5). Corresponding values for Hertzog soil were 0.57, 0.92, and 0.63 mm. These results indicated that both soils had low aggregate stability which, according to Le Bissonnais (1996), could be
classified as unstable. This could be attributed to their high fine sand and silt contents, and Mg for Hertzog soil (Table 2.2, Chapter 2). Inoculation of the soils with the Nostoc strain 9v increased the proportion of macro aggregates (Figure 4.4) and the proportion of big aggregates in both soils as reflected by their MWD values (Figure 4.6). These results indicated improved resistance to breakdown in the inoculation treatments, especially with respect to slow wetting and mechanical breakdown (Figure 4.5).

As reported in earlier studies on microbiotic soil crusts (Belnap and Gardner, 1993; Malam Issa et al., 2001) or in soils inoculated with cyanobacteria (Malam Issa et al., 2007), the observed improvement in the aggregation of inoculated soils could be related to increases in soil C and EPS that caused changes in the micromorphological characteristics of the aggregates. The present results showed positive relationship between the MWD and soil C ($R^2 = 0.485$) and EPS ($R^2 = 0.763$). The EPS was more strongly associated with MWD than soil C and indeed greater quantities of EPS were found in inoculated soils than in the non-inoculated ones (Table 4.2). Inoculated soils also exhibited EPS coatings and EPS bridges while non-inoculated samples had little or no EPS/filaments (Figures 4.1a – 4.1f). These observations suggest that the aggregation and improvement in structural stability of the experimental soils was largely due to the enmeshing effect of the inoculated cyanobacterium filaments and the gluing effect of excreted polysaccharides. Improvement in aggregation could also have been aided by the hydrophobic properties that EPS impart on soil aggregates, which retard the release of entrapped air (Kidron et al., 1999 as cited by Nisha et al., 2007) and thus breakdown of soil aggregates.
Nisha et al. (2007) showed that the EPS produced through biofertilization with cyanobacteria provided a substrate for the growth and enhanced activity of heterotrophic microflora which in turn produced more EPS, further amplifying its effect on soil structural stability. The effect of *Nostoc* strain 9v on other soil microflora was not investigated in the present study but it is possible that the observed increases in EPS following inoculation with cyanobacteria came from both the inoculated cyanobacteria and the heterotrophic microflora whose activity could have been enhanced by the EPS produced by the inoculated *Nostoc* strain 9v.

The MWD values for Guquka soil were lower than those reported by Malam Issa et al. (2007) for the same soil. This could be because the latter study was carried out under laboratory conditions where incubation conditions were more ideal than under the glasshouse conditions of the present study. The ideal laboratory incubation conditions could have ensured better cyanobacteria growth and consequently more soil C and EPS production, and thus greater impact on stability of aggregates.

The interactive effect of cyanobacteria inoculation and cropping on soil properties has hitherto received little attention. Results of this work showed that cropping with maize reduced the effectiveness of inoculation in improving aggregate stability of soils. This could have been partly due to the observed negative effect of cropping on *Nostoc* strain 9v establishment and subsequent lower production of EPS. It could also be attributed to other causes such as those highlighted by Reid and Goss (1981) and Reid et al. (1982). They reported that the growth and activities of living roots may control the overall direction and magnitude of changes in the aggregate stability under arable and ley crops. Roots of perennial ryegrass and lucerne were found to increase
aggregate stability while those of maize, tomato, and wheat decreased the stability of aggregates (Reid and Goss, 1981). In a subsequent study, Reid et al. (1982) presented evidence suggesting that poor aggregate stability in soils cropped with maize could be due to the destruction of (organic matter) – (Fe or Al) – (mineral particle) linkages. The destruction of the linkages was said to occur when the Fe and Al cations that link organic matter to mineral particles were removed by chelating agents released into the soil rhizosphere by maize roots. This possibility suggests a need for further studies to investigate the interaction effects of cyanobacteria with different plant species on soil aggregate stability.

4.6 CONCLUSIONS

The Nostoc strain 9v improved the soil C and EPS contents of the potted Guquka and Hertzog soils. The aggregate stability of the two soils was also improved as a result of increased production of EPS and soil C in the Nostoc strain 9v inoculated soils. Cropping with maize reduced the effectiveness of inoculation in improving aggregate stability. Cyanobacteria capable of EPS production could contribute to the amelioration of the structural stability of physically degraded soils in South Africa.
4.7 REFERENCES


Genstat Release 4.24DE (2005). Lawes Agricultural Trust (Rothamsted Experimental Station) UK.


CHAPTER FIVE

EFFECTS OF INOCULATION WITH INDIGENOUS CYANOBACTERIA STRAINS ON MAIZE YIELD AND FERTILITY STATUS OF A DEGRADED SOIL FROM THE EASTERN CAPE PROVINCE, SOUTH AFRICA

5.1 ABSTRACT

The ability of indigenous strains 3g and 7e to influence soil N and C content, and maize yield was tested on a degraded Hertzog soil. Cropped and non-cropped potted soils were inoculated with the cyanobacteria strains at the rate of 6 g (dry weight) per square meter in the glasshouse and periodically wetted with distilled water. The first maize harvest and soil sampling were undertaken after six weeks of inoculation. Thereafter, the top 25mm of the soil was mixed, replanted with maize, and after six weeks, a second harvest and soil sampling were done. Increases of 49%, 69% and 71% nitrate N were observed in cropped soils inoculated with strains 3g, 7e and 9v, respectively, when the first crop was harvested. In the second harvest corresponding increases in nitrate N were 41%, 39% and 33%, respectively while increases in ammonium – N were 32%, 55% and 26%, respectively, in soils inoculated with strains 3g, 7e and 9v, respectively. Inoculation with the two indigenous strains increased maize dry matter yields and N uptake both in the first and second harvests. However, strain 7e was more effective in improving dry matter yields and N uptake than was strain 3g.

Keywords: Ammonium – N; Degraded soil; Indigenous Nostoc cyanobacteria; Nitrate – N; Soil C and Soil N.
5.2 INTRODUCTION

Low soil fertility is partly responsible for declining food security in rural small-scale farming in South Africa (Laker, 1976; Mandiringana et al., 2005). Continued mining of nutrients including N and P without adequate replenishment has been reported to be the major yield limiting factor in most cases (Burns and Hardy 1975; Sanchez et al., 1997; van Averbeke and Yoganathan, 2003). Inoculation of soil with cyanobacteria is increasingly receiving attention as a potential, simple and low-cost method for restoring the productivity of degraded soils (Malam Issa et al., 2007; Nisha et al. 2008; Maqubela et al., 2008).

Cyanobacteria have been reported to improve soil productivity through their ability to fix atmospheric N (Evans and Barber, 1977; Stevenson, 1985; Harper and Marble, 1988; Belnap et al., 2001), and/or to improve the stability of soil aggregates (Vilenskii, 1960; Venkataraman 1975; Metting and Rayburn, 1983; Belnap and Gardner, 1993; Malam Issa et al., 2001).

A preliminary study utilizing a Nostoc cyanobacteria strain 9v isolated from a Tanzanian soil showed improved productivity of two nitrogen poor soils partly as a result of its ability to fix atmospheric N (Chapter 3; Maqubela et al., 2008). This prompted a need to investigate South African cyanobacteria strains that could improve the N content of soils and its availability to plants.

Two indigenous strains, coded as strains 3g and 7e, isolated from foils from the Eastern Cape Province, South Africa, were identified in laboratory screening studies in liquid cultures as
having the ability to fix N \(_2\) (Chapter 2). Strain 7e fixed higher quantities of N\(_2\) than any other strain whilst strain 3g had a dual effect of fixing N\(_2\) and producing exocellular polysaccharides. However, this potential remained to be established in soil under cropping conditions. The glasshouse study reported herein was, therefore, carried out to investigate the effects of cyanobacteria strains 3g and 7e on maize growth and N content, with and without cropping, in a nitrogen poor soil in the Eastern Cape Province, South Africa.

5.3 MATERIALS AND METHODS

Soil type and characteristics

The soil used in this study was from Hertzog village in Seymour, and was chosen because it is the source of strain 3g and was poor in nitrogen. It was classified as a Typic Haplustalf and its characteristics are summarised in Table 5.1. The soil was collected in bulk, air dried and sieved through a 2 mm mesh sieve before characterisation was done. The pH was determined at a soil: water ratio of 1: 2.5. Soil C and N were determined using a LECO C & N autoanalyser. Phosphorus was extracted following the Bray 1 extraction method (Bray and Kurtz, 1945) and P in the extract was determined by the molybdate/ascorbic blue method (Olsen and Dean, 1965). Ammonium acetate, buffered to pH 7, was used to extract the bases, which were determined by atomic absorption spectrophotometry, except for sodium which was determined by flame photometry.
Table 5.1: Selected chemical properties of Hertzog soil, and lower limits for assessing soil fertility status adapted from FSSA (1989) and Landon (1991).

<table>
<thead>
<tr>
<th>Soil characteristics</th>
<th>Hertzog soil</th>
<th>Lower critical limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (H$_2$O)</td>
<td>7.46</td>
<td>&lt; 5.0</td>
</tr>
<tr>
<td>Soil N (g kg$^{-1}$)</td>
<td>0.7</td>
<td>&lt; 1.0</td>
</tr>
<tr>
<td>Soil C (g kg$^{-1}$)</td>
<td>7.7</td>
<td>&lt; 10.0</td>
</tr>
<tr>
<td>P (g kg$^{-1}$)</td>
<td>29.8</td>
<td>&lt; 15</td>
</tr>
<tr>
<td>K (g kg$^{-1}$)</td>
<td>0.34</td>
<td>&lt; 8.0</td>
</tr>
<tr>
<td>Ca (g kg$^{-1}$)</td>
<td>1.61</td>
<td>&lt; 80.0</td>
</tr>
<tr>
<td>Mg (g kg$^{-1}$)</td>
<td>27.36</td>
<td>&lt; 4.0</td>
</tr>
<tr>
<td>Na (g kg$^{-1}$)</td>
<td>0.03</td>
<td>-</td>
</tr>
<tr>
<td>Al$^{3+}$ (g kg$^{-1}$)</td>
<td>&lt;0.005</td>
<td>-</td>
</tr>
<tr>
<td>Fe (g kg$^{-1}$)</td>
<td>0.029</td>
<td>-</td>
</tr>
<tr>
<td>H$^+$ (g kg$^{-1}$)</td>
<td>0.0</td>
<td>-</td>
</tr>
</tbody>
</table>

Cyanobacteria strains

Cyanobacteria strains isolated from natural soils from Qunu (strain 7e), Hertzog (strain 3g) both in South Africa, and Mkindo, Tanzania (strain 9v; used in Chapters 3 and 4) were used in this study. They were isolated following procedures described in section 2.3.2. Their nitrogenase activities are given in Table 5.2. Mass production of the cyanobacteria strains was done in liquid BG11$_0$ medium at 20 – 28 °C, under continuous normal light until a biomass of 1 g L$^{-1}$ was achieved. Details of the mass production are given in Chapter 3.
Table 5.2: Cyanobacteria strains, their sources of origin, nitrogenise activity and levels of EPS produced.

<table>
<thead>
<tr>
<th>Nostoc strain</th>
<th>Sources</th>
<th>Nitrogenase activity (nmol C$_2$H$_4$ µg chl$^{-1}$h$^{-1}$)</th>
<th>EPS produced</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Land use</td>
<td>Villages</td>
<td></td>
</tr>
<tr>
<td>7e</td>
<td>Virgin</td>
<td>Qunu (S.A)</td>
<td>16.08</td>
</tr>
<tr>
<td>3g</td>
<td>Virgin</td>
<td>Hertzog (S.A)</td>
<td>4.71</td>
</tr>
<tr>
<td>9v (Reference)</td>
<td>Cultivated</td>
<td>Mkindo, Tanzania</td>
<td>14.12</td>
</tr>
</tbody>
</table>

+, and +++ represent relative abundance of EPS as observed by the India ink method.

**Experimental design**

The experiment was carried out in a glasshouse using 36 cm diameter pots filled with 15 kg of soil. It was laid out as a factorial experiment with two cropping levels (cropped and non-cropped) and four inoculation treatments (3g, 7e, 9v, and non-inoculated control). The treatments were arranged in a completely randomized block design with five replications. Five maize seeds were sown in the cropped treatments and were thinned to two plants per pot at the two leaf stage. In the inoculated treatments, cyanobacteria strains were uniformly poured on the surfaces of potted soil at the rate of 6 g m$^{-2}$ (60 kg/ha) (dry matter basis) using a glass beaker. Watering was done with distilled water to maintain moisture at approximately field capacity whereas temperature was kept at an average of 25$^\circ$C during the day and 10$^\circ$C at night.

Plants were harvested (first harvest) after six weeks of growth by cutting the stems just above the soil surface, cleaned with distilled water and dried in an oven at 60$^\circ$C to constant weight. The
dried plant materials were ground to pass through a 2 mm sieve and analyzed for tissue N using
the LECO TRUSPEC C & N autoanalyser.

The top 5 mm of the soil was sampled by gently scooping from the centre and from four other
parts around the edges of each pot. The soil samples were uniformly mixed and a portion kept in
a refrigerator until it was used for inorganic N determination, whilst the other portion was dried,
ground to pass through a 2 mm sieve and analysed for C and N using a LECO TRUSPEC CN
autoanalyser. Inorganic N was extracted in fresh samples using potassium chloride solution
(Bremner, 1965). Ammonium-N and nitrate-N in the extracts were then analyzed
colorimetrically (Okalebo et al., 2002). The remaining soil in the pots was thoroughly mixed to
a depth of 25 mm and replanted with maize which was managed in the same way as the first
crop. The replanted maize was harvested after six weeks of growth (second harvest), dried,
ground and analysed as described for the first harvest. Soil samples were taken from each pot,
processed, and analyzed as described under the first harvest.

Analysis of variance was done by GenStat – Release 4.2 (Discovery edition 2). Mean separation
was by MSTAT – C using LSD at p=0.05.
5.4 RESULTS

5.4.1 Effects of Cropping and Inoculation on Soil C and N

There were no interaction effects between cropping and inoculation on soil C and N at both harvests whereas interaction effects were significant for nitrate N (first harvest) and ammonium N (second harvest) (Table 5.3).

Table 5.3: F – probabilities for soil C and N, ammonium N and nitrate N in Hertzog soil inoculated with indigenous strains of cyanobacteria

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Soil C</th>
<th>Soil N</th>
<th>NH$_4^+$-N</th>
<th>NO$_3^-$-N</th>
<th>Dry matter</th>
<th>Tissue N uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>First harvest</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inoculation</td>
<td>0.613$^{ns}$</td>
<td>0.460$^{ns}$</td>
<td>0.700$^{ns}$</td>
<td>0.001$^{***}$</td>
<td>0.032$^{*}$</td>
<td>0.002$^{**}$</td>
</tr>
<tr>
<td>Cropping</td>
<td>0.001$^{***}$</td>
<td>0.008$^{**}$</td>
<td>0.307$^{ns}$</td>
<td>0.428$^{ns}$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Inoculation*Cropping</td>
<td>0.809$^{ns}$</td>
<td>0.278$^{ns}$</td>
<td>0.187$^{ns}$</td>
<td>0.021$^{*}$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Second harvest</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inoculation</td>
<td>0.424$^{ns}$</td>
<td>0.481$^{ns}$</td>
<td>0.001$^{***}$</td>
<td>0.048$^{*}$</td>
<td>0.012$^{*}$</td>
<td>0.005$^{**}$</td>
</tr>
<tr>
<td>Cropping</td>
<td>0.634$^{ns}$</td>
<td>0.040$^{*}$</td>
<td>0.001$^{***}$</td>
<td>0.050$^{*}$</td>
<td>0.017$^{*}$</td>
<td>0.005$^{**}$</td>
</tr>
<tr>
<td>Inoculation*Cropping</td>
<td>0.309$^{ns}$</td>
<td>0.359$^{ns}$</td>
<td>0.011$^{*}$</td>
<td>0.326$^{ns}$</td>
<td>0.116$^{ns}$</td>
<td>0.128$^{ns}$</td>
</tr>
</tbody>
</table>

*; **; *** Denotes significance at P ≤ 0.05, 0.01 and 0.001, respectively; ns = non significant at P ≤ 0.05
The three *Nostoc* cyanobacteria strains increased nitrate N relative to that in the control in cropped and non-cropped soil but the extent of increase varied (Figure 5.1). Where soils were cropped, only strain 7e inoculation resulted in significantly higher nitrate N than the control, whereas in non-cropped soils strain 9v had the highest nitrate N followed by strain 7e (Figure 5.1). In both cropped and non-cropped treatments, soil nitrate N levels in the 3g inoculated soils were similar to the non-inoculated controls.

![Figure 5.1: Nitrate N content as affected by inoculation and cropping treatments in Hertzog soil six weeks after inoculation with cyanobacteria strains](image)

In the second harvest, the effect of inoculation treatment on ammonium-N levels was affected by the number of times the soil was cropped (Figure 5.2). Ammonium-N levels were higher in soils that were cropped once than those cropped twice (Figure 5.2). Soil inoculated with strain 7e had higher levels than those inoculated with the other two strains when the soil was cropped once. However, all inoculation treatments had comparable ammonium-N levels (i.e. non – significant
differences) where the soil was cropped twice. Only strain 7e inoculation resulted in significantly higher ammonium-N levels than the control whether the soils were cropped once or twice (Figure 5.2).

![Figure 5.2: Effect of cropping on the ammonium N content of soil inoculated with different cyanobacteria strains](image)

Inoculation with all three cyanobacteria strains tended to increase soil C and N in both harvests but the increases were not statistically significant (Table 5.4). Cropping reduced soil C from 0.77 to 0.55%, and soil N from 0.085 to 0.059% in the first harvest. In the second harvest, there were significant decreases in soil N (0.073 to 0.051%), and a non significant decrease on soil C (0.76 to 0.73) as a result of cropping the soil twice compared to cropping once (Table 5.5).
Table 5.4: Effects of inoculating Hertzog soil with cyanobacteria strains 3g, 7e, and 9v on the C and N contents of soils sampled after the first and second maize harvests

<table>
<thead>
<tr>
<th>Harvest</th>
<th>Inoculation</th>
<th>Soil C</th>
<th>Soil N</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>3g</td>
<td>0.66</td>
<td>0.066</td>
</tr>
<tr>
<td>Harvest</td>
<td>7e</td>
<td>0.69</td>
<td>0.082</td>
</tr>
<tr>
<td></td>
<td>9v</td>
<td>0.67</td>
<td>0.077</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.62</td>
<td>0.064</td>
</tr>
<tr>
<td>Second</td>
<td>3g</td>
<td>0.77</td>
<td>0.063</td>
</tr>
<tr>
<td>Harvest</td>
<td>7e</td>
<td>0.76</td>
<td>0.071</td>
</tr>
<tr>
<td></td>
<td>9v</td>
<td>0.79</td>
<td>0.066</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.65</td>
<td>0.049</td>
</tr>
</tbody>
</table>

Cropping reduced soil C from 0.77 to 0.55%, and soil N from 0.085 to 0.059% (Table 5.5). In the second harvest, there was a significant decrease in soil N (0.073 to 0.051%), and a non significant decrease on soil C (0.76 to 0.73) as a result of cropping the soil twice compared to cropping once (Table 5.5).
Table 5.5: Effect of cropping on soil C and soil N from Hertzog soil

<table>
<thead>
<tr>
<th>Harvest time</th>
<th>Cropping</th>
<th>Soil C</th>
<th>Soil N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>--------------</td>
<td>-----------</td>
<td>-----------</td>
</tr>
<tr>
<td>First Harvest</td>
<td>Cropped</td>
<td>0.55 b</td>
<td>0.059 b</td>
</tr>
<tr>
<td></td>
<td>Non-cropped</td>
<td>0.77 a</td>
<td>0.085 a</td>
</tr>
<tr>
<td>Second Harvest</td>
<td>Cropped x 2</td>
<td>0.73</td>
<td>0.051 b</td>
</tr>
<tr>
<td></td>
<td>Cropped x 1</td>
<td>0.76</td>
<td>0.073 a</td>
</tr>
</tbody>
</table>

5.4.2 Effects of Inoculation with Indigenous Strains and Cropping on Maize Dry Matter Yield, Tissue N and N Uptake

All three cyanobacteria treatments resulted in significantly higher dry matter yields than the control, both in the first and second harvests (Figures 5.3 a and b). Soil inoculation with cyanobacteria strains increased maize dry matter yields from 19.53 g/pot in non-inoculated soil to 22.91, 23.89 and 24.23 g/pot for 3g, 7e and 9v, respectively (Figure 5.3 a), in the first harvest and from 24.88 g/pot to 28.10, 28.55, and 29.61 g/pot, respectively, at the second harvest (Figure 5.3 b). Tissue N concentrations (Figure 5.3 c and d) and N uptake (Figure 5.3 e and f) followed the same trend as that of dry matter yields. Nitrogen uptake followed the same trend as dry matter yields, where all inoculated treatments had similar uptake values that were higher than those in the control (Figures 5.3 e and f).
Figure 5.3: Effects of inoculating Hertzog soil with cyanobacteria strains 3g, 7e, and 9v on maize dry matter yields (a & b), tissue N (c & d), and N uptake (e & f)
5.5 DISCUSSION

5.5.1 Strains 3g, 7e and 9v Inoculation Effects on Soil C and N Contents

Hertzog soil, despite its low C and N status, was quickly colonized by the inoculated *Nostoc* strains 3g, 7e and 9v. The microbiont “mat” formed by the inoculated strains resulted in increases of soil C and soil N contents in the top 5 mm of the soil (Table 5.4), although the increase was not significant. Lange *et al.* (1992) attributed the soil C and N increments to photosynthetic increases in both soil carbohydrate C (polysaccharide) and biomass C. The largest increases in both soil C and N occurred under strain 7e followed by 9v and 3g inoculated soil in the first harvest, and the trend was the same for soil N in the second harvest (Table 5.4), possibly because strains 7e and 9v showed high potential to fix atmospheric N\(_2\) than 3g (Chapter 2, section 2.4.3). Harper and Pendleton (1993), in a similar study, reported similar trends of soil N to those measured in 7e inoculated soil. Other workers also reported similar N levels as those of 7e inoculation even though their studies used the more accurate acetylene reduction method for assessing N-fixation (Skarpe and Henriksson, 1987; Buttars *et al.*, 1998). Lack of statistically significant increases in soil N following inoculation with strain 7e in the present study may be associated with the relatively more insensitive N test (total N) used here, as opposed to the more sensitive acetylene reduction test used by other workers. In the second harvest the largest increase in soil C occurred in 9v followed by 3g and 7e, possibly because 9v and 3g re-established faster than strain 7e.
The quantities of nitrate N produced in non-cropped and cropped soils inoculated with strain 7e were similar and higher than the non-inoculated soil in the first harvest (Figure 5.1), possibly caused by the growth of this strain. However, the nitrate N in the non-cropped soil was highest where strain 9v was applied. In the second harvest inoculation with strain 7e resulted in higher ammonium N levels than the controls both when the soil was cropped once and twice (Figure 5.2). The observed results could be explained by the high N fixing capability of strain 7e as reported in Chapter 2. The effects of strain 3g inoculation compared well with the non-inoculated control both in the cropped and non-cropped soil in the first harvest in terms of nitrate N, and in the second harvest in terms of ammonium N, when cropped once or twice. This could be explained by the observed low N\textsubscript{2} fixation ability of this strain (Chapter 2).

A positive relationship of soil C and soil N (R\textsuperscript{2}=0.667 and R\textsuperscript{2}=0.591) in first harvest and second harvests, respectively) (Figure 5.4) indicated close association of C and N dynamics in the inoculated and non-inoculated soils, in agreement with Nisha et al. (2007). Generally, the increases in N due to inoculation were proportionately greater than those of C, which resulted in a narrowing of C: N ratio in both harvests. In the first harvest, for example, the C: N ratio dropped from 9.7 in non-inoculated soils to 8.4 and 8.7, respectively when soil was inoculated with Nostoc strains 7e and 9v, which impacted positively on the mineralization of N (Table 5.4), except when the soil was inoculated with 3g in which the ratio was 10.0. In the second harvest the C: N ratio dropped from 13.3 in non-inoculated soil to 12.2, 10.7 and 12.0, respectively when soil was inoculated with 3g, 7e and 9v. Nisha et al. (2007) observed greater C loss from cyanobacteria – treated soils than non – treated ones and attributed it to greater microbial activity and respiration in the former. The greater increase in N relative to C observed in cyanobacteria –
treated soils in the present study could probably have been caused by the N – fixation of the cyanobacteria. Although the strains fix both C and N, the mineralization of C forms CO₂ which is easily lost whereas mineral N remains in the soil.

Figure 5.4: Relationship between soil C and soil N in non-inoculated soils and those inoculated with Nostoc strains 3g, 7e and 9v in the first harvest and second harvest

5.5.2 Cyanobacteria Inoculation Effects on Maize Dry Matter Yields, Tissue N, and N Uptake

Inoculation treatments resulted in higher dry matter yields (Figure 5.1 a-b) both in the first and second harvests. The increased maize dry matter yield can be explained by maize tissue N content and N uptake, which followed similar trends, as a result of N₂ fixation by the cyanobacteria. Increases in the dry matter yields and N uptake in 3g, 7e, and 9v treatments were higher in the first harvest than in the second harvest, indicating that maize plants benefited from 3g, 7e, and 9v strains before they were incorporated into the soil. Considering the higher levels of nitrate N and ammonium N in soils inoculated with 7e and the high N uptake in the same
treatment, it can be suggested that strain 7e fixed N which was converted to available forms for crop uptake. These findings are in agreement with those reported by Skujinš and Klubek (1978) and Nisha et al. (2007), that the fixed nitrogen is ultimately released in the soil as ammonium, where most of it is nitrified to nitrite (NO\textsubscript{2}−) and nitrate (NO\textsubscript{3}−) and influence plant growth.

5.6 CONCLUSIONS

Cyanobacteria strains 3g, 7e and 9v improved the soil nitrate N and ammonium N contents of Hertzog soil. Cyanobacteria strain 7e had a pronounced effect on the increase in soil ammonium N and nitrate N. Inoculation of the soil with strains 3g, 7e and 9v improved maize dry matter and N uptake. The effectiveness of inoculation, particularly on soil N and C was reduced by cropping with maize due to reduced cyanobacteria establishment particularly for strain 9v. Results from this study suggested that indigenous cyanobacteria strains screened for higher N\textsubscript{2} – fixing ability could improve the productivity of N poor soils in South Africa.
5.7 REFERENCES


CHAPTER SIX

EFFECTS OF INOCULATION WITH INDIGENOUS STRAINS ON THE AGGREGATE STABILITY OF A DEGRADED SOIL FROM THE EASTERN CAPE PROVINCE, SOUTH AFRICA

6.1 ABSTRACT

Two indigenous cyanobacteria strains selected for nitrogen N fixation (7e) and EPS production (3g), and a reference strain (9v), were evaluated for their ability to improve the aggregate stability of a semi-arid, silty loam soil which had a low content of organic C. The glasshouse study involved cropped and non-cropped treatments which were inoculated with the different cyanobacteria strains, with non-inoculated soil as a control. After 42 days the aggregate stability as indicated by FSD and MWD was measured under fast wetting, mechanical breakdown and slow wetting methods. Inoculation of cropped soil with strain 3g increased MWD by 85%, 33%, and 33% for fast wetting, mechanical breakdown and slow wetting, respectively, whereas strain 7e increased MWD by 64%, 41%, and 41%, respectively. Except for the slow wetting method, the increases in MWD following inoculation with the indigenous strains were greater than those observed in soil inoculated with Nostoc strain 9v. The increases in MWD for Nostoc strain 9v were 60% and 24%, for fast wetting and mechanical breakdown, respectively. In non-cropped soil, inoculation with strain 3g increased the MWD by 11%, 0%, and 7%, respectively, whereas 7e increased MWD by 21%, 11%, and 7%, respectively. However, strain 9v increased MWD better than 3g and 7e by 25%, 36% and 19%. High EPS, organic C content and cyanobacteria
filaments in inoculated treatments increased aggregate stability of Hertzog soil. Cropping reduced EPS content but it enhanced the aggregate stability of soil inoculated with strains 3g and 7e. By contrast, cropping with maize had a negative effect on the aggregate stability of soil inoculated with strain 9v. The results suggested that indigenous strains selected for EPS production and N fixation could improve the soil structure of degraded soils.

**Key words:** Aggregate stability, Degraded soil, EPS, Indigenous cyanobacteria, Inoculation, Mean Weight Diameter.
6.2 INTRODUCTION

Some soils in South Africa have high silt and low organic matter contents (Oldeman, 1994; Mills and Fey, 2003). When organic C content drops below the threshold value of 2%, and soil texture is loamy and silty – loam, water-stable aggregates are reported to quickly break down and become extremely erodible (Elwell, 1986; Kay and Angers, 2000). The breakdown of the aggregates usually occurs when under pressure on irrigation or high rainfall, resulting in surface sealing and crust formation and subsequently soil erosion (Davies et al., 1993; Woyessa and Bennie, 2004). Inoculation of soil with cyanobacteria is increasingly receiving attention as a potential, simple and low-cost method for restoring the productivity of degraded lands through improved nitrogen status (Belnap et al., 2001) and aggregate stability (Malam Issa et al., 2001).

Cyanobacteria have been reported to improve aggregate stability through enrichment of soil with organic matter, improvement of biological activity, and secretion of extracellular polysaccharides (EPS) (Marshall et al., 1996). The organic matter and EPS, which dominantly consists of polysaccharides, act as binding agents of soil particles (Tisdall and Oades, 1982; Belnap and Gardner, 1993; Malam Issa et al., 2001). A laboratory incubation study utilizing cyanobacteria strain 9v from Tanzania showed improved stability of aggregates of a soil from Guquka, in the Eastern Cape Province, within a few weeks of inoculation (Malam Issa et al., 2007). These results were confirmed in a subsequent glasshouse study which showed that strain 9v improved the aggregate stability of Guquka and Hertzog soils (Chapter 4; Maqubela et al., 2008). However, the presence of growing plants minimized the extent of aggregate stability improvement (Maqubela et al., 2008). Two indigenous Nostoc strains, coded as 3g and 7e,
isolated from soils in the Eastern Cape Province, South Africa, were shown to produce EPS and fix atmospheric nitrogen (Chapter 2).

Results of a glasshouse study (Chapter 5) demonstrated that strains 3g and 7e improved the N content of the soil and improved maize dry matter yields. However, their ability to improve aggregate stability remained to be established. The study reported here investigated the effects of cyanobacteria strains 3g and 7e on aggregate stability of a degraded soil in the Eastern Cape Province, South Africa, with and without cropping.

6.3 MATERIALS AND METHODS

Soil type and characteristics

The soil used in this study was from Hertzog village in Seymour. Its characteristics are described in detail in Chapter 2.

Cyanobacteria strains

Cyanobacteria strains isolated from virgin soils from Qunu (strain 7e), Hertzog (strain 3g) both in South Africa, and Mkindo (strain 9v), Tanzania, were used in this study. EPS production was in the order 7e < 3g = 9v based on the India ink test (Chapter 2). Details of mass production of strains 3g and 7e were the same as for strain 9v, (described in Chapter 3).
Experimental design

The glasshouse experiment described in Chapter 5 was used for the pursuance of the objectives of this study.

Soil sampling and analysis

Soil sampling was done after six weeks (i.e. first harvest) only and analyses of EPS, SOC, FSD and MWD were performed as described in Chapter 4 for strain 9v.

Analysis of variance of EPS, SOC, and MWD was done using GenStat – Release 4.2, Discovery edition 2 (2005). Means were separated using LSD at p < 0.05.

6.4 RESULTS

6.4.1 Effects of Cropping and Inoculation with Indigenous Cyanobacteria Strains on Soil Aggregate Stability and EPS Contents

The MWD of aggregates in the non-inoculated control as determined by three test methods were generally higher in the non-cropped than cropped soils (Table 6.1). The MWD of the non-inoculated and non-cropped soils were 0.44, 0.53 and 1.04 as determined by fast wetting, wet stirring and slow wetting methods, respectively (Table 6.1). They could be classified as very unstable, unstable and partly stable according to criteria suggested by Le Bissonnais (1996).
Inoculation with the three *Nostoc* strains significantly increased the MWD of the soil aggregates (Table 6.1). However, there was a significant interaction between inoculation and cropping (Table 6.1) in that aggregate MWD were increased by inoculation with all three strains (3g, 7e and 9v) in both cropped and non-cropped soils when all three test methods were used, except for the wet stirring method where the non-cropped 3g treatment was similar to the controls (Table 6.1). In the cropped soils the MWD, determined by the fast wetting method, were in the order 3g > 9v = 7e, whereas the order was 9v = 7e > 3g in non-cropped soils. However, when averaged MWD data for the three tests are considered, the results indicate that the MWD of soil aggregates in soils inoculated with the indigenous strains 3g and 7e were higher in cropped than non-cropped soils while the opposite was the case for aggregates in soils inoculated with strain 9v (Table 6.1).

Levels of EPS were increased by inoculation with all strains both in cropped and non-cropped soils, except in the 7e inoculated cropped soil which was comparable to the control. Higher EPS values were in 3g and 9v inoculations of non-cropped soils (Table 6.1). In the cropped soils the EPS content followed the order 3g=9v>7e=control whereas it was 9v=3g>7e=control in the non-cropped soils. Scanning Electron Microscope observations revealed EPS and intertwined cyanobacteria filaments covering surfaces of soil particles inoculated with strain 3g (Figure 6.1 a; c). No EPS and filaments were observed on the surfaces of non-inoculated soils (Figure 6.1 b and d).
Table 6.1: Cropping and inoculation treatment effects on soil mean weight diameter (MWD), and exocellular polysaccharides (EPS) contents in the first harvest

<table>
<thead>
<tr>
<th>Cropping</th>
<th>Inoculation</th>
<th>MWD&lt;sub&gt;FW&lt;/sub&gt;*</th>
<th>MWD&lt;sub&gt;WS&lt;/sub&gt;</th>
<th>MWD&lt;sub&gt;SW&lt;/sub&gt;</th>
<th>Average</th>
<th>EPS</th>
<th>Soil C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cropped</td>
<td>3g</td>
<td>0.61 b</td>
<td>0.68 b</td>
<td>1.06 c</td>
<td>0.78</td>
<td>3.2 b</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>7e</td>
<td>0.54 b</td>
<td>0.72 a</td>
<td>1.13 b</td>
<td>0.80</td>
<td>2.3 cd</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>9v</td>
<td>0.53 b</td>
<td>0.64 c</td>
<td>1.20 a</td>
<td>0.79</td>
<td>3.1 b</td>
<td>0.53</td>
</tr>
<tr>
<td>Control</td>
<td>3g</td>
<td>0.33 e</td>
<td>0.51 e</td>
<td>0.80 d</td>
<td>0.55</td>
<td>2.0 d</td>
<td>0.53</td>
</tr>
<tr>
<td>Non-cropped</td>
<td>7e</td>
<td>0.49 c</td>
<td>0.53 e</td>
<td>1.11 b</td>
<td>0.71</td>
<td>3.9 a</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>9v</td>
<td>0.53 b</td>
<td>0.59 d</td>
<td>1.11 b</td>
<td>0.74</td>
<td>2.4 c</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.44 d</td>
<td>0.53 e</td>
<td>1.04 c</td>
<td>0.67</td>
<td>2.0 d</td>
<td>0.70</td>
</tr>
</tbody>
</table>

* FW stands for Fast wetting, WS for wet stirring, and SW for slow wetting tests

* Average of MWD as determined by FW, WS, and SW methods
Figure 6.1 (a-b): Surfaces of non-cropped soils: (a) massive surface coating of exocellular polysaccharides (EPS) on soil inoculated with *Nostoc* strain 3g, and (b) soil particles on non-inoculated (control) soil. Figure 6.1 (c-d): Surfaces of cropped soils: (c) filaments and EPS coating binding soil particles and fragments in soil inoculated by *Nostoc* strain 3g, and (d) soil mineral particles and microaggregates with no observed filaments or EPS on the surfaces of non-inoculated (control) soil.
The cropped soil inoculated with strain 3g had greater proportion of macroaggregates than microaggregates whereas in soil inoculated with strains 7e and 9v, and the control, microaggregates constituted the greater proportion based on the fast wetting test method (Figure 6.2a). Non-cropped soils followed the opposite trend with smaller proportions of macroaggregates than microaggregates in the 3g inoculated soil whereas higher proportions of macroaggregates were observed in the other inoculation treatments. There were greater proportions of larger aggregates (0.5-1, and >2 mm) and lower proportions of smaller soil aggregates (< 0.2 mm) in the cropped soils inoculated with 3g, when compared to the other treatments (Figure 6.3a). In the non-cropped soils, there were greater proportions of larger aggregates (>2, 1.0-2.0, and 0.5-1.0 mm) for 7e and 9v treatments than the other treatments (Figure 6.3b).

When the wet stirring method was used MWD followed the order 7e > 3g > 9v in cropped soil, and 9v > 7e > 3g in the non-cropped soil. Soils inoculated with all three strains had greater proportions of macroaggregates than microaggregates, whereas proportions were similar in the control irrespective of cropping. The proportion of macroaggregates was also similar to that of microaggregates in the noncropped 3g inoculation (Figure 6.2b). The proportion of larger aggregates (>2 mm, and 1.0-2.0 mm), in cropped soil, was higher when inoculated with all three strains (3g, 7e, and 9v) (Figure 6.3c). In non-cropped soils, there were higher proportions of larger aggregates in soils inoculated with 9v (>2 mm, 1.0-2.0 mm, 0.5-1.0) and 7e (> 2mm, 1.0-0.5 mm, 0.50-0.25 mm) (Figure 6.3d).
Figure 6.2: Macro aggregates and micro aggregates from cropped and non-cropped soils treated with different strains of cyanobacteria as determined by (a) fast wetting, (b) wet stirring, and (c) slow wetting test methods
When the slow wetting method was used MWD followed the order 9v > 7e > 3g in cropped and 9v > 7e = 3g in the non-cropped soils. The macroaggregates constituted greater proportions of the soil in all inoculation treatments than microaggregates irrespective of cropping (Figure 6.2c). There were higher proportions of larger aggregates (>2 mm, 1.0-2.0 mm, and 1.0-0.5 mm) in the cropped soil inoculated with the three strains (3g, 7e, and 9v) (Figure 6.3e). In non-cropped soils the higher proportions of larger aggregates of different sizes were in 3g (1.0-0.5 mm), 7e (0.5-0.25 mm), and 9v (>2 mm, 1.0-2.0 mm, and 0.50-1.0 mm) treated soils (Figure 6.3f).

The relationship between EPS and MWD of soils, inoculated with strains 3g, 7e and 9v, was stronger with fast wetting \( R^2 = 0.659 \) followed by slow wetting \( R^2 = 0.468 \) and was poor with wet stirring \( R^2 = 0.284 \) (Figure 6.4). Soil C and MWD relationship in soil inoculated with 3g, 7e and 9v strains was poor for all test methods, with \( R^2 = 0.035 \), 0.180 and 0.071 for fast wetting, slow wetting and wet stirring, respectively (Figure 6.5).
Figure 6.3: Fragment size distribution (FSD) of cropped and non-cropped Hertzog soil treated with selected strains of cyanobacteria as determined by fast wetting (a&b), wet stirring (c&d) and slow wetting methods (e&f)
Figure 6.4: Relationship between EPS and mean weight diameter of aggregates from soil inoculated with strains 3g, 7e and 9v. FS, SW and WS represent fast wetting, slow wetting and wet stirring methods, respectively.

Figure 6.5: Relationship between soil C and mean weight diameter (MWD) of aggregates from soil inoculated with strains 3g, 7e and 9v. FS, SW and WS represent fast wetting, slow wetting and wet stirring methods, respectively. Soil C in the formula is represented by x.
6.5 DISCUSSION

The Hertzog soil had low aggregate stability which could be attributed to its high fine sand and silt contents (Table 2.2, Chapter 2). Inoculation of the soil with *Nostoc* strain 9v and the indigenous strains 3g and 7e improved the aggregate stability as reflected by the MWD data (Table 6.1) and the relative proportions of macro- and microaggregates (Figure 6.2). The improvement in aggregate stability was, however, influenced by cropping (Table 6.1).

The MWD was more strongly associated with EPS (Figure 6.4) than with soil C (Figure 6.5). Indeed greater quantities of EPS were found in 3g and 9v inoculated soils than in the non-inoculated ones both in cropped and non-cropped soils (Table 6.2). The presence of EPS was confirmed using SEM micrographs which revealed networks of filaments among which mineral particles were enmeshed and mineral particles and soil fragments coated by EPS presumed to have been secreted by the *Nostoc* 3g strain (Figure 6.1a and 1c). By contrast little or no EPS and/or filaments were observed on non-inoculated soils, especially on cropped ones (Figures 6.1b and 1d). These observations suggest that changes in organic matter content *per se*, due to inoculation, had limited effect on aggregation. It would seem that the observed improvement in aggregate stability of the experimental soil was largely due to the enmeshing effect of the inoculated cyanobacteria filaments and the gluing effect of excreted polysaccharides, with *Nostoc* strain 3g exhibiting a greater effect than strain 7e particularly in the cropped soils. These results are consistent with findings of earlier studies on microbiotic soil crusts (Belnap and Gardner, 1993; Malam Issa *et al.*, 1999, 2001 and 2007) or in soils inoculated with cyanobacteria (Malam Issa *et al.*, 2007) which also attributed improvements in the aggregation of inoculated
soils to increases in soil C and EPS that caused changes in the micromorphological characteristics of the aggregates. The contrasting effects of the two indigenous \emph{Nostoc} strains 3g and 7e on aggregate stability are consistent with their relative EPS production potential reported in Chapter 2, whereby strain 3g was a better producer of EPS as compared to untraceable EPS levels in the case of strain 7e.

The observed improvements in aggregation following inoculation with the \emph{Nostoc} strains could also have been aided by the hydrophobic properties that EPS impart on soil aggregates which retard the release of entrapped air (Kidron et al., 1999 as cited by Nisha et al., 2007) and thus breakdown of soil aggregates. This is supported by the stronger relationship between EPS and MWD determined using the fast wetting method which essentially determines the stability of aggregates against failure due to entrapped air caused by fast wetting. Inoculation of soils with strain 3g reduced the breakdown of soil aggregates by mechanisms of raindrop impact, and reduced soil surface sealing that cause water runoff and soil erosion especially in cropped soils. Strain 7e and the reference strain, \emph{Nostoc} 9v reduced the breakdown caused by mechanisms of mechanical breakdown (like ploughing) and slow wetting (like gentle rain or irrigation). This suggests that in order to address all possible stresses that cause aggregate breakdown it may be necessary to inoculate soils with multiple cyanobacteria strains. Therefore, work is needed to identify cyanobacteria strains that complement each other in improving soil aggregate stability.

Nisha \emph{et al.} (2007) showed that the EPS produced through biofertilization with cyanobacteria provided a substrate for the growth and enhanced activity of heterotrophic microflora such as saprophyses and symbiotics (\emph{Rhizobium}), which in turn produced more EPS, further amplifying
its effect on soil structural stability. The effect of indigenous *Nostoc* strains 3g and 7e on other soil microflora was not investigated in the present study.

Generally, cropping with maize reduced the effectiveness of inoculation in improving aggregate stability of soils inoculated with *Nostoc* strain 9v, whereas the effect of the indigenous *Nostoc* strains 3g and to lesser extent strain 7e was enhanced by cropping (Table 6.1 and Figure 6.2). The observed trend for *Nostoc* strain 9v is consistent with the results obtained in Chapter 4 and could partly be attributed to the observed negative effect of cropping on *Nostoc* strain 9v establishment and subsequent lower production of EPS. It could also be attributed to other causes such as those highlighted by Reid *et al.* (1982) who presented evidence suggesting that poor aggregate stability in soils cropped with maize could be due to the destruction of organic matter – (Fe or Al) – mineral particle linkages when the Fe and/or Al cations that link organic matter to mineral particles are removed by chelating agents released by maize roots into the soil rhizosphere.

The improved aggregate stability observed under cropping in soils inoculated with the indigenous strains 3g and 7e implies that the effectiveness of the two indigenous strains on aggregate stability improvement may not be affected by cropping with maize, at least. This effect cannot, however, be explained by the organic C or EPS levels as cropping had a negative effect on these parameters (Table 6.1). The contrasting effects on aggregate stability of cropping on strain 9v and the two indigenous strains 3g and 7e suggest a need for further studies to investigate the interaction effects of different cyanobacteria strains with different plant species on soil aggregate stability.
6.6 CONCLUSIONS

Inoculating the Hertzog soil with indigenous cyanobacteria *Nostoc* strains 3g, 7e, and the reference strain 9v, improved the soil’s aggregate stability. The improvement in aggregate stability was more related to EPS than soil C content. Unlike the reference *Nostoc* strain 9v, the two indigenous *Nostoc* strains 3g and 7e improved the aggregate stability of the cropped soil to a greater extent than in the non-cropped soil, indicating their compatibility with the maize cropping system. The results of this study suggest that indigenous *Nostoc* strains screened for higher EPS production could contribute to the amelioration of the structural stability of physically degraded soils in South Africa.
6.7 REFERENCES


**Genstat Release 4.24DE (2005).** Lawes Agricultural Trust (Rothamsted Experimental Station). UK.


CHAPTER SEVEN

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS FOR FOLLOW-UP STUDIES

7.1 GENERAL DISCUSSION

Cyanobacteria are known to be widespread in arid and semi-arid environments (Lange et al., 1992; Eldridge and Greene, 1994; Malam Issa et al., 1999), and to play critical ecological roles like atmospheric N fixation, nutrient enrichment and soil aggregate stability improvement (Evans and Ehleringer, 1993; Belnap and Harper, 1995; Nisha et al., 2007). The objective of this study was to identify cyanobacteria strains adapted to semi-arid environments of the Eastern Cape with ability to fix atmospheric N\(_2\), and produce EPS, and to test their effectiveness in improving soil N availability and aggregate stability.

7.1.1 Selection of Cyanobacteria with Biofertilization and Bioconditioning Potential

The ability of cyanobacteria strains to fix atmospheric N varies widely among different strains (Steppe et al., 1996; Henson et al., 2002) with some reported fixation rates ranging from 10 to 25 kg N per ha annually (Harper & Marble, 1988; West & Skujinš, 1977 as cited by Buttars et al., 1998). The ability to increase SOM, especially EPS, is known to be similarly variable among different cyanobacteria (Belnap and Gardener, 1993). Therefore, the first step in addressing the objective of this study was to identify cyanobacteria strains with ability to fix N\(_2\) and to produce
EPS in the Eastern Cape Province. This was done through the screening study reported in Chapter 2 whose results showed that, out of 97 cyanobacteria strains identified from selected sites, only three showed potential to fix N\(_2\), two of which had potential to produce EPS. The three strains were classified as *Nostoc* and were coded as strains 7e, 3g and 3v. The N\(_2\) fixation capabilities of the strains were 16.1, 4.7 and 1.3 nmol C\(_2\)H\(_4\) µg chl\(^{-1}\) h\(^{-1}\), for strain 7e, 3g and 3v, respectively. The greater capability of strain 7e to fix N\(_2\) could indicate that it had a high percentage of heterocysts which, according to Clark (2002), are cells devoted only to N\(_2\) fixation. In addition to N\(_2\) fixation capability, strain 3g was also capable of producing EPS (Table 2.4), and therefore it was chosen for testing because of its dual characteristics and origin. The fact that only three out of 97 cyanobacteria strains could be found to have bioconditioning and biofertilization potential underscores the importance of screening in order to identify suitable cyanobacteria. The underlying assumption is that such suitable strains of cyanobacteria would also be competitive in growth so as not to be overshadowed by the unsuitable ones.

The next phase of the study sought to evaluate the biofertilization and bioconditioning potential of the selected strains in glasshouse studies. The evaluation begun with preliminary glasshouse studies (Chapters 3 and 4) that utilized *Nostoc* strain 9v, which was the first one to be isolated from a tropical soil in Tanzania during the early stages of the EU funded project. This strain was found to have high potential to fix atmospheric N\(_2\) and to produce EPS. These studies were followed by the glasshouse studies that evaluated the effectiveness of the indigenous *Nostoc* strains 3g and 7e, with strain 9v as a positive control (Chapters 5 and 6). The biofertilization potential of the strains was evaluated in terms of their effect to improve soil C, total and mineral N as well as its availability to maize. The bioconditioning effects were evaluated in terms of the
effects of the strains on soil C, EPS, and aggregate stability as measured by mean weight diameters (MWD) of aggregates and their fragment size distribution (FSD). The results obtained are summarized and discussed in section 7.1.2 for biofertilization and section 7.1.3 for bioconditioning effects.

7.1.2 Inoculation Effects of Cyanobacteria Strains on Soil N Content and Maize Yield

Results of the preliminary study showed that inoculation with *Nostoc* strain 9v improved the soil C and soil N contents determined from the top 5 mm of Guquka and Hertzog soils (Table 3.1). The largest increases in soil C and soil N occurred in Guquka soil. Inoculation with the indigenous strains 3g and 7e also led to increases in soil C and soil N contents in the top 5 mm of Hertzog soil (Table 5.4). However, inoculation with strain 7e resulted in greater increases of both soil C and N relative to strain 3g and the reference strain 9v. During the second cropping, inoculation with strain 7e resulted in greater increases in soil N than did strain 9v (Table 5.4). The observed increases in soil C in both experiments could have been the result of photosynthetic increments as a result of cyanobacteria growth which contributed to both soil carbohydrate C (polysaccharide) and biomass C, as was also suggested by Lange *et al.* (1992).

A positive association of soil C and soil N was observed in the preliminary study ($R^2=0.838$, Figure. 3.1) and in the first and second harvests (Chapter 5) ($R^2=0.667$ and 0.591, respectively) (Figure 5.4). This indicated a close association of C and N dynamics in the inoculated and non-inoculated soils as also reported by Nisha *et al.* (2007). Generally, the increases in N due to inoculation were proportionately greater than those of C, and this resulted in a narrowing of C: N.
ratio in the preliminary study (Table 3.2) and in both croppings (Chapter 5, Table 5.4). In the second cropping, for example, the C: N ratio decreased from 13.3 in non-inoculated soil to 12.2, 10.7 and 12.0 when soil was inoculated with strains 3g, 7e, and 9v, respectively. This generally impacted positively on the mineralization of N and was reflected in increased levels of mineral N (Figures 5.1 and 5.2). The narrower C: N ratios following inoculation with the three *Nostoc* strains indicated greater increases in N relative to C in inoculated soils which could most likely have been as a result of $N_2$ fixation by the cyanobacteria. Harper and Pendleton (1993) reported increases in soil N comparable to those observed in the Guquka soil. Similar trends were also reported in studies that used the more accurate acetylene reduction method for assessing $N_2$ fixation (Skarpe and Henriksson, 1987; Buttars *et al.* 1998). The greater ability of strain 7e to increase soil N relative to the other tested strains could be attributed to its high potential to fix atmospheric $N_2$ (Chapter 2, section 2.4.3), and its ability to re-establish faster in the second cropping and, thus, to continue fixing $N_2$.

The increase in soil N observed in soil inoculated with *Nostoc* strain 9v resulted in improved maize growth in both Guquka and Hertzog soils in the preliminary study (Table 3.3) and in Hertzog soil inoculated with *Nostoc* strains 3g, 7e, and 9v (Figure 5.1 a-b) for both croppings. The increases in the dry matter yields and N uptake in 3g and 7e treatments (Chapter 5) were higher and comparable to those of the reference strain 9v in the first harvest than in the second harvest, indicating that maize plants benefited from N fixed by the *Nostoc* strains before the cyanobacteria biomass was incorporated into the soil. The higher levels of nitrate N and ammonium N in soils inoculated with the *Nostoc* strains and corresponding high N uptake from the different treatments suggested that the $N_2$ fixed by the inoculated indigenous strains of
cyanobacteria was converted to available forms for crop uptake. These findings are in agreement with those reported by Skujinš and Klubek (1978) and Nisha et al. (2007), which showed that fixed nitrogen was ultimately released in the soil as ammonium, where most of it was nitrified to nitrate influenced plant growth. The fact that increases in soil N and N uptake were observed in the first of two croppings (Chapter 5) implies that soil inoculation with these strains would not only benefit the subsequent crop but also the current crop. The results of this study showed that inoculating degraded soils with the indigenous \textit{Nostoc} strains 3g and 7e has the potential to improve the nitrogen nutrition of crops in nitrogen poor soils.

### 7.1.3 Effects of Cyanobacteria Strains Inoculation on Soil Organic Matter Content and Aggregate Stability.

The bioconditioning effects of the different \textit{Nostoc} strains were evaluated using MWD and FSD data. The MWD is qualitatively related to aggregate strength and increases with increasing aggregate stability (Horn and Baumgart, 2000). Le Bissonnais (1996) divided MWD into five classes and related them to soil aggregate stability as follows: $<0.4$ mm is very unstable, $0.4–0.8$ mm is unstable, $0.8–1.3$ mm is partly stable, $1.3–2$ is stable, and $>2$ mm is very stable. The non-inoculated Guquka soil had aggregates with MWD of 0.84, 0.88, and 0.62 mm as determined by the fast wetting, slow wetting, and wet stirring methods, respectively (Figure 4.5). Corresponding values for Hertzog soil were 0.57, 0.92, and 0.63 mm. These results indicated that both soils had low aggregate stability which, according to Le Bissonnais (1996), could be classified as unstable. This was attributed to their high fine sand and silt contents (Table 2.2, Chapter 2).
In Chapter 4, inoculation of Guquka and Hertzog soils with the *Nostoc* strain 9v increased the proportion of macroaggregates (Figure 4.4) in both soils, as reflected by their MWD values (Figure 4.5). Similarly (Chapter 6), inoculation of Hertzog soil with the indigenous *Nostoc* strains 3g and 7e increased the proportion of macroaggregates relative to the control as reflected by their MWD values (Table 6.1, Figures 6.2 and 6.3). However, the effect of strain 3g was greater than that of the reference strain 9v while strain 7e was at par with strain 9v in non-cropped soils. The results of the two experiments showed that the resistance of soil aggregates to breakdown was improved when Guquka and Hertzog soils were inoculated with the reference strain 9v, and when Hertzog soil was inoculated with the indigenous *Nostoc* strains 3g and 7e. These results indicated improved resistance to breakdown following inoculation, especially with respect to slow wetting and mechanical breakdown (Figure 4.5).

Regression analysis, in Chapter 4, showed positive relationship between MWD data and soil EPS ($R^2 = 0.763$) and soil C ($R^2 = 0.485$) (Figure 4.1). Similar trends were observed (Chapter 6), where strong positive relationships were found between the MWD and EPS with $R^2$ values of 0.659, 0.468 and 0.284 for fast wetting, slow wetting, and wet stirring, respectively, in contrast to the weaker association observed between MWD and soil C with corresponding $R^2$ values of 0.035, 0.180 and 0.071. These results indicate that observed increases in MWD were largely due to observed increases in EPS and to a less extent soil C in inoculated soils. It is noteworthy that in both Chapters 4 and 6, EPS was more strongly associated with MWD than soil with C. Indeed, scanning electron microscopy revealed that aggregates of inoculated soils exhibited EPS coatings and EPS bridges while non-inoculated samples had little or no EPS/filaments (Figures 4.1a – 4.1f; Figures 6.1b and 1d). The scanning electron microscope results observed in this
study were also similar to those reported from various soils collected from natural lands (Belnap and Gardner, 1993; Malam Issa et al., 2001), and to those reported on structurally degraded soils inoculated with indigenous cyanobacteria strains (Falchini et al., 1996). The observations suggest that the observed improvements in aggregation and structural stability of the experimental soils (Chapters 4 and 6) were largely due to the enmeshing effect of the inoculated cyanobacteria filaments and the gluing effect of excreted polysaccharides (EPS). However, improvement in aggregation could also have been aided by the hydrophobic properties that EPS impart on soil aggregates which retard the release of entrapped air (Kidron et al., 1999 as cited by Nisha et al., 2007) and thus slow breakdown of soil aggregates. This effect was not specifically evaluated in this study; however, the fact that EPS was most strongly associated with MWD (determined by fasting wetting) suggests that EPS minimized their slaking effect and thus contributed to sustenance of larger aggregates.

The interaction effects of cropping and cyanobacteria inoculation has not received much attention. The results of this study have shown that cropping with maize affected the effectiveness of the different Nostoc strains differently. Results (Chapter 4) showed that cropping with maize reduced the effectiveness of inoculation with strain 9v in improving aggregate stability of soils. In Chapter 6, cropping with maize was reported to reduce the effectiveness of inoculation in improving aggregate stability of soils inoculated with Nostoc strain 9v in a similar fashion as reported in Chapter 4. However, the effectiveness of Nostoc strain 3g, and to a lesser extent strain 7e, was enhanced by cropping (Table 6.1 and Figure 6.2). The observed negative effect of cropping with maize on the effectiveness of Nostoc strain 9v could have been partly due to the observed negative effect of cropping on the establishment of the strain and subsequent
lower production of EPS. It could also be attributed to other causes such as those highlighted by Reid and Goss (1981) and Reid et al. (1982) who presented evidence suggesting that poor aggregate stability in soils cropped with maize could be due to the destruction of (organic matter) – (Fe or Al) – (mineral particle) linkages. The destruction of the linkages was said to occur when the Fe and Al cations that link organic matter to mineral particles are removed by chelating agents released by maize roots into the soil rhizosphere.

The effect of cropping in enhancing the inoculation effect of the indigenous strains 3g, and to a lesser extent strain 7e, on aggregate stability, implies that the effectiveness of the two indigenous strains may not be affected by cropping with maize at least. This effect cannot, however, be explained by the organic carbon or EPS levels as cropping had a negative effect on these parameters (Table 6.1). The contrasting effects on aggregate stability of cropping on strain 9v and the two indigenous strains 3g and 7e suggest a need for further studies to investigate the interaction effects of different cyanobacteria strains with different plant species on soil aggregate stability.

7.2 GENERAL CONCLUSIONS

1. Screening for cyanobacteria at four selected sites in the Eastern Cape revealed a total of 97 cyanobacteria strains out of which only two strains were found to have capability to fix N₂ and to produce EPS. The two strains were classified as *Nostoc* and coded as strain 3g and 7e. This underscored the importance of screening studies in order to identify suitable cyanobacteria strains.
2. Inoculation of degraded Guquka and Hertzog soils with *Nostoc* strain 9v with dual ability to fix atmospheric N\textsubscript{2} and produce high EPS improved the structural stability of the soils, increased the soils’ N content and improved maize yields.

3. Inoculation of a degraded Hertzog soil with indigenous cyanobacteria strains 3g and 7e with ability to fix N\textsubscript{2} and produce EPS improved the aggregate stability of the soil, increased soil N and enhanced maize yields. Strain 7e was more effective than strain 3g in improving soil N and maize yields because it had greater capacity to fix N\textsubscript{2}.

4. Observed improvements in the aggregate stability of soils following inoculation with *Nostoc* strains 9v, 3g, and 7e were largely explained by increases in soil C but, most probably, also more EPS as a result of inoculation with the *Nostoc* strains.

5. The effectiveness of the cyanobacteria strains studied to improve soil aggregate stability was affected differently by cropping with maize. Cropping with maize had a negative impact on the effectiveness of strain 9v to improve aggregate stability but had a positive effect on strain 3g and to a limited extent strain 7e.

6. Generally the results of the different studies suggested that cyanobacteria screened for high N\textsubscript{2}-fixing ability and EPS production could improve the productivity of N poor soils and contribute to the amelioration of the structural stability of physically degraded soils in South Africa. These results suggest that cyanobacterial soil conditioners could be used
to enhance the yield and nutritional value of food crops, especially in areas where crop yields are marginal.

7.3 GENERAL RECOMMENDATIONS FOR FOLLOW-UP STUDIES

This study is the first of its kind in South Africa and as such many questions remain unanswered. The following are a few such areas which may need attention in the near future:

1. In view of the potential for cyanobacterial conditioners demonstrated by this study, there is a need for further screening and culturing of suitable cyanobacteria from other parts of South Africa for use in South Africa and other countries where crop yields are marginal and fertilizers are cost-prohibitive.

2. Only two cyanobacteria strains were identified and tested in this study. The screening process was, however, not exhaustive so there is need for further screening studies to search for more suitable strains capable of improving the N status of N-poor soils and the structural stability of degraded soils.

3. The effectiveness of the different strains in improving aggregate stability was influenced differently by cropping. There is a need to test this effect with different crops and their varieties as well as establish the mechanisms responsible for the differential responses.
4. There is a need to investigate the compatibility and complementarity of different cyanobacteria strains in improving aggregate stability with a view to determine strains that can be co-inoculated in degraded soils for greater effectiveness.

5. The studies were done under glasshouse conditions. So there is a need to evaluate the effectiveness of the strains under field conditions. Such studies should be used to establish realistic field application rates of the cyanobacteria.
7.4 REFERENCES


Appendix A: Exocellular polysaccharides (EPS) around the cells of cyanobacteria as shown by the surrounding white zone after being stained with India ink.
Appendix B: Preparation and production of three cyanobacteria strains in the laboratory (a and b) and in outdoor ponds (c).
Appendix C: Physical and chemical properties of used Guquka soil.

Profile description of soil at Guquka.

CLASSIFICATION: Soil Taxonomy: Typic Plinthustalf
FAO WRB: Ferric Luvisol
SA System: FORM: Griffin (Gf) FAMILY: 2200 Braeside

LOCALITY: Msizi Farm, Guquka Gridref.: Latitude: 32 deg. 39'05" S
Map no.: 3226DB SEYMOUR Longitude: 26 deg. 56'10" E

CLIMATE: Sub-humid (Ann. pptn. approx. 750 mm)

PARENT MATERIAL: Mode of Accumulation: Colluvial
No. & kinds: Mixed
Underlying material: Unknown
Weathering under, mat: Unknown

TOPOGRAPHY: Terrain morph. unit: 4 - Upper footslope
Slope: 7% Kind: Straight
Aspect: North West Altitude: 750m.a.s.l

VEGETATION/LAND USE: Maize field

<table>
<thead>
<tr>
<th>Horizon</th>
<th>Depth (cm)</th>
<th>Description of profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ap</td>
<td>0 – 16</td>
<td>Light yellow brown (10YR 6/4) dry; dark yellow brown (10YR 4/4) moist; horizon disturbed; sandy clay loam; moderate coarse subangular blocky; sightly hard; many coarse pores; few fine sesquioxide concretions; few fine roots; abrupt smooth transition</td>
</tr>
<tr>
<td>Bt1</td>
<td>16 – 41</td>
<td>Dark yellowish brown (10YR 4/6) dry; dark brown (7.5YR 3/4) moist; horizon undisturbed; sandy clay; moderate coarse subangular blocky; very hard; common biological channels and infillings; few fine round sesquioxide concretions; few fine roots; gradual smooth transition</td>
</tr>
<tr>
<td>Bt2</td>
<td>41 – 90</td>
<td>Strong brown (7.5YR 5/6) dry; yellowish red (2.5YR 4/6) moist; horizon undisturbed; clay; moderate coarse subangular blocky; very hard; common biological channels and infilllings; many fine pores; common fine round sesquioxide concretions; very few fine roots; clear smooth transition</td>
</tr>
<tr>
<td>Bt3</td>
<td>90 – 160+</td>
<td>Dark red (2.5YR 3/6) moist; horizon undisturbed; clay; moderate coarse subangular blocky; friable; many medium rounded sesquioxide concretions; distinct geogenic mottles; many sesquioxide cutans.</td>
</tr>
</tbody>
</table>

Described by: OT Mandiringana, PNS Mnkeni and MP Maqubela Date: 08 July 2002.
Appendix C continued

Analytical data of soil at Guquka.

<table>
<thead>
<tr>
<th>Horizon</th>
<th>Ap</th>
<th>Bt1</th>
<th>Bt2</th>
<th>Bt3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth (cm)</td>
<td>0 - 16</td>
<td>16 – 41</td>
<td>41 – 90</td>
<td>90 – 160+</td>
</tr>
<tr>
<td>Fine earth (%)</td>
<td>8.86</td>
<td>98.02</td>
<td>90.98</td>
<td>33.05</td>
</tr>
<tr>
<td>Particle size distribution (%)</td>
<td>Co. sand</td>
<td>0.85</td>
<td>0.30</td>
<td>1.65</td>
</tr>
<tr>
<td></td>
<td>Med. Sand</td>
<td>0.70</td>
<td>0.90</td>
<td>1.70</td>
</tr>
<tr>
<td></td>
<td>Fi. Sand</td>
<td>23.45</td>
<td>23.65</td>
<td>33.15</td>
</tr>
<tr>
<td></td>
<td>Silt</td>
<td>46.63</td>
<td>29.53</td>
<td>26.38</td>
</tr>
<tr>
<td></td>
<td>Clay</td>
<td>28.37</td>
<td>45.62</td>
<td>37.13</td>
</tr>
<tr>
<td>pH (water)</td>
<td>5.14</td>
<td>5.58</td>
<td>5.67</td>
<td>5.89</td>
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<tr>
<td>pH (KCl)</td>
<td>4.30</td>
<td>4.78</td>
<td>4.80</td>
<td>4.93</td>
</tr>
<tr>
<td>Exchangeable cations (cmol (+) kg⁻¹ soil)</td>
<td>Na</td>
<td>0.02</td>
<td>0.04</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>0.24</td>
<td>0.12</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Ca</td>
<td>1.65</td>
<td>2.65</td>
<td>1.61</td>
</tr>
<tr>
<td></td>
<td>Mg</td>
<td>0.16</td>
<td>0.26</td>
<td>0.60</td>
</tr>
</tbody>
</table>
Appendix D: Physical and chemical properties of Hertzog soil

Profile description of soil at Hertzog

**CLASSIFICATION:**
- Soil Taxonomy: Typic Haplustalf
- FAO WRB: Haplic Luvisol
- SA System: FORM; Valsrivier (Va) FAMILY: 1122 Aliwal

**LOCALITY:** Amphos Farm, Hertzog Gridref.: Latitude: 32 deg. 35'03" S
Map no.: 3226DB SEYMOUR Longitude: 26 deg. 43'14" E

**CLIMATE:** Semi-arid (Ann. pptn. approx. 550 mm)

**PARENT MATERIAL:**
- Mode of Accumulation: Alluvial
- No. & kinds: Mixed
- Underlying material: Sedimentary material
- Weathering under, mat: Advanced

**TOPOGRAPHY:**
- Terrain morph. unit: 4 – Lower footslope
- Slope: 2% Kind: Straight
- Aspect: North West Altitude: 655 m.a.s.l

**VEGETATION/LAND USE:** Fallow land

<table>
<thead>
<tr>
<th>Horizon</th>
<th>Depth (cm)</th>
<th>Description of profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ap</td>
<td>0 –27</td>
<td>Very dark greyish brown (10YR 3/2) moist; horizon undisturbed; clay loam; weak fine subangular blocky; friable; many medium pores; many medium black Mn nodules; very few lime nodules; many fine and few coarse roots; abrupt smooth transition</td>
</tr>
<tr>
<td>Bt1</td>
<td>27 – 80</td>
<td>Dark yellowish brown (10YR 3/6) moist; horizon undisturbed; clay; strong coarse angular blocky; extremely firm; distinct cutanic character; many medium black Mn nodules; few medium angular lime nodules; few fine roots; gradual smooth transition</td>
</tr>
<tr>
<td>Bt2</td>
<td>80 –120</td>
<td>Olive yellow (2.5YR 6/6) moist; horizon undisturbed; clay; strong coarse angular blocky; firm; many medium black Mn nodules; common medium mixed lime nodules; few fine roots; diffuse smooth transition</td>
</tr>
<tr>
<td>Bt3</td>
<td>120– 157+</td>
<td>Yellow (2.5YR 7/6) moist; slightly firm rock material weathering in – situ with very many clay cutans, few re iron oxide mottles, many black Mn mottles, and few medium lime nodules good rooting medium.</td>
</tr>
</tbody>
</table>

**Described by:** OT Mandiringana, PNS Mnkeni and MP Maqubela **Date:** 10 July 2002
Appendix D continued

Analytical data of soil at Hertzog

<table>
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<tr>
<th>Horizon</th>
<th>Ap</th>
<th>Bt1</th>
<th>Bt2</th>
<th>Bt3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth (cm)</td>
<td>0 – 27</td>
<td>27– 80</td>
<td>80 – 120</td>
<td>120– 157+</td>
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<tr>
<td>Fine earth (%)</td>
<td>95.86</td>
<td>97.95</td>
<td>88.62</td>
<td>83.34</td>
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<tr>
<td>Particle size distribution</td>
<td>Co. sand</td>
<td>0.75</td>
<td>0.40</td>
<td>1.75</td>
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<tr>
<td>(%)</td>
<td>Med. Sand</td>
<td>0.80</td>
<td>0.80</td>
<td>1.60</td>
</tr>
<tr>
<td></td>
<td>Fi. Sand</td>
<td>24.45</td>
<td>24.65</td>
<td>35.15</td>
</tr>
<tr>
<td></td>
<td>Silt</td>
<td>45.73</td>
<td>28.73</td>
<td>26.38</td>
</tr>
<tr>
<td></td>
<td>Clay</td>
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<td>45.42</td>
<td>35.13</td>
</tr>
<tr>
<td>pH (water)</td>
<td>7.91</td>
<td>9.08</td>
<td>9.00</td>
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<td>pH (KCl)</td>
<td>6.81</td>
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<td>Exchangeable cations</td>
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<tr>
<td>(cmol (+) kg⁻¹ soil)</td>
<td>K</td>
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<td></td>
<td>Ca</td>
<td>10.98</td>
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<td>6.88</td>
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<tr>
<td></td>
<td>Mg</td>
<td>4.43</td>
<td>7.14</td>
<td>6.47</td>
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</table>
Appendix E: Properties of Guquka and Hertzog soils taken from the top 5 cm of cultivated soil

<table>
<thead>
<tr>
<th>Soil property</th>
<th>Units</th>
<th>Guquka</th>
<th>Hertzog</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clay minerals</td>
<td></td>
<td>Illite</td>
<td>Mica</td>
</tr>
<tr>
<td>pH (H₂O)</td>
<td></td>
<td>5.4</td>
<td>7.9</td>
</tr>
<tr>
<td>pH (KCl)</td>
<td></td>
<td>4.2</td>
<td>6.9</td>
</tr>
<tr>
<td>Soil N (%)</td>
<td>(%)</td>
<td>0.04</td>
<td>0.07</td>
</tr>
<tr>
<td>Soil C</td>
<td></td>
<td>0.61</td>
<td>0.77</td>
</tr>
<tr>
<td>C/N</td>
<td></td>
<td>15.3</td>
<td>10.2</td>
</tr>
<tr>
<td>P</td>
<td>mg kg⁻¹</td>
<td>0.8</td>
<td>30</td>
</tr>
<tr>
<td>K</td>
<td></td>
<td>105</td>
<td>370</td>
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<tr>
<td>Mg</td>
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<td>260</td>
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<td>Ca</td>
<td></td>
<td>280</td>
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<td>Fe</td>
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<td>53</td>
</tr>
<tr>
<td>Mn</td>
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<td>46</td>
<td>310</td>
</tr>
<tr>
<td>Zn</td>
<td></td>
<td>0</td>
<td>0.8</td>
</tr>
<tr>
<td>Cu</td>
<td></td>
<td>1.0</td>
<td>2.4</td>
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<tr>
<td>ECEC</td>
<td>cmol c kg⁻¹</td>
<td>20</td>
<td>102</td>
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<tr>
<td>SiO₂</td>
<td>g 100g⁻¹</td>
<td>74</td>
<td>79</td>
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<tr>
<td>Al₂O₃</td>
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<td>10</td>
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<tr>
<td>Fe₂O₃</td>
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<tr>
<td>MnO</td>
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<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>
Nostoc cyanobacterial inoculation in South African agricultural soils enhances soil structure, fertility, and maize growth

M. P. Maqubela • P. N. S. Mnkeni • O. Malam Issa • M. T. Pardo • L. P. D’Acqui

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Abstract Many soils in South Africa have low nutrient supply, poor structural stability and are prone to soil erosion due to susceptibility to surface sealing and crusting. Two crusting soils from the Eastern Cape Province, South Africa were used to evaluate the effects of inoculation with a strain of Nostoc on soil structure, fertility and maize growth. The Nostoc suspension was uniformly applied over potted soils at a rate of 6g (dry weight) per square meter soon after maize germination. Nostoc inoculation increased soil N by 17% and 40% in Hertzog and Guqqua soils, respectively. Soil C was also increased significantly and this increase was strongly associated with that of soil N ($R^2 = 0.838$). The highest contents of soil C, soil N and mineral N, however, were found in non-cropped Nostoc inoculated soils. Nostoc inoculation increased maize dry matter yields by 49% and 40% in Hertzog and Guqqua soils, respectively. Corresponding increases in maize tissue N were 23% and 14%, respectively. Scanning electron microscopy (SEM) revealed that soil particles and fragments of non-cropped inoculated soils had coatings of extracellular polymeric substances (EPS) with other particles enmeshed in networks of filaments, whilst by contrast little or no EPS and/or filaments were observed on cropped and/or non-inoculated soils. This was consistent with chemical analysis which showed that Nostoc caused significant increases in the EPS and soil C contents of non-cropped soils. The proportion of very stable aggregates was increased by inoculation with Nostoc possibly due to the greater quantities of soil C and EPS observed in inoculated soils. Inoculated soils cropped with maize had a lower proportion of stable aggregates presumably due to their low soil C and EPS contents compared to non-cropped soils. The results suggested that Nostoc could improve the fertility and structural stability of the studied degraded soils.

Keywords Aggregate stability • Cyanobacteria • Exo-cellular polymeric substances • Inoculation • Nostoc • South Africa
Abbreviations
SEM scanning electron microscopy
EPS extracellular polymeric substances
FSD fragment size distribution
MWD mean weight diameter
FW fast wetting
WS wet stirring
SW slow wetting

Introduction
Most soils used for crop production in South Africa have low soil fertility (Mills and Fey 2003; Mandirirangana et al. 2005), as well as high silt and low soil organic matter (SOM) contents (Oldeman 1994). The aggregates of these soils therefore quickly collapse under pressure on irrigation or high rainfall resulting in surface sealing and physical crust formation and hence soil erosion (Woyessa and Bennie 2004). The tendency for soil surface crusting can be reduced by improving the resistance of aggregates to withstand physical and physico-chemical mechanisms of breakdown that operate during rainfall and irrigation events. According to Le Bissonnais (1996) aggregate breakdown is caused by one or more of the following mechanisms: (1) slaking, caused by the compression of entrapped air during wetting; (2) breakdown by differential swelling related to the behaviour of clay material after swelling; (3) breakdown by raindrop impact, and (4) physico-chemical dispersion due to osmotic stress. Methods proposed by Le Bissonnais (1996) used to measure these mechanisms are fast wetting, mechanical breakdown, and slow wetting.

Soil organic matter content has been reported to be of utmost importance in improving degraded soils (Domini and Haynes 2002; Graham et al. 2002). Adding organic matter to soil increases the stability of aggregates to breakdown (Baldock and Nelson 2000) by reducing the rate of wetting and increasing the resistance to stresses generated during wetting (Caron et al. 1996). Previous results indicated that below the threshold soil organic carbon content of 2%, water-stable aggregates quickly broke down and became extremely erodible (Elwell 1989). Filamentous cyanobacteria can contribute to macro-aggregation and result in improved resistance to soil erosion (Aspiras et al. 1971; Fulchini et al. 1996). As primary producers, they contribute to the enrichment of soil with SOM and to the improvement of biological activity (Aceca et al. 2003). Cyanobacterial EPS secretions are dominated by polysaccharides which can bind soil particles (Belnap and Gardner 1993; Eldridge and Greene 1994; Malam Issa et al. 2001), in addition to their role in the protection of the cyanobacteria against environmental conditions (DeWinder 1990) and in assisting cyanobacteria motility (Stal 1995).

Inoculating soil with cyanobacteria has been reported to improve the aggregation of the top soil (Rao and Burns 1990; Malam Issa et al. 2007) and to increase water retention, and ecosystem regeneration (Eldridge and Greene 1994). The potential positive effects of cyanobacteria, however, are not restricted to soil physical properties. Soil inoculation with N2-fixing cyanobacteria, has also been shown to induce increases in SOC, total N and available nutrients in the topsoil. Thus, Rogers and Burns (1994) demonstrated that inoculation of a poorly structured silt loam soil with Nostoc muscorum led to a pronounced effect on soil chemical properties, with total C increasing by 50–63% and total N increasing by 111–120%. In a laboratory experiment, Aceca et al. (2003) also showed that soil inoculation with different cyanobacterial strains induced great microbial proliferation as well as high increases in SOC and available nutrients, the efficacy of the treatment depending on the type of soil.

Observed increases in soil total N following cyanobacteria inoculation is attributed to the ability of cyanobacteria to fix atmospheric nitrogen (Harper and Marble 1988). Soil inoculation with cyanobacteria with these attributes may therefore represent a simple and low-cost method for improving the productivity of degraded lands in developing countries where very little or no inorganic fertilizers are usually applied. So far no research has been undertaken with regard to the possibility of using N2-fixing cyanobacteria in South African soils.

The work presented here was part of a European Union (EU) funded project (Cyanosols) on the use of local strains of cyanobacteria to improve resilience and overall soil fertility in arid and semi-arid soils. The purpose of this study was to assess the potential use of cyanobacteria for the enhancement of the structure and fertility of two degraded arable soils from the Eastern Cape Province, South Africa. Therefore, we evaluated (1) the effect of inoculation on soil C, soil N, mineral N and EPS content (2) the effect of inoculation on soil structural stability, and
(3) the interactive effect of inoculation and cropping on the above mentioned soil characteristics under tunnel house conditions.

Materials and methods

The studied sites were located in the Eastern Cape Province of South Africa near the villages of Guquka (32°39' S, 26°57' E) and Hertzog (32°35' S, 26°43' E). The soils were classified according to Soil Survey Staff (1975) as a Typic Plinthustalf (Guquka) and a Typic Haplustalf (Hertzog). Guquka soil had been cropped with maize in the previous summer season but was on fallow during the time of survey. Hertzog soil was on its second fallow year at the time of the survey. Bulk soil samples were collected from a depth of 15 cm at the two sites then air-dried, and sieved through a 4-mm sieve for use in the tunnel house study. Samples used in the laboratory for chemical and physical analysis were further sieved through a 2-mm sieve. The pH was measured in a 1:2.5 (v/v) soil:water suspension. Available P was determined following the Bray 1 procedure (Bray and Kurtz 1945). Soil N and C were analysed using a LECO C/N analyzer (LECO Corporation 2003). These characteristics are given in Table 1. The soil C contents for both Guquka and Hertzog soils were very low, whilst the silt contents were relatively high compared to sand and clay and therefore were highly susceptible to crusting.

Pots with a diameter of 20 cm were filled with 4 kg of the sieved soils and used as the experimental units. The treatments were arranged in a split split-plot design with four replications in which soils (Hertzog and Guquka) were the main plots, cropping treatments (cropped and non-cropped with maize) were the subplots and inoculation treatments (inoculated and non-inoculated with a Nostoc strain) were the sub sub-plots. A basal rate of 40 mg P kg⁻¹ as potassium phosphate was applied to each pot to provide P and K before five maize seeds were sown in each. The maize plants were thinned to two plants per pot 7 days after germination.

The cyanobacterium strain was isolated from a tropical soil from Tanzania, another site studied during the EU funded Cyanosols project. It was isolated from colonies that developed on solid and selective growth medium BG 110 which lacks chemically combined nitrogen. Isolation involved removing colonies that resembled cyanobacteria by colour and micromorphology when observed under light microscope and serially streaking them on fresh growth medium with 0.16 mM of cyclohexamide (a growth inhibitor of eukaryotes) to purify them from fungi and other eukaryotic microorganisms. A strain of the genus Nostoc was selected because it showed a high growth rate on culture medium, and had high ability to produce EPS and to fix nitrogen. A suspension of this strain at a concentration of 1 g (dry weight) per liter was uniformly poured over the potted soils soon after maize germination to provide an equivalent dry biomass of 6 g m⁻², whilst non-inoculated soils were irrigated with water equivalent to the liquid introduced by inoculation in the Nostoc treated pots. The C and N contents of the inoculated Nostoc strain were 359 and 35 g kg⁻¹, respectively. Pots were watered as necessary to compensate for water loss due to evapo-transpiration.

Six weeks after Nostoc inoculation the maize plants were harvested by cutting the stems just above the soil surface, cleaned of dirt and dried to constant weight in an oven at 60°C. The dried plant materials were weighed, ground and analysed for tissue N using a LECO C/N analyzer (LECO Corporation 2003).

Soil sampling for chemical analysis and aggregate stability determination was done in the top 5 mm of the soils since Rao and Burns (1990) demonstrated that the effects of surface phototrophic growth on soil properties were restricted to the surface layer (0-0.7 cm). The sampling was done by gently scooping the top 5 mm of soil from the centre and from four positions along the edges of each pot. Samples intended for chemical analysis were uniformly mixed and a portion was kept in the refrigerator until it was used for inorganic N

<table>
<thead>
<tr>
<th>Soil characteristics</th>
<th>Guquka</th>
<th>Hertzog</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coarse sand (%)</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>Medium sand (%)</td>
<td>0.7</td>
<td>0.5</td>
</tr>
<tr>
<td>Fine sand (%)</td>
<td>33.5</td>
<td>32.2</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>49.9</td>
<td>45.4</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>15.7</td>
<td>21.8</td>
</tr>
<tr>
<td>MWD* (mm)</td>
<td>0.56</td>
<td>0.51</td>
</tr>
<tr>
<td>Soil C (%)</td>
<td>0.42</td>
<td>0.58</td>
</tr>
<tr>
<td>Soil N (%)</td>
<td>0.04</td>
<td>0.06</td>
</tr>
<tr>
<td>P (mg kg⁻¹)</td>
<td>7.4</td>
<td>28.9</td>
</tr>
<tr>
<td>pH (H₂O)</td>
<td>5.01</td>
<td>7.99</td>
</tr>
</tbody>
</table>

MWD: Mean weight diameter

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determination, whilst another portion was dried, ground and used for soil C and N determination using the LECO C/N analyser. Inorganic N was determined colorimetrically as described by Olalebo et al. (2002). Exocellular polymeric substances (EPS) sugars were analysed by extracting 10g of soil with 30ml of 0.5M NaOH and then hydrolysing overnight in 5ml of 12M H₂SO₄. Samples were then centrifuged at 11,000 rpm for 30 min and the extracted EPS was determined spectrophotometrically using the phenol-sulphuric acid method (Dubois et al. 1956). A blank which did not contain the phenol was prepared to correct for contamination.

Soil samples for aggregate stability determination were placed in rigid containers immediately after sampling to avoid further breakage of the aggregates. Microscopic investigations of soil structure and aggregate stability measurements were simultaneously run for inoculated and non-inoculated samples. The micromorphological characteristics of the samples were investigated with a scanning electron microscope (SEM) model Quanta 200 Phillips.

Aggregate stability measurements were performed in triplicate using 5g soil samples according to the method of Le Bissonnais (1996). The samples were first sieved between 3 and 5mm mesh and dried at 40°C for 48h. Samples were then subjected to fast wetting, slow wetting, or mechanical breakdown treatments according to Attou et al. (1998). Fast wetting tests forces related to entrapped air within the aggregates and differential swelling of clays (Chenu et al. 2000). It was done by gently immersing soil aggregates in 50ml deionised water for 10min. The water was drawn off by pipette leaving behind the slaked aggregates. Slow wetting tests the stability of aggregates under low moisture conditions such as those subjected to moderate rains. It measures aggregate stability under conditions in which air entrainment and differential swelling are minimized. This was performed by placing aggregates on a filter paper maintained at a matric potential of ~0.3kPa for 30min and the residual aggregates were collected thereafter. The mechanical breakdown method tests the cohesion of soil aggregates independently of slaking by air entrainment and the effect of differential swelling. Air was removed from the aggregates before energy was applied, by immersing soil aggregates into 50ml ethanol. After 10min the ethanol was drawn by pipette and aggregates were transferred to another flask with 50ml of deionised water. The flask was filled with 200ml of distilled water, corked and agitated end over end 20 times and left to stand for 30min to allow coarse particles to settle. Suspended material and excess water was removed by pipette and the residual aggregates collected.

Following each test method, the residual aggregates were transferred to a 50μm sieve immersed in ethanol. The aggregates which were retained on the sieve were transferred to evaporation dishes and dried at 40°C for 24h. The fragment size distribution (FSD) was measured by dry sieving the aggregates with a set of six sieves of 2, 1, 0.5, 0.2, 0.1 and 0.05mm in diameter. The weight of aggregates collected on each sieve was determined and expressed as a percentage of the initial sample dry mass. Aggregate stability was described using the resulting fragment size distribution in the seven granulometric classes and the mean weight diameter (MWD) calculated as follows:

\[ MWD = \frac{\sum \bar{T}_i \times w_i}{100} \]

Where \(\bar{T}_i\) is the mean intersieve size and \(w_i\) the percentage particles left on each sieve.

The results of the fragment classes were also grouped into macro (>0.2mm in diameter) and micro (<0.2mm in diameter) aggregates. Statistical analysis was done using the Genstat statistical software (Genstat Release 4.24DE 2005) but mean separation was done using the M Stat C software.

Results

Soil surface observations

Three weeks after inoculation about 90% of the entire surfaces of inoculated soils were observed to be uniformly covered with layers of Nostoc in combination with other cyanobacteria, whilst only thin patches of cyanobacteria were found on the surface of non-inoculated soils. These observations suggested that the inoculated Nostoc strain established well on the surfaces of the two experimental soils. Further observations under SEM showed great differences between inoculated and non-inoculated samples (Fig. 1a–f). Inoculated soil samples had networks of...
Fig. 1 SEM micrographs of the soil surface: a Non-cropped inoculated Guqua soil—cyanobacteria filaments and EPS coating are observed. Notice soil particles entangled among the network of filaments. b Non-cropped, inoculated Guqua soil—soil particles enclosed in the network of filaments. Notice mineral particles and soil fragments trapped in the network and superficial pores resulting from the intertwining of filaments. c Non-cropped, inoculated Guqua soil—detailed view showing massive accumulation of EPS. d Non-cropped, inoculated Hitzog soil—EPS coating and forming organic bridges between soil particles. e Cropped, non-inoculated Guqua soil—only mineral particles and fragments of soil are observed. f Cropped and non-inoculated Hitzog soil—organic material is absent.
filaments among which mineral particles were enmeshed (Fig. 1a and b), and mineral particles and soil fragments were coated by EPS presumed to have been secreted by the *Nostoc* strain (Fig. 1a and d). By contrast little or no EPS and/or filaments were observed on non-inoculated soils, especially on cropped ones (Fig. 1c and f).

**Effect of Nostoc inoculation on selected soil chemical properties**

Application of *Nostoc* significantly increased soil C (p < 0.001), soil N (p = 0.006), mineral N (p < 0.05) and EPS (p < 0.001) contents in the surface soils (0–5mm) studied (Table 2). The percentage increases when averaged across the two soils were 14%, 50%, 6% and 37% for soil C, soil N, mineral N, and EPS, respectively. There was a significant interaction (p = 0.049) between soil type and inoculation with *Nostoc* in regard to soil C content (Table 2) in that the largest increase in soil C of about 27% occurred in Guquka soil as compared to only 8% in Hertzog soil. The interaction between inoculation and cropping was not significant (p = 0.212) but a greater increase in soil C following inoculation was observed in non-cropped soils (16%) than in cropped soils (11%). There was no significant interaction (p = 0.199) between inoculation with the *Nostoc* strain and soil type on soil N but the increase in soil N following inoculation (Table 2) was highest (67%) in Guquka soil compared to Hertzog soil (20%).

Production of EPS was significantly (p < 0.001) favoured in *Nostoc* treated soils as reflected by observed increases in EPS production of 31% and 42% in Guquka and Hertzog soils, respectively (Table 2). The interaction between soil type and inoculation was not significant (p = 0.256) on EPS but the interaction between cropping and inoculation was significant (p = 0.013). Inoculation with *Nostoc* increased EPS by 58% (2.05 to 3.24mg g\(^{-1}\)) in non-cropped soils whereas in cropped soils it increased EPS by only 13% (1.83 to 2.07mg g\(^{-1}\); Table 2).

**Effect of cyanobacteria application and cropping on soil aggregate stability**

**Fragment size distribution (FSD)**

Results of FSD of materials collected after aggregate stability tests are shown in Figs. 2 and 3. The percentage of fragments >2mm in non-cropped inoculated soils from Guquka ranged from 22–32% and were significantly higher than those obtained on non-cropped and non-inoculated soils from Guquka that ranged 19–24% (Fig. 2a). The FSD of non-cropped soils from Hertzog exhibited lower values of coarse aggregates compared to that of Guquka. The proportion of aggregate fragments >2mm collected after fast wetting (FW), wet stirring (WS) and slow wetting (SW) on non-cropped soils from Hertzog ranged from 13% to 21% and 13% to 20% when inoculated and non-cropped, respectively (Fig. 2b).

The FSD of cropped and inoculated Guquka soil showed the proportion of fragments >2mm to range from 14% to 18% which was higher than the 5–15% observed in the cropped but non-inoculated Guquka

**Table 2 Intercation effects of a *Nostoc* inoculation with soil type and cropping on selected surface soil (0–5 mm) chemical properties**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Soil</th>
<th>Guquka</th>
<th>Hertzog</th>
<th>Cropping</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inoculated</td>
<td>Non-inoculated</td>
<td>Inoculated</td>
<td>Non-inoculated</td>
</tr>
<tr>
<td>Soil C (%)</td>
<td>0.57±0.03</td>
<td>0.45±0.00</td>
<td>0.80±0.02</td>
<td>0.74±0.03</td>
</tr>
<tr>
<td>Soil N (%)</td>
<td>0.05±0.008</td>
<td>0.03±0.005</td>
<td>0.06±0.005</td>
<td>0.05±0.002</td>
</tr>
<tr>
<td>C/N</td>
<td>11.4</td>
<td>15.0</td>
<td>13.3</td>
<td>14.8</td>
</tr>
<tr>
<td>Mineral N (mg kg(^{-1}))</td>
<td>48.8±1.1</td>
<td>46.1±1.2</td>
<td>50.9±1.1</td>
<td>48.1±1.4</td>
</tr>
<tr>
<td>EPS (mg g(^{-1}))</td>
<td>2.52±0.13</td>
<td>1.92±0.20</td>
<td>2.80±0.30</td>
<td>1.97±0.06</td>
</tr>
</tbody>
</table>

For each parameter (except C/N), means followed by the same letter or none at all, are not significantly different at P≤0.05

*E&PS* exo-cellular polymeric substances

*Standard error of means*
soils (Fig. 3a). The proportion of aggregates >2mm for Hertzog soil were 11% to 14% when cropped and inoculated and 7% to 14% when cropped and non-inoculated (Fig. 3b).

When grouped into macro aggregates (>0.2mm) and micro aggregates (<0.2mm) the results showed that inoculating with the Nostoc strain increased the proportion of macro-aggregates with a corresponding decrease in the proportion of micro-aggregates in both Guquka and Hertzog soils (Fig. 4). This effect was greater in non-cropped than in cropped soils. The mass percentage of macro-aggregates and micro-aggregates for non-cropped and inoculated soils from Guquka ranged between 50% and 71% and between 29% and 50%, respectively while corresponding values for non-cropped and non-inoculated soils ranged between 42% and 65% and between 35% and 58%, respectively. A similar pattern was observed on Hertzog soil but the negative effect of cropping on aggregate stability was less pronounced compared to Guquka soil (Fig. 4).

**Mean weight diameter (MWD)**

Inoculation with Nostoc significantly increased the MWD of soil aggregates as determined by fast wetting ($p = 0.005$), wet stirring ($p = 0.002$) and slow wetting ($p < 0.001$) methods. However, this effect was interactive with soil type ($p = 0.012$) for the slow wetting method in that it was more pronounced for Guquka than for Hertzog soil (Fig. 5a). Cropping resulted in significantly lower aggregate MWD determined by all three test methods (Fig. 5b). Values measured in samples from cropped
soils ranged from 0.5 to 0.9, 0.4 to 0.8, 0.8 to 0.9mm when determined by fast wetting, wet stirring, and slow wetting methods, respectively which were lower than corresponding values obtained in non-cropped soils which ranged from 0.7 to 1.2, 0.7 to 1.2 and 1.0 to 1.4mm, respectively. There was, however, a significant interaction between cropping and inoculation for MWD\text{soil} (\(\rho = 0.008\)) in that inoculation increased MWD\text{soil} significantly where the soils were non-cropped but not when cropped (Fig. 5b). A positive association between MWD and soil C (\(R^2 = 0.485\)) and EPS (\(R^2 = 0.763\)) was observed (Figs. 6a and b) indicating that aggregate stability increased with increases in the levels of soil C and EPS in the soils.

Effect of \textit{Nostoc} application on maize growth and nitrogen uptake

Application of \textit{Nostoc} caused improvement in the growth and N uptake of maize (Table 3). In Gaqua soil, dry matter (DM) yield was increased by 49% (8.1 to 12.1g per pot) while in Hertzog soil DM yield was increased by 40% (5.3 to 7.4g per pot). A similar trend was observed for maize tissue N concentration and N uptake in both soils (Table 3). The observed increases in maize tissue N concentration and N uptake following \textit{Nostoc} application mirrored observed improvements in soil N and mineral N content of the soils following inoculation with \textit{Nostoc} (Table 2).
**Discussion**

Effect of *Nostoc* inoculation on selected soil chemical properties

The inoculated *Nostoc* strain showed outstanding ability to colonize Guquala and Hertzog soils very quickly, irrespective of their physical and chemical characteristics. This good establishment of *Nostoc* in the two soils was responsible for the observed improvement in the soil C and soil N contents of these C and N poor soils (Table 2). This increase was presumably the result of the photosynthetic increment in both soil carbohydrate C (polysaccharides) and biomass C (Lange et al. 1992). Treatment performance, however, depended on soil type with the largest increases in both soil C and N occurring in Guquala soil possibly because it had much lower levels of these nutrients to start with (Table 2). The observed increases in soil N particularly in Guquala soil were comparable to those reported by Harper and Pendleton (1993) in a similar study. The observed trends in soil N were also similar to those reported by Skarpe and Henriksson (1987) and Buttars et al. (1998) even though their studies used the more accurate acetylene reduction method for assessing N-fixation.

A strong positive association of soil C and soil N \( R^2 = 0.838, \) Fig. 7 \) indicated close association of C and N dynamics in the inoculated and non-inoculated soils as also reported by Nisha et al. (2007). Generally, the increases in N due to inoculation were proportionately greater than those of C which resulted in a narrowing of C:N ratio (Table 2). In Guquala soil, for example, the C:N ratio dropped from 15.0 in non-inoculated soils to 11.4 when soil was inoculated with *Nostoc* which impacted positively on the mineralization of N (Table 2). These results clearly indicated that inoculation with *Nostoc* enriched the experimental soils with N as a result of N-fixation.

Effect of *Nostoc* inoculation on soil aggregate stability

The MWD is qualitatively related to aggregate strength and increases with increasing aggregate stability (Horn and Baumgart 2000). Le Bissonnais (1996) divided MWD into five classes and related them to soil aggregate stability as follows: <0.4mm is very unstable, 0.4–0.8mm is unstable, 0.8–1.3mm is partly stable, 1.3–2 is stable, and >2mm is very stable. The non-inoculated Guquala soil had aggregates with MWD of 0.84, 0.88, and 0.62mm as determined by the fast wetting, slow wetting, and wet stirring methods, respectively (Fig. 5). Corresponding values for Hertzog soil were 0.57, 0.92, and 0.63mm. These results indicated that both soils had low aggregate stability which according to Le Bissonnais (1996)
could be classified as unstable. This could be attributed to their high silt contents (Table 1). Inoculation of the soils with the *Nostoc* strain increased the proportion of macro aggregates (Fig. 4) and the proportion of big aggregates in both soils as reflected by their MWD values (Fig. 6). These results indicated improved resistance to breakdown following inoculation, especially with respect to slow wetting and mechanical breakdown (Fig. 5).

As reported in earlier studies on microbiotic soil crusts (Belnap and Gardner 1993; Malam Issa et al. 2001) or in soils inoculated with cyanobacteria (Malam Issa et al. 2007), the observed improvement in the aggregation of inoculated soils could be related to increases in soil C and EPS that caused changes in the micromorphological characteristics of the aggregates. Our results showed positive associations between the MWD and soil C ($R^2 = 0.485$) and EPS ($R^2 = 0.763$). The EPS was more strongly associated with MWD than soil C and indeed greater quantities of EPS were found in inoculated soils than in the non-inoculated ones (Table 2). Inoculated soils also exhibited EPS coatings and EPS bridges while non-inoculated samples had little or no EPS/filaments (Fig. 1a-f). These observations suggest that the aggregation and improvement in structural stability of the experimental soils was largely due to the enmeshing effect of the inoculated cyanobacterium filaments and the gluing effect of excreted polysaccharides. Improvement in aggregation could also have been aided by the hydrophobic properties that EPS impart on soil aggregates which retard the release of entrapped air (Kidron et al. 1999 as cited by Nisha et al. 2007) and thus breakdown of soil aggregates.
Nisha et al. (2007) showed that the EPS produced through biofertilization with cyanobacteria provided a substrate for the growth and enhanced activity of heterotrophic microflora which in turn produced more EPS, further amplifying its effect on soil structural stability. The effect of Nostoc on other soil microflora was not investigated in the present study but it is possible that the observed increases in EPS following

Table 3 Dry matter yield and nitrogen uptake of maize in response to inoculation with a Nostoc strain in Hertzog and Guquka soils

<table>
<thead>
<tr>
<th>Inoculation</th>
<th>Hertzog</th>
<th>Guquka</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry matter (g per pot)</td>
<td>Tissue N (%)</td>
</tr>
<tr>
<td>Inoculated</td>
<td>12.1±1.35*</td>
<td>0.53±0.06</td>
</tr>
<tr>
<td>Non-inoculated</td>
<td>8.1±0.40</td>
<td>0.43±0.03</td>
</tr>
</tbody>
</table>

Means in a column followed by the same letter are not significantly different at $P \leq 0.05$

*Standard error of means.
inoculation with cyanobacteria came from both the inoculated cyanobacteria and the heterotrophic microflora whose activity could have been enhanced by the EPS produced by the inoculated Nostoc.

The MWD values for Gaquka soil were lower than those reported by Malam Issa et al. (2007). This could be because the latter study was carried out under laboratory conditions where incubation conditions were more ideal than under the glasshouse conditions of the present study. The ideal laboratory incubation conditions could have ensured better cyanobacteria growth and consequently more soil C and EPS production, and thus greater impact on stability of aggregates.

The interactive effect of cyanobacteria inoculation and cropping on soil properties has hitherto received little attention. Our results showed that cropping with maize reduced the effectiveness of inoculation in improving aggregate stability of soils. This could have been partly due to the observed negative effect of cropping on Nostoc establishment and subsequent lower production of EPS. It could also be attributed to other causes such as those highlighted by Reid and Goss (1981) and Reid et al. (1982). They reported that the growth and activities of living roots may control the overall direction and magnitude of changes in the aggregate stability under arable and ley crops. Root growth of perennial ryegrass and lucerne was found to increase aggregate stability while growth of maize, tomato, and wheat roots decreased the stability of aggregates (Reid and Goss 1981). In a subsequent study, Reid et al. (1982) presented evidence suggesting that poor aggregate stability in soils cropped with maize could be due to the destruction of (organic matter)-(Fe or Al)-(mineral particle) linkages. The destruction of the linkages was said to occur when the Fe and Al cations that link organic matter to mineral particles are removed by chelating agents released by maize roots into the soil rhizosphere. This possibility suggests a need for further studies to investigate the interaction effects of cyanobacteria with different plant species on soil aggregate stability.

Effect of Nostoc inoculation on maize yield and nitrogen uptake

Inoculation with Nostoc resulted in improved maize growth in both Gaquka and Hertzog soils. The improved growth appeared related to the observed increases in soil N in the soils and favourable mineralization of the same in inoculated soils even though these parameters were only assessed in the top 5 mm of the potted soils. Soils that had higher dry matter had higher tissue N. These results were in agreement with those of Nisha et al. (2007) who also observed improvements in the growth of a pearl millet-wheat sequence in response to cyanobacteria biofertilization.

The observed increases in soil C and aggregate stability could have improved the water holding and infiltration capacities of the soils, and potentially the plant water use efficiency from the soils. However, water was not a limiting factor in the tunnel house experiment, so the observed improvement in maize dry matter yields could not be attributed to improved water use efficiency but rather to improved soil N...
levels. Such an effect could, however, translate to improved water retention and use efficiency under water scarce field conditions that often prevail in the semi-arid environment of the Eastern Cape, South Africa. Generally, our results indicate that cyanobacteria screened for EPS production and N-fixing ability could improve the productivity of nitrogen poor and physically degraded soils in the Eastern Cape Province of South Africa.

The cyanobacteria application rate of 6 g (dry weight) per square meter used in the present study was on the high side but considered necessary in order for the inoculated cyanobacteria to multiply and have impact in the short-term pot study. However, such a high rate of application can not be recommended for field scale application as the amount of inoculum required would be unrealistically high. Future studies should therefore investigate realistic application rates of the *Nostoc* strain for use under field conditions.

Conclusions

The *Nostoc* strain established well on the potted Guquka and Hertzog soils and improved the soil C, soil N and EPS contents of the two soils. The increases in soil N translated to improved maize growth and N uptake in both soils. The aggregate stability of the two soils was also improved as a result of increased production of EPS and soil C in the *Nostoc* inoculated soils. Cropping with maize reduced the effectiveness of inoculation due to its negative effect on *Nostoc* establishment and associated reduction in EPS and OM production, as well as possible destructive effect of maize roots on soil aggregates. Nevertheless, the results suggested that cyanobacteria screened for EPS production and N$_2$-fixing ability could improve the productivity of N poor soils and contribute to the amelioration of the structural stability of physically degraded soils in South Africa. These results and others suggest that by using cyanobacterial soil conditioners the yield and nutritional value of food crops could be enhanced without costly fertilizers. Therefore there is a need to develop low-cost techniques for screening and culturing of suitable cyanobacteria for use in South Africa and other countries where crop yields are marginal and fertilizers are cost-prohibitive.

Acknowledgement

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