REFORESTATION EFFECTS OF A FORMER SUGARCANE LAND ON SOIL FAUNA INVERTEBRATE COMMUNITY AND ENZYME ACTIVITY

By

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DECLARATION

I, Sibusisiwe Jenifer Mnembe declare that:

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Dr. P. Chivenge

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Prof. P.L. Mafongoya

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GENERAL ABSTRACT

Globally, there is pressure to reduce greenhouse gas emissions and reforestation or afforestation of degraded land could aid in mitigating these emissions and sequester carbon. The eThekwini Municipality in Durban, South Africa hosted the 2010 FIFA World Cup and the event generated approximately 307.21 tCO_2e. This study evaluated the effects of reforestation on soil fauna invertebrate community, enzyme activities and soil physico-chemical properties. Two land-use types were studied, chronosequence of land under reforestation (aged 2-, 4- and 6 –year-old forest stands) and sugarcane (representing degraded land which was used as the control). Sampling was carried out from two dominant soil types (Acrisols and Leptosols) in the study area. Soil fauna samples were collected in January 2016 (beginning of rainy season) and March 2016 (middle of rainy season) using 25x25x30 cm monoliths. Insects were hand sorted and identified to order level. Soil samples were collected from the 0-20 cm depth and analysed for enzyme activities and physico-chemical properties. Soil fauna abundance, Shannon diversity index and richness were affected by rainfall amount and soil type. Soil fauna abundance in the beginning of the rainy season (January) was 76% higher than in the middle of the rainy season. Hymenoptera and Isoptera were the most abundant groups of fauna in the study. Abundance of Isoptera was only higher at the oldest forest stand (6-year-old), suggesting that the land was in good repair. Reforestation resulted in higher activities of β-glucosaminidase, dehydrogenase and fluorescein diacetate hydrolase compared to sugarcane. Reforestation increased soil pH by ± 0.45 units in the 6-year-old forest stand compared to sugarcane. Decline in calcium and magnesium concentration, infiltration rate and aggregate stability and some enzyme activities were observed in the 2-year-old forest stand compared to sugarcane. Reforestation improved soil infiltration rate in both soils and aggregate stability especially in Leptosols and resulted in significant decreases in bulk density compared to sugarcane under Leptosols. The study concluded that, even though there were no significant differences in soil fauna abundance, diversity and richness; reforestation improved soil physical properties and enzyme activity.

Keywords: reforestation, enzyme activity, soil fauna, soil physical properties, soil chemical properties

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE: GENERAL INTRODUCTION

1.1 Background

Reforestation of degraded agricultural land is increasingly being promoted globally for sequestering carbon and mitigating greenhouse gas emissions, and improving ecosystem functioning (Vesterdal et al., 2002). The eThekwini Municipality in South Africa undertook a reforestation project to promote carbon sequestration and reduce greenhouse gas emissions to compensate for the increased emissions during the 2010 FIFA world cup. A total number of 673 021 indigenous tree species from tree-preneurs have been planted on the buffer zone of the Buffelsdraai landfill site, which had been under continuous sugarcane cultivation for more than 100 years. The site is characterized by steep slopes which makes it highly susceptible to erosion.

Like all vegetation, sugarcane provides cover but due to the general practice of burning prior to harvesting it results in reduced soil aggregate stability and organic matter (Dominy, 2000), thus increasing the erosivity of the soils (Dominy et al., 2001). In green cane residues protect the soil from erosion, whereas pre-harvest burning results in the accumulation of ash which is susceptible to erosion (Davies, 1998). While loss of fertile topsoil is considered irreversible and detrimental to the long-term productivity of a site, reforestation has been shown to reverse soil erosion and increase soil organic matter contents and enhance nutrient cycling (Cunningham et al., 2015). The permanent surface cover in forest soils reduces raindrop impact and thus reduces soil erosion compared to agricultural soils (Cunningham et al., 2015). Additionally, forest ecosystems have higher and continuous litter additions, leading to a development of a nutrient rich surface that provides energy for soil biota, giving rise to an increase in soil organic matter and nutrient cycling (Sauer et al., 2012). Unlike agricultural soils, forest ecosystems have higher soil quality due to reduced soil disturbance and compaction (Cunningham et al., 2015, Sauer et al., 2012). This creates favorable soil physical properties for soil biota, with greater aggregate stability and pore size distribution. Additionally, permanent soil cover in forest ecosystems reduces microclimate fluctuations thus creating conditions that are conducive for biological activity (Cunningham et al., 2015).

According to Oldfield et al. (2014) most reforestation projects soils are given minimal attention, yet tree growth is largely dependent on soil health for the provision of nutrients. Soil health

refers to the ability of the soil to fulfil the necessary function; it is characterized by biological, chemical; and physical properties (Anderson, 2003). According to Cardoso et al. (2013) soil biological indicators respond quickly to environmental change(s) than chemical and physical indicators. Soil biological indicators include macro fauna and microorganisms, such as bacteria and fungi. The continual burrowing and feeding activity of macrofauna results in reduced compaction, increased soil aeration, improved nutrient cycling and organic matter decomposition, and hence increase organic matter content in soils (Chen et al., 2013, Menta, 2012, Stork and Eggleton, 1992). A study on the role of soil organisms in nutrient cycling demonstrated that soil saphrophages accumulate elements such as calcium and potassium in their exoskeleton (Krivolutzky and Pokarzhevsky, 1977). Soil organisms are abundant in healthy soils; their abundance is affected by many factors including litter fall and its quality (Witt, 1997). The quality of litter fall has indirect effects on its decomposition rate, the chemical composition of litter itself may retard decomposition (Kooch et al., 2016).

Higher litter fall inputs usually result in more soil organic carbon (SOC), thus increasing the abundance and activity of soil organisms (Liu et al., 2012, Ndaw et al., 2009). Litter consists of nutrients and carbon which serve as energy source for microbial communities; this promotes their activity in the soil (Mukhopadhyay and Joy, 2010). Soil microbial biomass carbon (SMBC) contains approximately 1 to 5 % of SOC, which is labile and responds quickly to environmental change(s) compared to SOC (Xiaojun et al., 2013). As a result, SMBC is used as an early indicator for SOC change. According to Trumbore (1997) the turnover rate of SOC ranges from 14 to 400 years, depending on the ecosystem where a study was carried. Raich and Schlesinger (1992) estimated the mean global turnover time for SOC as 32 years, by dividing total carbon stocks by the average carbon dioxide flux from the soil.

In South Africa, there have been studies on land use change and management, exploring effects of commercial afforestation on bird species and native vegetation. Armstrong and Van Hensbergen (1995) assessed the effects of afforestation and clear-felling of pine plantations on birds and small mammals at Grootvadersbosch. Allan et al. (1997) studied the effects of grassland afforestation with alien plants on avifauna in Mpumalanga province. Mugwedi et al. (2017) assessed the success of Buffelsdraai reforestation project, by measuring vegetation structure, tree species diversity and richness, invasive alien species and ecological processes. In general, South Africa lacks information regarding soil microbial characteristics and fauna in afforestation systems. Since microorganisms and soil macrofauna are sensitive to management practices, quantifying them may serve as an early indicator land use change may have on soil.

This information may assist the eThekwini Municipality management, since it contributes in monitoring the progress of the reforestation project.

1.2 Objectives and hypothesis

The main goal of this research was to assess the effects of reforestation on soil microbial characteristics and fauna. The main objectives of the study were (a) to evaluate changes on soil fauna abundance, diversity and richness and (b) to assess changes in soil enzyme activity after reforestation of former sugarcane land. It was hypothesized that soil faunal community composition and enzyme activity would increase with reforestation age. As a result of food and substrate availability for fauna and microbes provided by litter from trees; changes in soil fauna community composition and microbial content should be expected as time progresses on reforested sites. In this research, the effects of land use change on soil biological functioning were evaluated.

The thesis has 5 chapters; chapter one is the general introduction, the second chapter is the literature review of soil physical properties, macrofauna and microbial characteristics of soils and changes that occur due to reforestation .Chapter three is an evaluation of soil invertebrate community and chemical properties under different forest stands (aged 2-, 4- and 6-years-old) compared to sugarcane, which serves as a control. Chapter four is an assessment of reforestation effects on soil enzymatic activities and physical properties under the above-mentioned land uses. Chapter five is the general discussion, conclusion and recommendations.

References

ALLAN, D. G., HARRISON, J. A., NAVARRO, R., VAN WILGEN, B. W. & THOMPSON, M. W. 1997. The impact of commercial afforestation on bird populations in Mpumalanga Province, South Africa—insights from bird-atlas data. *Biological Conservation,* 79**,** 173-185.

ANDERSON, T. 2003. Microbial eco-physical indicators to assess soil quality. *Agriculture, Ecosystems and Environment,* 98**,** 285-293.

ARMSTRONG, A. & VAN HENSBERGEN, H. 1995. Effects of afforestation and clearfelling on birds and small mammals at Grootvadersbosch, South Africa. *South African Forestry Journal,* 174**,** 17-21.

CARDOSO, E. J. B. N., VASCONCELLOS, R. L. F., BINI, D., MIYAUCHI, M. Y. H., SANTOS, C. A. D., ALVES, P. R. L., PAULA, A. M. D., NAKATANI, A. S., PEREIRA, J. D. M. & NOGUEIRA, M. A. 2013. Soil health: looking for suitable indicators. What should be considered to assess the effects of use and management on soil health? *Scientia Agricola,* 70**,** 274-289.

CHEN, F., ZHENG, H., ZHANG, K., OUYANG, Z., LAN, J., LI, H. & SHI, Q. 2013. Changes in soil microbial community structure and metabolic activity following conversion from native Pinus massoniana plantations to exotic Eucalyptus plantations. *Forest Ecology and Management,* 291**,** 65-72.

CUNNINGHAM, S. C., MAC NALLY, R., BAKER, P. J., CAVAGNARO, T. R., BERINGER, J., THOMSON, J. R. & THOMPSON, R. M. 2015. Balancing the environmental benefits of reforestation in agricultural regions. *Perspectives in Plant Ecology, Evolution and Systematics,* 17**,** 301-317.

DAVIES, J. 1998. The causes and consequences of cane burning in Fiji's sugar belt. *The Journal of Pacific Studies,* 22**,** 5-12.

DOMINY, C., HAYNES, R. & VAN ANTWERPEN, R. 2001. Long-term effects of sugarcane production on soil quality in the South Coast and Midlands areas of KwaZulu-Natal. Proc S Afr Sug Technol Ass, Citeseer, 222-227.

KOOCH, Y., ROSTAYEE, F. & HOSSEINI, S. M. 2016. Effects of tree species on topsoil properties and nitrogen cycling in natural forest and tree plantations of northern Iran. *CATENA,* 144**,** 65-73.

KRIVOLUTZKY, D. & POKARZHEVSKY, A. 1977. The role of soil animals in nutrient cycling in forest and steppe. *Ecological Bulletins***,** 253-260.

LIU, Y., WEI, X., GUO, X., NIU, D., ZHANG, J., GONG, X. & JIANG, Y. 2012. The long-term effects of reforestation on soil microbial biomass carbon in sub-tropic severe red soil degradation areas. *Forest ecology and management,* 285**,** 77-84.

MENTA, C. 2012. *Soil fauna diversity-function, soil degradation, biological indices, soil restoration*, INTECH Open Access Publisher.

MUGWEDI, L. F., ROUGET, M., EGOH, B., RAMDHANI, S., SLOTOW, R. & RENTERÍA, J. L. 2017. An Assessment of a Community-Based, Forest Restoration Programme in Durban (eThekwini), South Africa. *Forests,* 8**,** 255.

MUKHOPADHYAY , S. & JOY, V. C. 2010. Influence of leaf litter types on microbial functions and nutrient status of soil: ecological suitability of forest trees for afforestation in tropical laterite wastelands. *Soil Biology and Biochemistry,* 42**,** 2306-2315.

NDAW, S., GAMA-RODRIGUES, A., GAMA-RODRIGUES, E., SALES, K. & ROSADO, A. 2009. Relationships between bacterial diversity, microbial biomass, and litter quality in soils under different plant covers in northern Rio de Janeiro State, Brazil. *Canadian journal of microbiology,* 55**,** 1089-1095.

OLDFIELD, E. E., FELSON, A. J., WOOD, S. A., HALLETT, R. A., STRICKLAND, M. S. & BRADFORD, M. A. 2014. Positive effects of afforestation efforts on the health of urban soils. *Forest Ecology and Management,* 313**,** 266-273.

RAICH, J. & SCHLESINGER, W. H. 1992. The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. *Tellus B,* 44**,** 81-99.

SAUER, T. J., JAMES, D. E., CAMBARDELLA, C. A. & HERNANDEZ-RAMIREZ, G. 2012. Soil properties following reforestation or afforestation of marginal cropland. *Plant and soil,* 360**,** 375-390.

STORK, N. E. & EGGLETON, P. 1992. Invertebrates as determinants and indicators of soil quality. *American journal of alternative agriculture,* 7**,** 38-47.

TRUMBORE, S. E. 1997. Potential responses of soil organic carbon to global environmental change. *Proc. Natl. Acad. Sci. USA,* 94**,** 8284-8291.

VESTERDAL, L., RITTER, E. AND GUNDERSEN, P. 2002. Change in soil organic carbon following afforestation of former arable land. *Forest ecology and management, 169*(1-2),137-147.

WITT, B. 1997. Using soil fauna to improve soil health. *Student On-Line Journal,* 2**,** 1-5.

XIAOJUN, N., JIANHUI, Z. & ZHENGAN, S. 2013. Dynamics of soil organic carbon and microbial biomass carbon in relation to water erosion and tillage erosion. *PloS one*, 8**,** e64059.

CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

Land-use change is a process whereby human activities modify a certain land cover type for purposes such as agriculture and forestry (Noble et al., 2000). Land-use change comes at a price; for example conversion of forest to agriculture is associated with soil quality reduction and increased soil erosion (Siqueira et al., 2014, Wu, 2008). It also has negative effects on ecosystem processes and services such as trapping gases, more particularly CO_2 thus affecting greenhouse gas emissions at a global scale (Wu, 2008). Recently there has been an increasing trend globally of reforestation driven by increased stress of climate change and variability (Cunningham et al., 2015). Afforestation aids in mitigating climate change, conserving biodiversity and improving soil quality (Adams and Fiedler, 2015, Elmarsdottir et al., 2008, Lydiate and Helm, 2014).However, afforestation is associated with loss of land for food production and might lead to increased food prices due to competition for land between forestry and food production (Kreidenweis et al., 2016). The Intergovernmental Panel on Climate Change defined afforestation as the establishment of forest on land that has not been forested for approximately 20 to 50 years or more and was previously under a different land-use type (Noble et al., 2000). Afforestation of agricultural soils comes with many benefits, including soil aggregate stability improvement, reduction of bulk density since disturbance from planting and harvesting is reduced (Cunningham et al., 2015).

According to Rosario et al. (2014) forest systems are associated with the accumulation of organic debris, these residues offer a favourable habitat for soil fauna and microbes and also increases their diversity in soil. The canopy cover provided by trees is advantageous to soil biota. Due to shading, variability in soil moisture and temperature is reduced thus providing soil biota with an environment that enhances their activity and favours their growth and development (Adams and Fiedler, 2015, Cunningham et al., 2015). Research has shown that fungal diversity increases due to increased organic material associated with afforestation. Afforestation with birch (*Betula pendula* Roth) on former arable soils after 30 years in central Finland showed that fungal communities changed when compared to original site (i.e. arable soil) (McLean and Huhta, 2002). Fungal communities were more similar to old deciduous forest due to improved litter quality , increased organic matter and earthworms (they disperse fungal spores in soil) (McLean and Huhta, 2002). Both soil fauna and microbes are important

in the ecosystem for functions that are associated with their activities in the soil which include litter decomposition, nutrient cycling, aggregate stability, carbon sequestration and breakdown of toxic compounds (Bot and Benites, 2005, Islam and Wright, 2003). However, little is known regarding the effect of afforestation on soil fauna and microbes in South Africa. This review summarizes the role of soil fauna and microbes in the soil environment and the effects of afforestation on soil fauna and microbes.

2.2 Soil physical properties

The capability of soil for agricultural and environmental uses is regulated by physical properties, since they control factors such as nutrient availability and water flow (Phogat et al., 2015). Soil physical properties include soil structure, texture, porosity, infiltration and bulk density (Cardoso et al., 2013, Phogat et al., 2015). According to Cardoso et al. (2013), soil is said to be physically poor when it has low infiltration rate, aeration and root density and enhanced surface runoff. Dominy et al. (2001) reported that long term cultivation of sugarcane has negative impacts on soil physical properties. In general, soil under sugarcane production have low soil organic matter status due to removal and burning of crop residues (Dominy et al., 2001). This practice enhances soil structural breakdown, as a result soil aggregate stability decreases. Amongst physical properties, soil structure influences other physical properties such as bulk density since soil structure affects pore size distribution in soil. Soil structure is considered as a good indicator of soil quality (Podrazsky et al., 2015, Vopravil et al., 2014). Soil structure is the arrangement of soil particles into aggregate, which can be measured by mean weight diameter and water stable aggregates (Podrazsky et al., 2015).

Research has shown that reforestation is associated with formation of stable soil aggregates and reduction of bulk density (Podrazsky et al., 2015, Vopravil et al., 2014). This may be due to increased soil organic matter inputs in the soil. Organic matter improves soil aggregation through production of cementing agents that help bind the soil particles (Cardoso et al., 2013). Formation of stable soil aggregates is important in restoring degraded land, consequently soil quality increases. Abunyewa et al. (2015) and Mapa (1995) have reported that reforestation improves soil infiltration. The formation of stable soil aggregates due to more organic matter in reforested areas creates more macro-pores, as a result soil infiltration rate increases.

2.3 Soil fauna

2.3.1 Soil environment and soil fauna

The soil environment has organic matter, nutrients and other minerals and microclimate conditions are highly buffered in soil (Lavelle and Spain, 2005). Thus, it supports fauna through the provision of habitats and food, in turn the fauna decomposes litter in the soil surface thus improving soil fertility (Balvanera et al., 2015, Lavelle and Spain, 2005). Soil fauna plays various roles in the ecosystem, and based on their ecosystem functions they are grouped as decomposers, nutrient cyclers, ecosystem engineers (they modify soil physical properties due to their activity) and bio-controllers (Kibblewhite et al., 2008). However, these organisms are negatively affected by human practices such as intensive tillage practices, pesticide application and conversion of native vegetation. Thus it is important to manage land in a manner that will maximise ecological benefits provided by soil organisms (Ayuke et al., 2009). Soil fauna are divided into 3 groups based on their body size: microfauna (<0.2 mm, includes nematodes), mesofauna (>0.2mm, includes pot worms) and macrofauna (2-10mm, includes millipedes) (Bot, 2005; Lavelle, 2005). According to (Bot and Benites, 2005) characteristics of soil organisms vary, some spend their whole lifecycle in the soil (e.g. earthworms) whereas others only inhabit the soil during early stage development (e.g. larvae). Out of the three fauna groups, the emphasis of this review will be on soil macrofauna since they were used as biological indicators for soil health in this study.

Macrofauna are invertebrates that are visible to the naked eye and they include ants, beetles, centipedes, earthworms and termites (Bot and Benites, 2005) . The most abundant groups of invertebrate fauna are ants, earthworms and termites and they have been termed ecosystem engineers due their activities that alter the soil structure (Lavelle and Spain, 2005). The activity of these invertebrates is influenced by factors such as moisture content, pH and temperature. In general soil moisture, can limit the activity and distribution of fauna, groups such as earthworms and isopods require moist environments, with water stress their activity is reduced and it can lead to mortality (Lavelle and Spain, 2005). A study by Collison et al. (2013) showed that macrofauna survived under low soil moisture conditions (gravimetric moisture content of 10 – 30 %), but a decline in decomposition rate of litter was observed due to declined activity associated with low moisture content in soil. For organisms to survive in soils with low moisture contents they developed strategies (such as descending to below 30 cm depth in the soil) to protect themselves from desiccation. A study by Collison et al. (2013) also showed that millipedes (*Glomeris marginata*- Villers) were found occupying deep soil layer in soil with

low soil moisture content compared to soil with high moisture content. This behaviour was a strategy to avoid dehydration, soil organisms migrate down the soil when moisture is reduced. Soil pH is another important factor for the survival of fauna in the soil. Response of soil fauna to acidity varies with groups. Termites are more abundant at pH 4-6, whereas beetles and earthworms are mostly abundant at high soil pH (Lavelle et al., 1995). Thus, the overall activity and composition of macrofauna is affected by soil pH.

2.3.2 The role of macrofauna in soil

Soil macrofauna provides various benefits, including decomposition and modification of soil structure (Barrios, 2007).

(a) Ants

Ants happen in more prominent numbers in the soil environment, however little attention has been paid to their effects on soil properties when compared to earthworms and termites due to their feeding habits (Bot and Benites, 2005, Frouz and Jilková, 2008). Unlike other ants that feed on soil arthropods, leaf cutting ants (*Tribe Attini*) from America have crucial ecosystem engineering functions in the soil (Lavelle and Spain, 2005). Ants harvest plant material and store it in their nests ,as a result organic matter and nutrients are incorporated in the soil (Frouz and Jilková, 2008, Lavelle and Spain, 2005). Thus, enhancing water holding capacity nutrient cycling and improvement of soil structure, and this is beneficial for microbes and plants in the soil. Activity of ants may alter soil pH. Studies have reported that ants may increase pH in acidic soils and the opposite is true on alkaline soils (Frouz and Jilková, 2008). Ant nests have higher cation and nutrient content than adjacent soil due to food residues and excreta which may increase base cations in the soil, thus resulting in increased soil pH (Frouz and Jilková, 2008, Jilkova et al., 2010). On the other hand, the accumulation of organic matter may result in decreased pH, through production of organic acids during decomposition (Frouz and Jilková, 2008). According to Vasconcellos et al. (2013), ants are usually associated with recovering environments. Rosario et al. (2014) studied the effects of reforestation with *Schizolobium parahyba* Barneby in Amazonia at different reforestation ages (i.e. 2, 3, 4 and 5-year-old) in dry and rainy seasons. The findings showed that ants were the most abundant group compared with beetles, termites and other faunal groups; they represented 92.02 % of insects collected in the dry season (Rosario et al., 2014). Due to their high abundance and sensitivity to environmental change, they are commonly used as bioindicators in forestry impact

assessments, land management and in environmental monitoring work (Pacheco et al., 2009, Ribas et al., 2011). Using leaf-litter ants as bioindicators, Pacheco et al. (2009) found that secondary forest (30-year-old) had more leaf-litter ant species (82 species) compared to the 30-year-old *Pinus allioti* afforestation (60 species). The secondary forest had higher tree species richness compared to *Pinus allioti* plantations. As a result, ants had more availability of food and nesting sites and relatively low microclimate fluctuation thus supporting more ants (Pacheco et al., 2009).

(b) Beetles

Beetles are very diverse in soil, most of them occupy the surface litter and can either be phytophagous, predators or saphrophagus (Bot and Benites, 2005). Phytophagous beetles feed on plants including shrubs and trees, whereas predators prey on other insects (Coleman et al., 2004). Saphrophagus beetles serve as decomposers, they feed on decaying organic matter (Coleman et al., 2004). In forest systems ,the saphrophagus Bess beetles are responsible for decomposing wood, thus important in forest nutrient cycling (Kattan et al., 2010). One of the most important beetles are dung beetles from family *Scarabeidae* which play an important role in savannahs and grasslands mainly used for grazing in Africa (Bot and Benites, 2005, Lavelle and Spain, 2005) . They dig galleries which can go down to 70 cm in the soil, by burrowing in the soil they lead to reduced bulk density, increased porosity, aeration, infiltration, thus reduced run-off (Forgie et al., 2013) . Beetles are sensitive indicators of environmental quality. They are influenced by microclimatic conditions and vegetation cover, thus useful in assessing recovery of an environment after disturbance (Luo et al., 2013, Rosario et al., 2014, Ueda et al., 2015). In reforestation studies by Vasconcellos et al. (2013) beetles were important for differentiating the undisturbed site from reforested sites (aged 5, 10 and 20-years-old). The abundance of beetles also separated the 20-year-old site from the other reforested sites, showing that the site is in good repair (Vasconcellos et al., 2013).

(c) Earthworms

In the soil earthworms are important for decomposition and SOM dynamics; they are grouped into 3 categories (i.e. epigeic, endogeic and anecic) (Bhadauria and Saxena, 2010, Bot and Benites, 2005). Epigeic worms occupy and feed on litter, whereas endogeic occupy and feed on the soil and anecic feed on surface litter and live in the soil (Bhadauria and Saxena, 2010, Bot and Benites, 2005, Cardoso et al., 2013). As earthworms ingest the soil and plant material

they produce casts rich in nutrients and have binding agents (i.e. earthworm mucilage and mucus) which aid in binding soil particles thus forming water stable soil macro aggregates (Bhadauria and Saxena, 2010, Cardoso et al., 2013). According to Kumar et al. (2013), within the macro-aggregates microbial activity is triggered due to high organic matter content. The plant material that was incorporated during macro aggregate formation is further decomposed. The further decomposed material becomes enclosed with mineral and microbial products, creating micro-aggregates within macro-aggregates (Kumar et al., 2013). With time, macro-aggregates may breakdown as production of microbial binding agents lessen (Kumar et al., 2013). However, micro-aggregates remain stable, thus stabilizing carbon in the soil (Bhadauria and Saxena, 2010, Bossuyt et al., 2005, Kumar et al., 2013). Earthworm casts serves as a source of nutrients. Fresh casts have high contents of calcium, potassium, nitrogen and phosphorus which are obtained from earthworm urine and mucus and are readily available to plants and enhance microbial activity in the soil (Bhadauria and Saxena, 2010, Witt, 1997).

Earthworms are the most important detrivores in forest soils (Kooch and Jalilvand, 2008). However, acidification of forest soils may decrease earthworm activity, thus retarding nutrient cycling since most earthworm species do not tolerate very acidic soils (Hlava and Kopecký, 2013). Due to their important role in soil, sensitivity to soil properties and vegetation; earthworms have been used as soil quality indicators (Sigurdsson and Gudleifsson, 2013). In Hawaii, earthworms were used as biological indicators for soil quality after reforestation of abandoned sugarcane fields (Zou and Bashkin, 1998). Eucalypt plantations (aged 0, 0.5, 2, 4 and 10 years-old) were compared with secondary plant communities. Sugarcane had no earthworms and recently after establishment of eucalypt plantations earthworm density increased indicating that the environment is in good repair (Table 2.1). However, there were no anecic and epigeic worms in sugarcane and plantations of all ages, suggesting that they only appeared in secondary plant communities. The absence of anecic and epigeic worms shows that the chemical composition of litter is important on worms (Zou and Bashkin, 1998). Since eucalypt tree produces litter with high phenols, it was not edible for anecic and epigeic worms (Zou and Bashkin, 1998). This shows that the effectiveness of earthworms as biological indicators for reforestation studies varies with earthworm species since they respond differently to litter of trees used.

Table 2.1: Earthworm density and fresh weight in abandoned sugarcane fields, eucalypt plantations (aged 0.5, 2, 4 and 10 years) and secondary plant communities (aged 1 and 4 years , containing sugarcane, grass and legume) in Hawaii (Zou and Bashkin, 1998)

	Endogeic worms		Anecic–epigeic worms	
Age since abandonment	Density (number m^{-2})	Fresh weight (g m^{-2})	Density (number m^{-2})	Fresh weight (g m^{-2})
Sugarcane field				
0	0 a (0)	0 a (0)	0 a (0)	0 a (0)
Tree plantation				
0.5	12 a (12)	4.2 a (4.4)	0 a (0)	0 a (0)
2	151 a (8)	48 a (7)	0 a (0)	0 a (0)
4	154 a (29)	53 a (12)	0 a (0)	0 a (0)
10	398 b (92)	136 b (29)	0 a (0)	0 a (0)
Secondary community				
1	140 a (50)	34 a (9)	33 b (8)	12 a (6)
4	126 a (50)	38 a (13)	21 b (10)	8 a (3)

Note: common letters within a column indicate no significant difference (Scheffe's multi-range test, α- 0.10) among treatments. Numbers in brackets indicate standard error.

(d) Termites

Termites are social insects (they live in colonies) and most of them are soil eaters (Lavelle and Spain, 2005). Termites build mounds by collecting soil particles from various depths and the material is deposited in mounds, thus altering texture, increasing C, base cations and pH when compared to surround soil (Asawalam et al., 1999, Kaschuk et al., 2010) . In acidic soils where termites occur in substantial numbers, if mounds could be incorporated with the adjacent soil or of the rain erodes mounds and distributes nutrients on nutrient poor surface, both physical and chemical properties could be enhanced (Asawalam et al., 1999, Kaschuk et al., 2010). According to Schaefer (2001), due to the repeated biological activity of termites the soil could have been re-deposited to the galleries dug by these organisms after rainfall, thus they are associated with the formation of Latosols in the tropics which can reach depths of 30 m or more. A couple of studies using termites as a subject in degraded land have shown that these organisms are good bioindicators for habitat quality (Bhavana et al., 2015, Pribadi et al., 2011). However, termites are not widely use as bioindicators in restoration studies compared to ants (de Paula et al., 2016). According to Harris (1966) afforestation with eucalyptus in less humid areas in Africa was negatively affected by termites, since they fed on seedlings . However, a study by Debelo and Degaga (2017) in Ethiopia showed that termites caused minimal damage

on tree seedlings. Termites were used as a subject of study in Sao Nicolau farm, Brazil. Sites reforested (both 10-years-old) with exotic teak trees (*Tectona grandis*) and native plantations of fig trees (*Ficus sp*) were compared with three control sites (primary forest, secondary forest and active pasture) (de Paula et al., 2016). Species abundance did not vary between primary forest and both reforested sites (Fig. 2.1,) showing recovery of termites (de Paula et al., 2016). Overall species richness of termites in native plantations was more similar to the primary forest (Fig. 2.1) (de Paula et al., 2016). The study also showed that the abundance and diversity of humivorous termites was higher in reforested area compared with pasture or secondary forest (de Paula et al., 2016). Humivorous termites feed on highly decomposed organic matter mixed with soil and they are important for availability of nutrients to plants and microbial biomass (de Paula et al., 2016). The presence of humivorous termites will enhance the restoration of the reforested site and influence the reforested site to provide ecosystem functions that are almost similar to that of primary forest (de Paula et al., 2016).

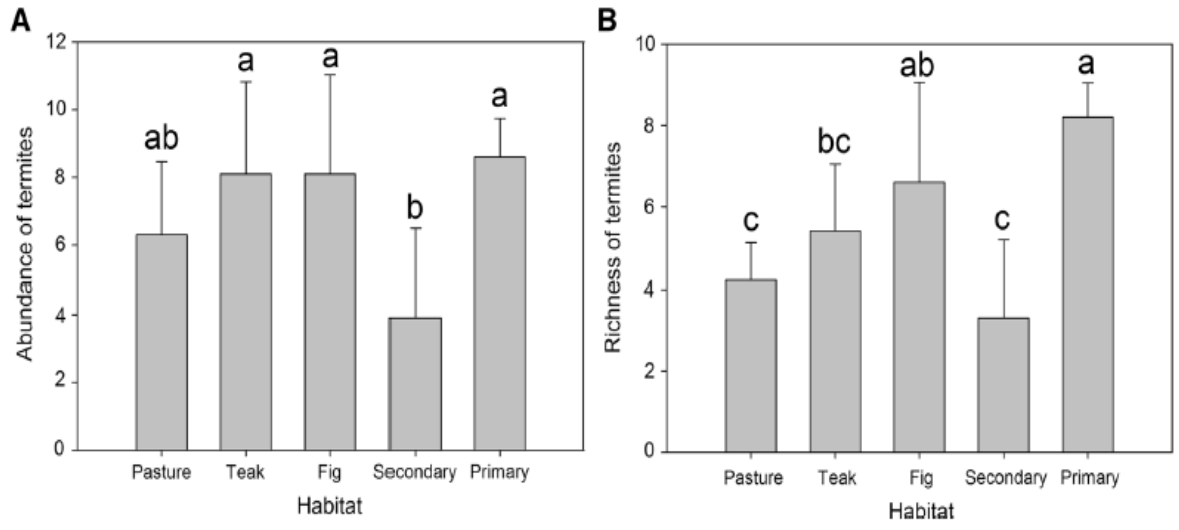

Figure 2.1: The mean abundance (A) and species richness (B) of termites in pasture, teak , fig reforestation and secondary and primary forests of Sao Nicolau farm, Brazil (de Paula et al., 2016). Error bars show standard error and different lower-case letters above bars show statistical difference.

2.4 Soil microbial activity

Nannipieri et al. (1990) defined microbial activity as "a general term that includes all the metabolic reactions and interactions conducted by the microflora and macrofauna in the soil".

Microbial activity in the soil is important for nutrient cycling and decomposition of OM. When examining soil microbial activity, soil respiration and enzyme activities are good indicators (Vinhal-Freitas et al., 2010) . In this review enzyme activities in the soil are discussed.

2.4.1 Soil enzymes and their activities

Soil enzymes are proteins that microorganisms use to catalyse essential biochemical reactions in the soil (Bakshi and Varma, 2011). Enzymes are produced by microorganisms, plant residues and roots and soil animals. They are categorized as intracellular and extracellular (Gianfreda and Rao, 2014, Lavelle and Spain, 2005) . Majority of soil enzymes are intracellular, meaning that they function within living cells which they were produced from (Gianfreda and Rao, 2014). Intracellular enzymes include dehydrogenase. Extracellular enzymes are produced by living cells as well, however they are secreted externally, such as in gram positive bacteria which produces extracellular enzymes for external digestion (Lavelle and Spain, 2005). Extracellular enzymes include β-glucosidase. When these enzymes are released in the soil they are immobilized clay colloids, organic and inorganic constituents through adsorption, cross-linking, polymerization and other mechanisms (Knight and Dick, 2004, Lavelle and Spain, 2005, Tietjen and Wetzel, 2003). Thus, resulting in decreased activity and stabilization of their structure and can be used to detect management effects on soil (Das and Varma, 2011) . Soil enzymes are important for decomposition of organic waste, N fixation, detoxification of xenobiotics and stabilization of structure (Bakshi and Varma, 2011, Mukumbareza et al., 2015) . Due to their relationship with soil biology, they respond rapidly to land use change and management, thus their activities are used as biological indicators for soil quality (Mukumbareza et al., 2015, Rajper et al., 2016, Utobo and Tewari, 2015). Soil enzyme activities vary with soil type due to differences in their properties, they are also affected and controlled by factors such as agricultural management, organic matter properties and content, moisture and temperature (Das and Varma, 2011, Gianfreda and Rao, 2014). A study by Borowik and Wyszokowska (2016) showed that soil moisture has an influence on enzyme activity. The activity was high at moisture content of 20% MWC (maximum water capacity) compared to 40 and 60% MWC suggesting that the 20% MWC was more suitable for growth and development of microbes, thus leading to increased microbial activity.

Studies have also shown that microbial activity can be affected by SOC content, which is related to clay type since it influences sorption capacity of SOC (Borowik and Wyszkowska, 2016, Merino et al., 2016, Schnecker et al., 2014). Soil with high organic carbon content had

the highest enzymatic activity compared to soils with lower organic carbon content (Borowik and Wyszkowska, 2016) . Microbes use organic carbon as a source of energy, thus reduction in availability of organic carbon may result in decreased microbial activity. Measurement of soil enzyme activities aid in assessing degree of pollution in the soil, assessing successional stages of an ecosystem and determining soil microbial activity which is important for soil fertility (Das and Varma, 2011) .

2.4.2 Enzymes and their roles in soil

Enzymes released in the soil solution play 3 main roles (1) modification of the environment for the survival of microorganisms, (2) detoxification of the surrounding environment and (3) hydrolysis of insoluble substrates (Dick, 1997). Soil enzymes are useful in assessing impacts of land use and management on soil fertility. (Srivastava (2010)) noted a relationship between enzymatic activity, nutrient status and forestry. The results of the study showed that replacement of oak forest by pine has negative effects on microbial activity and this was associated with reduced soil fertility. Some enzyme activities and their potential roles are described below, namely dehydrogenase, FDA, β-glucosaminidase and β-glucosidase.

(a) Dehydrogenase

Dehydrogenase is an intracellular enzyme responsible for oxidising soil organic matter through electron transfer reactions (Das and Varma, 2011, Wolińska and Stępniewska, 2012). Dehydrogenase affects respiration pathways of micro-organisms, thus indicating the oxidative activity of the soil microbial biomass (Pereira et al., 2013, Velmourougane et al., 2013, Wolińska and Stępniewska, 2012). Dehydrogenase is sensitive and responds quickly to land use change and management, as a result it is used as an indicator for soil quality (Wolińska and Stępniewska, 2012). According to Gonnety et al. (2012) the use of enzyme activity for soil quality monitoring studies on forests in Africa is rare. However, several studies in Brazil have shown that dehydrogenase is a good indicator for monitoring soil quality in reforested areas (Bini et al., 2013, da Silva et al., 2012, Pereira et al., 2013). Dehydrogenase was used as an indicator of the recovery of regenerated forests (aged 10 and 20-years-old), which were previously under sugarcane production (da Silva et al., 2012). Regenerated forests were compared with conventional tillage plantation and native forest in dry and wet season. Dehydrogenase activity was higher in the 20-year-old forest, followed by 10-year-old forest, native forest and sugarcane plantation (native forest and sugarcane plantation were not

significantly different) in wet season (Fig. 2.2) (da Silva et al., 2012). Dehydrogenase activity in summer indicates that regenerated forests are in good recovery. In the dry season, there were no differences among sites (Fig. 2.2). This study showed that dehydrogenase activity is affected by moisture. According to Wolińska and Stępniewska (2012) water availability is essential for survival and activity of microbes. With increased moisture, microbial activity is enhanced through increased intracellular water potential (Wolińska and Stępniewska, 2012). Thus, increasing enzymatic activity; this could explain the increased activity of dehydrogenase in wet season.

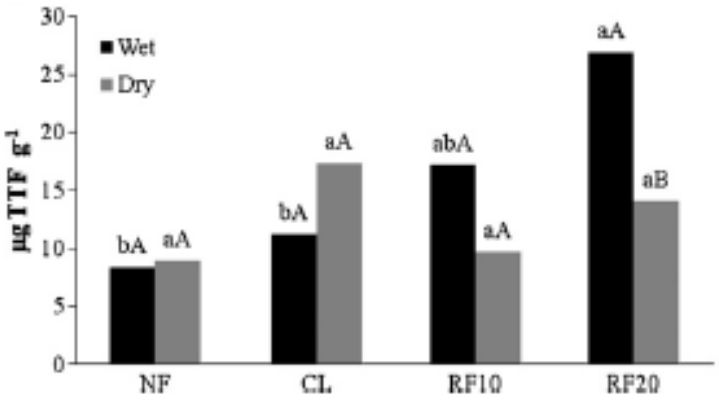

Figure 2.2: Dehydrogenase activity in sites under native forest (NF), conventional sugarcane plantation (CL) and regenerated forests (aged 10: RF10 and 20-year-old: RF20) in wet and dry season at Igarassu city, Brazil (da Silva et al., 2012). Different lower-case and upper-case letters above bars show statistical difference.

(b) β-glucosaminidase

β-glucosaminidase is the enzyme that drives the hydrolysis of glucosamine residues from the terminal non-reducing ends of chitooligosacharrides (Ekenler, 2002, Ekenler and Tabatabai, 2002). The international Union of Biochemistry grouped this enzyme as β-hexosaminidase since it splits the amino acid galactosamine (Ekenler, 2002, Ekenler and Tabatabai, 2002). This enzyme hydrolyses glycosidic bonds in chitin, which is a major structural component in insects and fungal cell wall (Acosta-Martinez et al., 2007, Parham and Deng, 2000). As a result of the hydrolysis, chitin is converted to amino sugars in soil which comprises of 5-10% of the organic nitrogen in the surface of almost all soils (Acosta-Martinez et al., 2007, Ekenler and Tabatabai, 2002) . The hydrolysis is important in the cycling of both carbon (C) and nitrogen (N) in the

soil since the production of amino sugars enhances the availability of N and also serves as a source of easily mineralizable C in humid soils (Acosta-Martinez et al., 2007, Rajper et al., 2016) .

β-glucosaminidase activity in soil may reflect long term changes in N availability (Parham and Deng, 2000). β-glucosaminidase activity is sensitive to land use and management practices such as tillage, crop rotation and residue management, thus it could be used as a soil quality indicator and can also be used as an index of nitrogen mineralization in soils (Ekenler and Tabatabai, 2002, Rajper et al., 2016). β-glucosaminidase activity was used as an indicator to investigate soluble organic nitrogen pools in natural secondary forests and larch plantations (34- 45 years-old) (Yang et al., 2012). Results showed that secondary forests had more β-glucosaminidase activity than larch plantations (Yang et al., 2012). β-glucosaminidase activity correlated positively with soluble organic nitrogen. Differences in β-glucosaminidase activity among the two forests could have been affected by quality and quantity of litter to the soil , which affects soluble organic nitrogen (Yang et al., 2012).

(c) β-glucosidase

In general, β-glucosidases are widely-distributed enzyme in the soil (Das and Varma, 2011, Makoi and Ndakidemi, 2008, Martinez-Salgado et al., 2010) environment. They are responsible for the hydrolysis of celluboise in soil and the final product of the hydrolysis is glucose. Glucose serves as an important C energy source for microorganisms, thus affecting C cycle (Das and Varma, 2011, Makoi and Ndakidemi, 2008, Martinez-Salgado et al., 2010). In the soil solution, these enzymes can either be degraded rapidly or stabilized, due to stabilization these enzymes may reflect past biological activity; thus showing effects of past management strategies on soil quality (Das and Varma, 2011, Rajper et al., 2016, Utobo and Tewari, 2015); . β-glucosidases are sensitive to changes soil pH, metal toxicity and management, thus they are used as biochemical indicators for detecting ecological changes (Das and Varma, 2011, Makoi and Ndakidemi, 2008). According to Makoi and Ndakidemi (2008), measuring the activity of these enzymes aids in detecting changes in OC turnover in a shorter period (1-3years) before routine measurements of OC could be applied. β-glucosidases were used to investigate the effects of *Eucalyptus* plantation in the soil. β-glucosidase activity was compared between *Eucalyptus* plantations (aged 2. 3 and 5-years-old) and native forest (Lino et al., 2016). The 2-year-old plantation had the highest β-glucosidase activity followed by 5-year-old, 3-year-old and native forest (Fig. 2.3) (Lino et al., 2016). Higher β-glucosidase activity in the 2-year-old

plantation was associated with rapid decomposition of plant residues, thus providing microbes with substrate which enhances enzyme activity (Lino et al., 2016). The results of the study by Lino et al. (2016) were in correspondence with Singh et al. (2012). Singh et al. (2012) found significant difference between β-glucosidase activity in degraded land, reforested land and rehabilitated cropland. The activity was ordered as reforested land > rehabilitated cropland degraded land p (Singh et al., 2012). High activity of β-glucosidase in reforested land was associated with high litter input which results in high carbon turnover, thus enhancing enzyme activity (Singh et al., 2012).

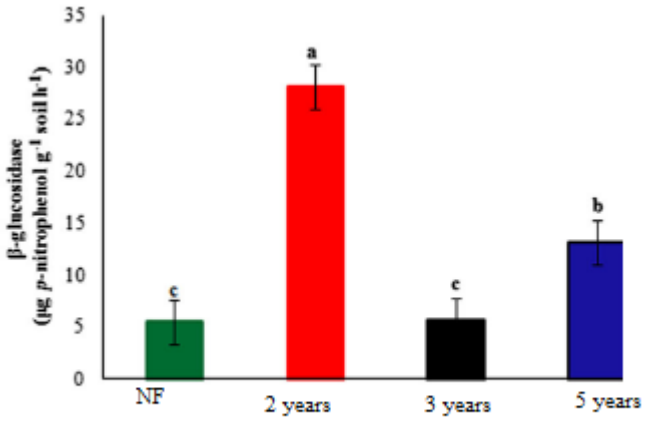

Figure 2.3: β-glucosidase activity in sites under native forest (NF) and reforested land (aged 2, 3 and 5-year-old) in Cabo de Santo Agostinho, Brazil (Lino et al., 2016). Different lower-case letters above bars show statistical difference.

(d) Fluorescein diacetate hydrolase

Fluorescein diacetate (FDA) in the soil has been used as a substrate to measure enzymatic activity (Adam and Duncan, 2001, Alkorta et al., 2003, Green et al., 2006, Schnürer and Rosswall, 1982). In the soil, FDA is hydrolysed by intracellular and extracellular enzymes including esterases, lipases and proteases, as a result the hydrolysis of FDA is more likely to depict soil enzymatic activity (Alkorta et al., 2003, Green et al., 2006, Rajper et al., 2016, Schnürer and Rosswall, 1982) . FDA hydrolysis has been used to measure the activity of soil microbial decomposers (bacteria and fungi), thus serving as a good indicator for total microbial activity (Adam and Duncan, 2001, Alkorta et al., 2003, Schnürer and Rosswall, 1982). A study by Silva et al. (2004) showed that FDA hydrolysis activity could be used as an indicator for soils undergoing reforestation. In the study, 5 treatments were evaluated (i.e. soils reforested

with native species, exotic species, neem and pigeon, native forest (reference) and non-forested soils). The results showed that using native species for reforestation improves biological functioning of degraded soils, thus making FDA activity useful in environmental monitoring (Silva et al., 2004) . FDA hydrolysis activity was used as an indicator for forest succession in Brazil. Two regenerated forests (aged 10 and 20-years-old) were compared to sugarcane and native forest, in dry and wet season (da Silva et al., 2012). In both seasons hydrolysis of FDA was higher in 10-years-old forest followed by 20-years-old forest, native forest and lastly sugarcane (Fig. 2.4). The trend was similar in both seasons (Fig. 2.4) and FDA hydrolysis activity was associated with high organic matter content (da Silva et al., 2012). Organic matter provides substrate for microbes, higher organic matter content can support higher microbial biomass thus enhancing enzyme production (Wolińska and Stępniewska, 2012)

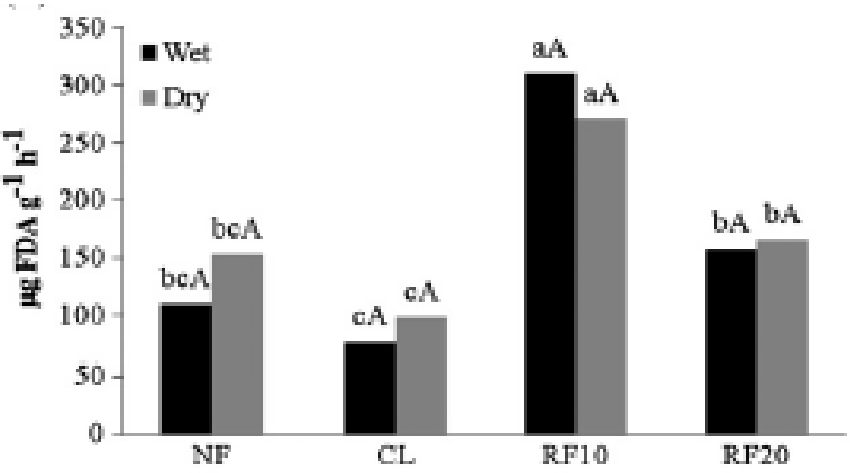

Figure 2.4: Hydrolysis of fluorescein diacetate (FDA) in sites under native forest (NF), conventional sugarcane plantation (CL) and regenerated forests (aged 10: RF10 and 20-year-old: RF20) in wet and dry season at Igarassu city, Brazil (da Silva et al., 2012). Different lower-case and upper-case letters above bars show statistical difference.

2.6 Effects of management on soil properties, fauna and microbial activity

In general, soils under native forests support more fauna taxonomic groups than agricultural soils (Rosario et al., 2014). The rate of litter input and decomposition in forests is balanced, thus more favourable for soil fauna and microbial activity (Cardoso et al., 2013). According to (Rosario et al. (2014)) , forests have a humus layer associated with leaf litter, diverse soil fauna groups live within the humus layer and it supports both litter and wood feeding fauna such as

beetles and termites. The large amounts of litter fall and root exudates associated with reforestation provide microbes with substrates and residues maintain soil microclimate, thus enhancing microbial activity (Kuwano et al., 2014) . Forest conversion to agriculture leads to loss of SOC which reduces microbial activity; increases carbon dioxide emissions to the atmosphere and may also reduce fauna severely within a few weeks of clearing (Cardoso et al., 2013) . However afforestation has a potential to reverse effects of agricultural practices.

Recently there is a growing global pressure to reduce greenhouse gas emissions, as a result degraded areas previously under agriculture are being reforested (Adams and Fiedler, 2015, Sauer et al., 2012, Vasconcellos et al., 2013). Agricultural practices induce soil compaction due to machinery and livestock (Cunningham et al., 2015) .As a result soil porosity is reduced thus affecting microhabitats of soil microbes, which may reduce microbial activity (Vasconcellos et al., 2013). This also affects earthworm activity, burrowing in soils with high bulk density can be difficult (Birkás et al., 2004) . However, reforestation has a great potential of improving soil quality, since there is less soil disturbance and more residue cover than in soils under intensive agriculture (Sauer et al., 2012). Due to high litter fall and reduced soil disturbance, reforested soils are less compacted (thus lower bulk density) and higher soil porosity (Cunningham et al., 2015, Sauer et al., 2012). Improvement in soil properties may appear after a long period following afforestation (Cunningham et al., 2015, Sauer et al., 2012). Significant decrease in soil bulk density may appear after at least three decades following afforestation (Cunningham et al., 2015). This is because impacts of previous land use may persist in soil for years, thus influencing soil characteristics (Cunningham et al., 2015, Podrazsky et al., 2015). Study by Podrazsky et al. (2015) revealed that changes in soil bulk density after afforestation may be gradual. Soils cultivated with *Eucalyptus regnans* in Australia aged 10, 25 and 61-year-old had similar bulk density. However, bulk density decreased significantly in the eldest stand (aged 250+ years) (Podrazsky et al., 2015).

Litter inputs under reforested land increases soil organic matter content and microhabitats for microbes, as a result reforestation is associated with healthy soil biology (Sauer et al., 2012). In Brazil, insect fauna associated with reforestation with Parica was evaluated. The comparison of 2, 3 and 5-years-old after reforestation showed that the 5-year-old stands were richer according to families and more abundant in sampled individuals (Rosario et al., 2014) . This was associated with canopy cover by trees which reduced solar radiation and moisture loss, thus proving an environment that is more suitable for fauna (Rosario et al., 2014). According to (Korboulewsky et al., 2016), tree species litter quality rather than tree species richness is

the most important factor that influences soil fauna community response to afforestation. Litter quality is an important factor on soil fauna communities, the addition of broadleaved species on conifers reduced polyphenol content, thus leading to higher decomposition rates and higher abundance and activity of soil organisms (Korboulewsky et al., 2016). During the initial phase of reforestation, SOC content is low and microbes will assimilate the labile SOC, upon completion microbial activity is reduced (Liu et al., 2012). With more litter input as the forest recovers, microbes may be increased due to increased food availability and microhabitats (Liu et al., 2012).

Research has shown that reforestation improves soil quality of degraded land, however, the negative impacts of intensive agriculture can be long-lived in soil (Filser et al., 1995). According to Cardoso et al. (2013) intensive agriculture reduces SOC in soil and the recovery of SOC back to original levels found in native forest may take decades and this influences soil microbial activity negatively. According to Vopravil et al. (2014), the main contributor to soil quality improvement after afforestation is the formation of stable aggregates. Formation of stable aggregates is crucial in restoring degraded land and carbon sequestration. Soil organic carbon sequestration is dependent on soil aggregate stability (Kumar et al., 2013). Soil macro-aggregates consist of younger organic material, whereas micro-aggregates stabilize old soil organic carbon (Kumar et al., 2013). Afforestation has a potential to increase soil aggregate stability (Vopravil et al., 2014), thus enhancing carbon sequestration. However, the improvement of aggregate stability following afforestation is dependent on the quality of organic matter, determined by the traits of litter (Podrazsky et al., 2015, Vopravil et al., 2014). . Working with spruce, pine, silver birch and Douglas-fir showed that tree species influence the response of soil properties to afforestation (Vopravil et al., 2014). The results showed that afforestation with spruce and pine increased aggregate stability as indicated by higher mean weight diameter and water stable aggregates compared to other trees.

2.7 Conclusions

In this review, soil macrofauna and microbial activity under afforested and reforested lands was discussed. Afforestation improves soil quality, thus enhancing soil biological functioning. However, negative effects of intensive tillage may remain for years in the soil after afforestation. The response of soil organisms to afforestation is dependent on litter quality of the tree species used for afforestation, since they affect soil properties differently. In Southern Africa, there is a general lack of information regarding effects of afforestation on soil fauna

and microbial characteristics. Most of the literature used was from Brazil, there isn't much in South Africa and other African countries. In the following chapters of this thesis, effects of afforestation on soils previously under sugarcane production in KwaZulu-Natal on soil fauna and microbial characteristics are explored.

References

ABUNYEWA, A.A., AGYARKO, K. AND ASIEDU, E.K., 2015. Effects of reforestation on degraded soils in the moist semi deciduous zone of West Africa. *Journal of Science and Technology (Ghana)*, *35*(1), 1-9.

ACOSTA-MARTINEZ, V., CRUZ, L., SOTOMAYOR-RAMIREZ, D. & PÉREZ-ALEGRÍA, L. 2007. Enzyme activities as affected by soil properties and land use in a tropical watershed. *Applied Soil Ecology,* 35**,** 35-45.ADAM, G. & DUNCAN, H. 2001. Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils. *Soil Biology and Biochemistry,* 33**,** 943-951.

ADAMS, M.-O. & FIEDLER, K. 2015. The value of targeted reforestations for local insect diversity: A case study from the Ecuadorian Andes. *Biodiversity and conservation,* 24**,** 2709-2734.

ALKORTA, I., AIZPURUA, A., RIGA, P., ALBIZU, I., AMEZAGA, I. & GARBISU, C. 2003. Soil enzyme activities as biological indicators of soil health. *Reviews on environmental health,* 18**,** 65-73.

ASAWALAM, D. O., OSODEKE, V. E., KAMALU, O. J. & UGWA, I. K. 1999. Effects of termites on the physical and chemical properties of the acid sandy soils of southern Nigeria. *Communications in Soil Science & Plant Analysis,* 30**,** 1691-1696.

AYUKE, F., KARANJA, N., MUYA, E., KIBBERENGE, M., MUNGATU, J. & NYAMASYO, G. 2009. Macrofauna diversity and abundance across different land use systems in Embu, Kenya. *Tropical and Subtropical Agroecosystems,* 11**,** 371-384.

BAKSHI, M. & VARMA, A. 2011. Soil enzyme: the sate of art. *In:* SHUKLA, G. & VARMA, A. (eds.) *Soil Enzymology.* Heidelburg: Springer.

BALVANERA, P., QUIJAS, S., MARTIN-LOPEZ, B., BARRIOS, E., DEE, L., ISBELL, F., DURANCE, I., WHITE, P., BLANCHARD, R. & DE GROOT, R. 2015. The links between biodiversity and ecosystem services. *Handbook of Ecosystems Services.* Earthscan-Routledge.

BARRIOS, E. 2007. Soil biota, ecosystem services and land productivity. *Ecological Economics* **,**64(2), 269-285.

BHADAURIA, T. & SAXENA, K. G. 2010. Role of earthworms in soil fertility maintenance through the production of biogenic structures. *Applied and Environmental Soil Science,* 2010.

BHAVANA, K., POOVOLI, A., RAJMOHANA, K. & SHWETA, M. 2015. A Comparison on termite assemblages in coffee & teak plantations and semi-evergreen forest-a case study in North Wayanad, Kerala, India. *Tropical Agricultural Research,* 26.

BINI, D., DOS SANTOS, C. A., DO CARMO, K. B., KISHINO, N., ANDRADE, G., ZANGARO, W. & NOGUEIRA, M. A. 2013. Effects of land use on soil organic carbon and microbial processes associated with soil health in southern Brazil. *European Journal of Soil Biology,* 55**,** 117-123.

BIRKÁS, M., JOLÁNKAI, M., GYURICZA, C. & PERCZE, A. 2004. Tillage effects on compaction, earthworms and other soil quality indicators in Hungary. *Soil and Tillage Research,* 78**,** 185-196.

BOROWIK, A. & WYSZKOWSKA, J. 2016. Soil moisture as a factor affecting the microbiological and biochemical activity of soil. *Plant, Soil and Environment,* 62**,** 250-255.

BOSSUYT, H., SIX, J. & HENDRIX, P. F. 2005. Protection of soil carbon by microaggregates within earthworm casts. *Soil Biology and Biochemistry,* 37**,** 251-258.

BOT, A. & BENITES, J. 2005. *The importance of soil organic matter: Key to drought-resistant soil and sustained food production,* Food & Agriculture Org.

CARDOSO, E. J. B. N., VASCONCELLOS, R. L. F., BINI, D., MIYAUCHI, M. Y. H., SANTOS, C. A. D., ALVES, P. R. L., PAULA, A. M. D., NAKATANI, A. S., PEREIRA, J. D. M. & NOGUEIRA, M. A. 2013. Soil health: looking for suitable indicators. What should be considered to assess the effects of use and management on soil health? *Scientia Agricola,* 70**,** 274-289.

COLEMAN, D. C., CROSSLEY, D. A. & HENDRIX, P. F. 2004. *Fundamentals of soil ecology,* Amsterdam; Boston, Elsevier Academic Press.

COLLISON, E., RIUTTA, T. & SLADE, E. M. 2013. Macrofauna assemblage composition and soil moisture interact to affect soil ecosystem functions. *Acta oecologica,* 47**,** 30-36.

CUNNINGHAM, S. C., MAC NALLY, R., BAKER, P. J., CAVAGNARO, T. R., BERINGER, J., THOMSON, J. R. & THOMPSON, R. M. 2015. Balancing the environmental benefits of reforestation in agricultural regions. *Perspectives in Plant Ecology, Evolution and Systematics,* 17**,** 301-317.

DA SILVA, D. K. A., DE OLIVEIRA FREITAS, N., DE SOUZA, R. G., DA SILVA, F. S. B., DE ARAUJO, A. S. F. & MAIA, L. C. 2012. Soil microbial biomass and activity under natural and regenerated forests and conventional sugarcane plantations in Brazil. *Geoderma,* 189**,** 257-261.

DAS, S. K. & VARMA, A. 2011. Role of enzymes in maintaining soil health. *In:* SHUKLA, G. & VARMA, A. (eds.) *Soil enzymology*. Berlin: Springer.

DE PAULA, R. C., SILVEIRA, R. D. M. L., DA ROCHA, M. M. & IZZO, T. J. 2016. The restoration of termite diversity in different reforestated forests. *Agroforestry systems,* 90**,** 395-404.

DEBELO, D. G. & DEGAGA, E. G. 2017. Study on termite damage to different species of tree seedlings in the Central Rift Valley of Ethiopia. *African Journal of Agricultural Research,* 12**,** 161-168.

DICK, R. P. 1997. Soil enzyme activities as integrative indicators of soil health. *In:* PANKURST, C. E., DOUBE, B. M. & GUPTA, V. V. S. R. (eds.) *Biological indicators of soil health.* Wellingford: CABI.

DOMINY, C.S., Haynes, R.J. and Van Antwerpen, R., 2001. Long-term effects of sugarcane production on soil quality in the South Coast and Midlands areas of KwaZulu-Natal. *Proc S Afr Sug Technol Ass* ,75, 222-227.

EKENLER, M. 2002. Enzyme activities in soils as affected by management practices.

EKENLER, M. & TABATABAI, M. 2002. β-Glucosaminidase activity of soils: effect of cropping systems and its relationship to nitrogen mineralization. *Biology and Fertility of Soils,* 36**,** 367-376.

ELMARSDOTTIR, A., FJELLBERG, A., HALLDORSSON, G., INGIMARSDOTTIR, M., NIELSEN, O. K., NYGAARD, P., ODDSDOTTIR, E. S. & SIGURDSSON, B. D. 2008. Effects of afforestation on biodiversity. *AFFORNORD. Effects of afforestation on ecosystems, landscape and rural development. TemaNord,* 562**,** 37-47.

FILSER, J., FROMM, H., NAGEL, R. F. & WINTER, K. 1995. Effects of previous intensive agricultural management on microorganisms and the biodiversity of soil fauna. *The Significance and Regulation of Soil Biodiversity.* Springer.

FORGIE, S. A., PAYNTER, Q., ZHAO, Z., FLOWERS, C. & FOWLER, S. V. 2013. The impact of tunnelling and dung burial by new exotic dung beetles (Coloptera: Scarabaeinae) on surface run-off, survivorship of a cattle helminth, and pasture foliage biomass in New Zealand pastures.

FROUZ, J. & JILKOVÁ, V. 2008. The effect of ants on soil properties and processes (Hymenoptera: Formicidae). *Myrmecological News*, 11**,** 191-199.

GIANFREDA, L. & RAO, M. A. 2014. *Enzymes in agricultural sciences*, OMICS Group International.

GONNETY, J. T., ASSÉMIEN, E. F., GUÉI, A. M., AYA, A. N., DJINA, Y., KONÉ, A. W. & TONDOH, J. E. 2012. Effect of land-use types on soil enzymatic activities and chemical properties in semi-deciduous forest areas of Central-West Côte d'Ivoire. *Biotechnologie, Agronomie, Société et Environnement,* 16**,** 478.

GREEN, V., STOTT, D. & DIACK, M. 2006. Assay for fluorescein diacetate hydrolytic activity: optimization for soil samples. *Soil Biology and Biochemistry,* 38**,** 693-701.

HARRIS, W. 1966. The role of termites in tropical forestry. *Insectes Sociaux,* 13**,** 255-265.

HLAVA, J. & KOPECKÝ, O. 2013. Earthworm population responses to deciduous forest soil acidity and vegetation cover. *Scientia Agriculturae Bohemica,* 44**,** 133-137.

ISLAM, K. & WRIGHT, S. 2003. Microbial communities. *Encyclopedia of Soil Science. Marcel Dekker. Published online. http://www. dekker. com/servlet/product/DOI/101081EESS120014249 [May 3, 2005]*.

JILKOVA, V., FROUZ, J., DOMISCH, T. & FINER, L. 2010. The effect of wood ants (Formica s. str.) on soil chemical and microbiological properties. *Published on DVD*.

KASCHUK, G., ALBERTON, O. & HUNGRIA, M. 2010. Three decades of soil microbial biomass studies in Brazilian ecosystems: lessons learned about soil quality and indications for improving sustainability. *Soil Biology and Biochemistry,* 42**,** 1-13.

KATTAN, G. H., MURCIA, C. & GALINDO-CARDONA, A. 2010. An evaluation of bess beetles (Passalidae) and their resource base in a restored Andean forest. *Tropical Conservation Science,* 3**,** 334-343.

KIBBLEWHITE, M., RITZ, K. & SWIFT, M. 2008. Soil health in agricultural systems. *Philosophical Transactions of the Royal Society B: Biological Sciences,* 363**,** 685-701.

KNIGHT, T. R. & DICK, R. P. 2004. Differentiating microbial and stabilized b-glucosidase activityrelative to soil quality. *Soil Biology and Biochemistry,* 36**,** 2089-2096.

KOOCH, Y. & JALILVAND, H. 2008. Earthworms as ecosystem engineers and the most important detritivors in forest soils. *Pakistan Journal of Biological Sciences,* 11**,** 819-825.

KORBOULEWSKY, N., PEREZ, G. & CHAUVAT, M. 2016. How tree diversity affects soil fauna diversity: A review. *Soil Biology and Biochemistry,* 94**,** 94-106.

KREIDENWEIS, U., HUMPENÖDER, F., STEVANOVIĆ, M., BODIRSKY, B. L., KRIEGLER, E., LOTZE-CAMPEN, H. & POPP, A. 2016. Afforestation to mitigate climate change: impacts on food prices under consideration of albedo effects. *Environmental Research Letters,* 11**,** 085001.

KUMAR, R., RAWAT, K. S., SINGH, J., SINGH, A. & RAI, A. 2013. Soil aggregation dynamics and carbon sequestration. *J Appl Nat Sci,* 5**,** 250-267.

KUWANO, B. H., KNOB, A., FAGOTTI, D. S. L., MELÉM JÚNIOR, N. J., GODOY, L., DIEHL, R. C., KRAWULSKI, C. C., ANDRADE FILHO, G., ZANGARO FILHO, W. & TAVARES-FILHO, J. 2014. Soil quality indicators in a Rhodic Kandiudult under different uses in Northern Paraná, Brazil. *Revista Brasileira de Ciência do Solo,* 38**,** 50-59.

LAVELLE, P., CHAUVEL, A. & FRAGOSO, C. 1995. Faunal activity in acid soils. *Plant-Soil Interactions at Low pH: Principles and Management.* Springer.

LAVELLE, P. & SPAIN, A. V. 2005. *Soil ecology,* Dordrecht; Boston, Kluwer Academic Publishers.

LINO, I. A. N., SANTOS, V. M., ESCOBAR, I. E. C., SILVA, D. K. A., ARAÚJO, A. S. F. & MAIA, L. C. 2016. Soil enzymatic activity in Eucalyptus grandis plantations of different ages. *Land Degradation & Development,* 27**,** 77-82.

LIU, Y., WEI, X., GUO, X., NIU, D., ZHANG, J., GONG, X. & JIANG, Y. 2012. The long-term effects of reforestation on soil microbial biomass carbon in sub-tropic severe red soil degradation areas. *Forest ecology and management,* 285**,** 77-84.

LUO, T.-H., YU, X.-D. & ZHOU, H.-Z. 2013. Effects of reforestation practices on staphylinid beetles (Coleoptera: Staphylinidae) in southwestern China forests. *Environmental entomology,* 42**,** 7-16.

LYDIATE, A. & HELM, C. V. 2014. A soil quality comparison from agriculture to afforestation in Heartwood Forest. *Trans. Herts. Nat. Hist. Soc,* 46**,** 141.

MAKOI, J. H. & NDAKIDEMI, P. A. 2008. Selected soil enzymes: examples of their potential roles in the ecosystem. *African Journal of Biotechnology,* 7.

MAPA, R.B., 1995. Effect of reforestation using Tectona grandis on infiltration and soil water retention. *Forest Ecology and Management, 77*(1-3), 119-125.

MARTINEZ-SALGADO, M., GUTIÉRREZ-ROMERO, V., JANNSENS, M. & ORTEGA-BLU, R. 2010. Biological soil quality indicators: a review. *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology,* 1**,** 319-328.

MCLEAN, M. & HUHTA, V. 2002. Microfungal community structure in anthropogenic birch stands in central Finland. *Biology and Fertility of Soils,* 35**,** 1-12.

MERINO, C., GODOY, R. & MATUS, F. 2016. Soil enzymes and biological activity at different levels of organic matter stability. *Journal of Soil Science and Plant Nutrition,* 16**,** 14-30.

MUKUMBAREZA, C., CHIDUZA, C. & MUCHAONYERWA, P. 2015. Effects of oats and grazing vetch cover crops and fertilisation on microbial biomass and activity after five years of rotation with maize. *South African Journal of Plant and Soil,* 32**,** 189-197.

NANNIPIERI , P., GREGO, S. & CECCANTI, B. 1990. Ecological significance of the biologicalactivity in soil. *In:* BOLLANG, J. M. & STOTZKY, G. (eds.) *Soil Biochemistry.* New York: Marcel Dekker,Inc.

NOBLE, I., BOLIN, B., RAVINDRANATH, N., VERARDO, D. & DOKKEN, D. 2000. *Land Use, Land-Use Change, and Forestry*, Cambridge University Press.

PACHECO, R., SILVA, R. R., MORINI, M. S. D. C. & BRANDÃO, C. R. 2009. A comparison of the leaf-litter ant fauna in a secondary atlantic forest with an adjacent pine plantation in southeastern Brazil. *Neotropical Entomology,* 38**,** 55-65.

PARHAM, J. & DENG, S. 2000. Detection, quantification and characterization of β-glucosaminidase activity in soil. *Soil Biology and Biochemistry,* 32**,** 1183-1190.

PEREIRA, J. D. M., BARETTA, D., BINI, D., VASCONCELLOS, R. L. D. F. & CARDOSO, E. J. B. N. 2013. Relationships between microbial activity and soil physical and chemical properties in native and reforested Araucaria angustifolia forests in the state of São Paulo, Brazil. *Revista Brasileira de Ciência do Solo,* 37**,** 572-586.

PHOGAT, V. K., TOMAR, V. S. & DAHIYA, R. 2015. Soil physical properties. In: RATTAN, R. K., KATYAL, J. C., DWIVEDI, B. S., SARKAR, A. K., BHATTACHATYYA, T., TARAFDAR, J. C. & KUKAL, S. S. (eds.) Soil Science: An Introduction. Indian Society of Soil Science.

PODRAZSKY, V., HOLUBÍK, O., VOPRAVIL, J., KHEL, T., MOSER, W. & PRKNOVA, H. 2015. Effects of afforestation on soil structure formation in two climatic regions of the Czech Republic.

PRIBADI, T., RAFFIUDIN, R. & HARAHAP, I. S. 2011. Termites community as environmental bioindicators in highlands: a case study in eastern slopes of Mount Slamet, Central Java. *Biodiversitas Journal of Biological Diversity,* 12.

RAJPER, A. M., UDAWATTA, R. P., KREMER, R. J., LIN, C.-H. & JOSE, S. 2016. Effects of probiotics on soil microbial activity, biomass and enzymatic activity under cover crops in field and greenhouse studies. *Agroforestry Systems,* 90**,** 811-827.

RIBAS, C. R., CAMPOS, R. B., SCHMIDT, F. A. & SOLAR, R. R. 2011. Ants as indicators in Brazil: a review with suggestions to improve the use of ants in environmental monitoring programs. *Psyche: A Journal of Entomology,* 2012.

ROSARIO, V. S. V., BATISTA, T. F. V., PROVENZANO, R., LEMOS, L. J. U., DOS SANTOS, J. D. V. & LUNZ, A. M. 2014. Edaphic insect fauna associated with reforestation with Schizolobium parahyba Barneby in Amazonia. *Amazonian Journal of Agricultural and Environmental Sciences,* 57**,** 373-381.

SAUER, T. J., JAMES, D. E., CAMBARDELLA, C. A. & HERNANDEZ-RAMIREZ, G. 2012. Soil properties following reforestation or afforestation of marginal cropland. *Plant and soil,* 360**,** 375-390.

SCHNECKER, J., WILD, B., HOFHANSL, F., ALVES, R. J. E., BÁRTA, J., ČAPEK, P., FUCHSLUEGER, L., GENTSCH, N., GITTEL, A. & GUGGENBERGER, G. 2014. Effects of soil organic matter properties and microbial community composition on enzyme activities in cryoturbated arctic soils. *PLoS One,* 9**,** 1-10.

SCHNÜRER, J. & ROSSWALL, T. 1982. Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. *Applied and environmental microbiology,* 43**,** 1256-1261.

SIGURDSSON, B. D. & GUDLEIFSSON, B. E. 2013. Impact of afforestation on earthworm populations in Iceland. *Icelandic Agricultural Sciences,* 26**,** 21-26.

SILVA, M., SIQUEIRA, E. R. & COSTA, J. L. D. S. 2004. Flurescein diacetate hydrolysis as a bioindicator of microbial activity in soil under reforestation. *Ciência Rural,* 34**,** 1493-1496.

SINGH, K., PANDEY, V. C., SINGH, B. & SINGH, R. 2012. Ecological restoration of degraded sodic lands through afforestation and cropping. *Ecological Engineering,* 43**,** 70-80.

SIQUEIRA, G., SILVA, E. & PAZ-FERREIRO, J. 2014. Land use intensification effects in soil arthropod community of an Entisol in Pernambuco State, Brazil. *The Scientific World Journal,* 2014.

SRIVASTAVA, P. K. 2010. Microbial Activity and Nutrient Status in Oak and Pine Oriented ForestSoil of Mid Altitude Central Himalaya. *Geneconserve,* 9**,** 1-10.

TIETJEN, T. & WETZEL, R. G. 2003. Extracellular enzyme-clay mineral complexes: enzyme adsorption, alteration of enzyme activity, and protection from photodegradation. *Aquatic Ecology,* 37**,** 331-339.

UEDA, A., DWIBADRA, D., NOERDJITO, W. A., KON, M., OCHI, T., TAKAHASHI, M. & FUKUYAMA, K. 2015. Effect of habitat transformation from grassland to Acacia mangium plantation on dung beetle assemblage in East Kalimantan, Indonesia. *Journal of insect conservation,* 19**,** 765-780.

UTOBO, E. & TEWARI, L. 2015. Soil enzymes as bioindicators of soil ecosystem status. *Appl. Ecol. Environ. Res,* 13**,** 147-169.

VASCONCELLOS, R. L., SEGAT, J. C., BONFIM, J. A., BARETTA, D. & CARDOSO, E. J. 2013. Soil macrofauna as an indicator of soil quality in an undisturbed riparian forest and recovering sites of different ages. *European Journal of Soil Biology,* 58**,** 105-112.

VELMOUROUGANE, K., VENUGOPALAN, M., BHATTACHARYYA, T., SARKAR, D., PAL, D., SAHU, A., RAY, S., NAIR, K., PRASAD, J. & SINGH, R. 2013. Soil dehydrogenase activity in agro-ecological sub regions of black soil regions in India. *Geoderma,* 197**,** 186-192.

VINHAL-FREITAS, I. C., WANGEN, D. R. B., FERREIRA, A. D. S., CORRÊA, G. F. & WENDLING, B. 2010. Microbial and enzymatic activity in soil after organic composting. *Revista Brasileira de Ciência do Solo,* 34**,** 757-764.

VOPRAVIL, J., PODRÁZSKÝ, V., KHEL, T., HOLUBÍK, O. & VACEK, S. 2014. Effect of afforestation of agricultural soils and tree species composition on soil physical characteristics changes. *Ekologia,* 33**,** 67-80.

WITT, B. 1997. Using soil fauna to improve soil health. *Student On-Line Journal,* 2**,** 1-5.

WOLIŃSKA, A. & STĘPNIEWSKA, Z. 2012. *Dehydrogenase activity in the soil environment*, INTECH Open Access Publisher.

WU, J. 2008. Land use changes: economic, social, and environmental impacts. *Choices,* 23**,** 6-10.

YANG, K., ZHU, J., YAN, Q. & ZHANG, J. 2012. Soil enzyme activities as potential indicators of soluble organic nitrogen pools in forest ecosystems of Northeast China. *Annals of forest science,* 69**,** 795-803.

ZOU, X. & BASHKIN, M. 1998. Soil carbon accretion and earthworm recovery following revegetation in abandoned sugarcane fields. *Soil Biology and Biochemistry,* 30**,** 825-830.

CHAPTER THREE: RESPONSES OF SOIL FAUNA AND CHEMICAL PROPERTIES TO REFORESTATION IN BUFFELSDRAAI, DURBAN

Abstract

Reforestation effects on soil fauna remains unknown in South Africa, yet soil invertebrates are essential in assessing soil quality. The aim of the study was to evaluate the changes in soil invertebrate community and chemical properties in a chronosequence (2-, 4- and 6-year-old) of reforested land compared to sugarcane field (representing a degraded land). The collection of fauna was carried out in January 2016 (beginning of rainy season) and March 2016 (middle of rainy season) using 25x25x25 cm monoliths. The sampling was carried out in Acrisols and Leptosols in the buffer zone of Buffelsdraai landfill site. Five sampling points were randomly selected on uniform soils for each forest age stand and sugarcane. Insects were hand sorted and identified to order level. Soil fauna abundance, Shannon diversity index and richness were determined and were all affected by rainfall amount and soil type. The results demonstrated that soil fauna abundance in the beginning of the rainy season in January was 76 % higher than in the middle of the rainy season in March. Hymenoptera and Isoptera were the most abundant groups of fauna in the study. Abundance of Isoptera was only higher at the oldest forest stand (6-year-old), suggesting that the land was in good repair. Reforestation increased soil pH, in the 6-year-old forest stand it was ± 0.45 units higher than in sugarcane.

3.1 Introduction

The eThekwini Municipality was one of the venues that hosted the FIFA 2010 World Cup in South Africa (SA). While this was going to generate income for the KwaZulu-Natal province, it was estimated that hosting the event would generate a total carbon footprint of 307.208 tCO_2e (eThekwini Municipality, 2011). To offset these emissions and sequester carbon a community reforestation project was established in the buffer zone of the Buffelsdraai Landfill Site in 2008 (eThekwini Municipality, 2011). The project involved planting indigenous trees on areas which were previously under sugarcane farming, which were degraded. While reforestation is expected to increase C sequestration, in plant biomass and soil organic C, it is also expected to increase biodiversity and ecosystem functioning. Already, earlier studies on the site have shown increased numbers of bird species over the 5-year-old period of reforestation (Douwes et al., 2015). However, the influence of reforestation at the site on soil invertebrates remains unknown. Measuring soil fauna may serve as an early indicator of change in soil organic matter

(SOM) and recovery of a degraded ecosystem. Soil organic matter has an effect on biological, chemical and physical soil properties, it may improve structure, water holding capacity (Bot and Benites, 2005) and also acts as an adsorbent of pesticides and herbicides thus reducing negative impacts they may on soil organisms . These properties are important for the survival and activity of soil fauna; fauna is important in soil for litter decomposition and improving soil structure (Witt, 1997). With increased organic matter in the soil, populations of faunal groups such as earthworms may increase (Bot and Benites, 2005). Earthworms require a moist environment and with increased organic matter content, soil's capacity to hold water increases, thus favouring their survival (Lavelle and Spain, 2005). Fauna activity in the soil (litter breakdown and modification of soil structure) has an effect on microbial activity, as a result fauna regulates organic matter dynamics (Lavelle et al., 1994). Since fauna regulates soil organic matter dynamics, changes in their communities may have influence on SOM, thus they can serve as an indicator of SOM change.

The most abundant species of macrofauna in soil are ants, earthworms and termites and they are well known for their biological and pedogenic roles in soils (Barrios, 2007, Lavelle and Spain, 2005) . Soil macrofauna are vital for breaking down and distributing organic material (Barrios, 2007, Muchane et al., 2012) and this has a positive influence on microbial processes. The effectiveness of micro-organisms is dependent on the activity of macrofauna (Cardoso et al., 2013). Soil fauna breaks down (mechanically) organic material, thus increasing the surface area and making it easy for microbes to degrade organically bound compounds (Winsome, 2005).

Macrofauna also contribute to the physical structure of the soil. For example, macro-aggregates in the soil can be formed through earthworm activity, they ingest soil and organic matter forming aggregates in their gut which is then excreted into the soil (Balvanera et al., 2015, Bossuyt et al., 2005). During digestion, biochemical transformation occurs within the earthworm gut, resulting in the production of mucilage (released by enzymes in the digestive tract) and polysaccharides which serve as binding agents that stabilize aggregates hence protecting soil organic carbon (SOC) (Whalen and Sampedro, 2010). Soil organic carbon is the main component of SOM and SOM can be estimated by determining SOC content (Bianchi et al., 2008). Formation of aggregates improves C storage and protects SOC which directly increases soil microbial activity. A study on protection of soil C by micro-aggregates within earthworm casts has shown that earthworm activity leads to protection of soil C within micro aggregates and this further improves preservation of C (Bossuyt et al., 2005). Burrowing

activity of fauna in the soil leads to increased porosity thus improving gaseous exchange (Dajoz, 1998)), which is essential for healthy soil. Soil fauna directly influence soil physical properties, due to their burrowing activities, they increase soil infiltration and decrease bulk density (Lavelle et al., 1992). However, they can also influence soil chemical properties indirectly. Earthworm casts are rich in calcium (Ca), magnesium (Mg), potassium (K); mineral forms of nitrogen and phosphorus and high content of soluble carbon readily available for microbes and plant uptake (Winsome, 2005, Witt, 1997). Ant nests also have higher cation and nutrient content compared to adjacent soil, resulting in increased soil pH (Jilkova et al., 2010).

Soil macrofauna have been used as bio-indicators of soil quality to compare different land uses and land management practices due to their sensitivity to environmental stresses and variations to land management (Lavelle and Spain, 2005, Siqueira et al., 2014). According to Cardoso et al. (2013) soil organisms may live in the soil throughout their life cycle or at a certain phase of their life cycle. Soil organisms are sensitive to land use change and land management practices such as tillage, which affect food supply, litter quality and quantity and shelter, influencing their survival and reproduction in soil (Cunha Neto et al., 2012). Due to their response to environmental changes, they serve as good biological indicators of soil health (Cunha Neto et al., 2012). It has been reported that tillage in annual cropping systems has negative impacts on the abundance and diversity of soil macrofauna communities (Lavelle and Spain, 2005). Intensive tillage leads to disturbance in the soil environment by breaking down aggregates, this increases soil's sensitivity to erosion and it also leads to loss of carbon which serves as an energy source for fauna (Lavelle and Spain, 2005, Whalen and Sampedro, 2010). In agricultural systems, there is lack of permanent soil cover due to harvesting of crops and removal of their residues. Thus, population of organisms such as beetles decrease, surface residues are food source for beetles and aid in avoiding desiccation (Lavelle and Spain, 2005, Whalen and Sampedro, 2010). Unlike agricultural systems, in forests there is no use of machinery which leads to destruction of soil aggregates and reduction of organic matter. Rather they provide insects with a stable environment which reduces climatic variation, hence forests have been reported as a good system for preserving soil macrofauna communities (Barros et al., 2002, Lavelle and Spain, 2005).

Since soil fauna is a sensitive indicator of land use change and management effects on soil health, their evaluation can be insightful to the managers on the direction of change associated with a change in land use or land management. In SA, there is a general lack of information on soil invertebrate community in forest ecosystems and following land use change. Studies in

landuse change focused on hydrology and avifauna. Warburton et al. (2012) modelled the hydrological impacts of land use change in three South African catchments. Cooper (2015) evaluated the effects of landuse changes on the distribution of forest dependent bird species in South Africa. Petersen et al. (2017) assessed the effects of land use change on streamflow and stream water quality of a coastal catchment southwestern Cape, South Africa . The eThekwini Municipality hypothesized that with reforestation there will be an increase in biodiversity.

3.2 Objectives and hypothesis

The aim of this study was to evaluate the effects of reforestation on soil biological functioning using soil fauna (macrofauna: ants, beetles, earthworms and termites and mesofauna: pot worms) as an indicator. Abundance, diversity and richness of soil fauna communities was measured and compared in reforested soils with forest stands aged 2, 4 and 6-years-old and degraded land in Buffelsdraai. Soil pH, base cations, phosphorus, SOC, and total nitrogen (TN) were also measured. The distribution patterns of soil fauna were characterized by sampling in the beginning and in the middle of the rainy season. In this study, it was hypothesized that soil faunal abundance, diversity and richness would increase with reforestation age.

3.3 Materials and methods

3.3.1 Study area

The study area of approximately 620 ha was in the buffer zone of the Buffelsdraai Landfill Site in Verulam, KwaZulu-Natal province (29.63261° S, 30.98717° E) , South Africa (Fig. 3.1). The topography of the study area is characterized by undulating terrain, and it has an average slope of 11.91° and an average altitude of 228.07 m. According to the Köppen Geiger classification, the climate of the area is classified as humid subtropical (Cfa) (Climate-data n.d.) and with an average rainfall of 776 mm $year^{-1}$ and an annual average temperature 20.8 °C .Monthly rainfall and temperature are shown are shown in Fig. 3.2 . Approximately 100 years ago, more than 500 ha of the land were converted to sugarcane farming (Winn, 2016). Prior to sugarcane cultivation, the land was under natural vegetation, which included forest (riverine and scarp), thicket and woodland and grassland. The farmer sold the land to the Municipality more than 10 years ago, due to decreasing yields of sugarcane, largely associated with soil degradation and reduced rainfall. The study area has a wide range of soils including Sepane, Swartland and Valsrivier (Soil Classification WorkingGroup, 1991). The predominant soils fall under duplex and lithic South African soil groups and named Acrisols and Leptosols in the

World Reference Base for Soil Resources (Fey, 2010). Leptosols were found in steep slopes, underlain by Ecca shale and are generally shallow (0-30 cm), young and sensitive to erosion (Fey, 2010). According to Fey (2010) these soils are commonly found in steep slopes and are associated with convexity due to divergent water flow. Water flow erodes soil material from higher up and deposits it down the slope and if the main slope it too steep erosion rate will be in balance with deposition (Fey,2010). Acrisols were found in gentle slopes, underlain by dwyka tillite. Compared to Leptosols, Acrisols are less sensitive to erosion, have an increase of clay in the B horizon and are slightly deeper (up to 60 cm) which is more likely due to deposition of material from the upland area (Fey, 2010). The buffer zone of Buffelsdraai landfill site was divided into wet and dry areas; this was due to moisture differences in relation to topography. Water is drained from upper to lower slopes, resulting in concentration of water in lower parts of the slope. Leptosols dominated dry areas, whereas Acrisols were found in wet areas mostly located on lowest points of the slope where water drains.

Two land uses found were used for investigations, reforested soils and sugarcane plantation. Upon reforestation, abandoned sugarcane was cleared. The soils previously under sugarcane cultivation were then reforested with a mix of indigenous tree species (Table 3.1). As of October 2016, a total of 677 300 trees of various species (more than 72) had been planted by tree-preneurs in over 600 ha of land which was previously under sugarcane farming. The trees were planted in 2009-2010 (6-year-old), 2011-2012 (4-year-old) and 2014-2015 (2-year-old), and. Sugarcane site was approximately 800, 360 and 1000 m from the 2, 4 and 6-year-old forest stands respectively. Unlike the 6-year-old forest stand, the vegetation for both 2 and 4-year-old forest stands had grass (*Cynodon dactylon*); the grass in the 4-year-old forest stand was taller when compared to other sites. Sugarcane field was used as a control site, representing degraded land. This allowed for assessment of changes that could have occurred in the soil due to reforestation.

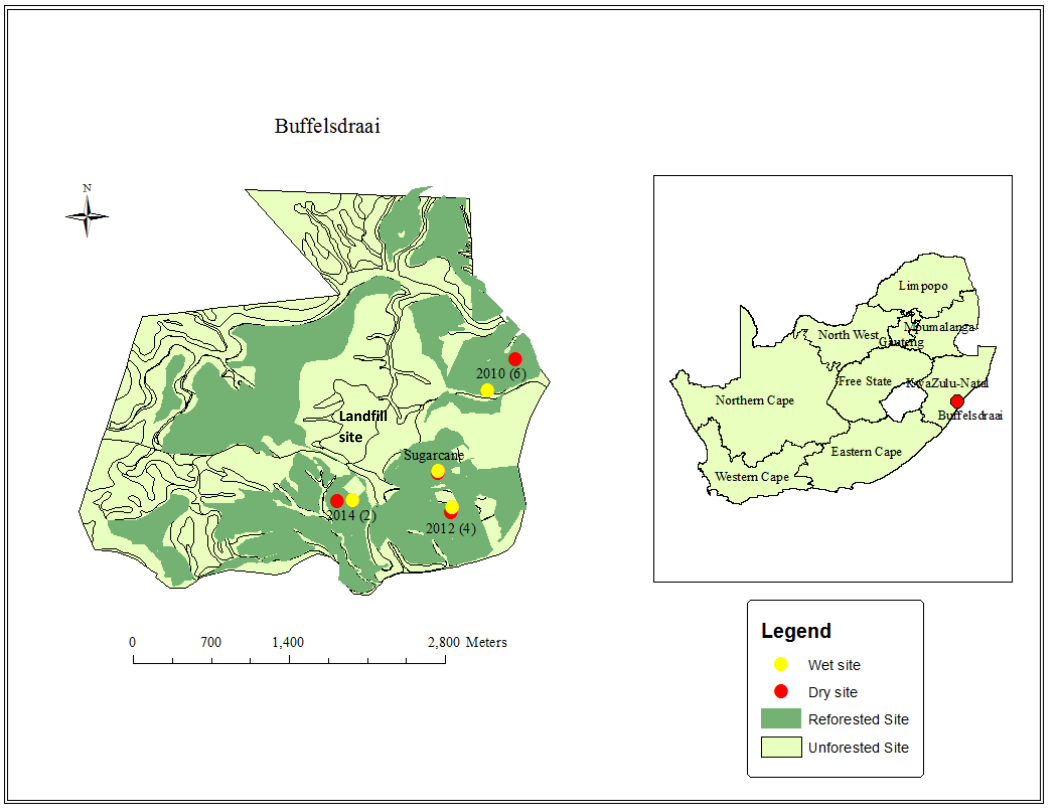

Figure 3.1: Map showing the four studied sites of forest stands aged 2-, 4-, and 6-year-old (trees were planted in 2014-2015, 2011-2012 and 2009-2010) and sugarcane within dry and wet sites in Buffelsdraai, Durban.

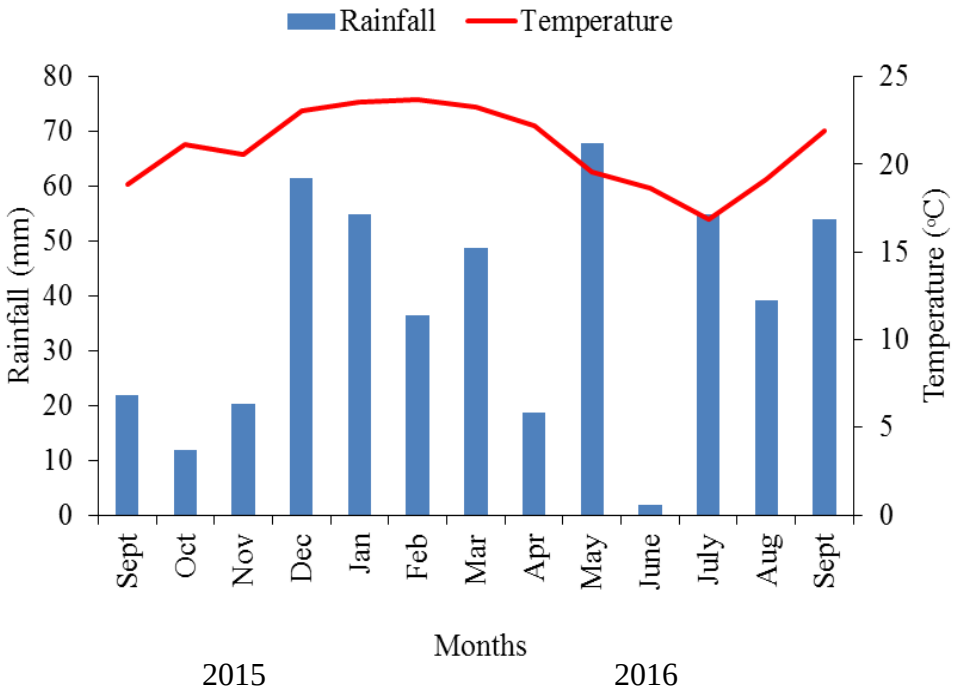

Figure 3.2: Monthly rainfall (mm) and temperature (°C) received in 2015-2016 at Buffelsdraai Research Station

Table 3.1: Most common tree species used for reforestation in the study area

Site	Acrisols	Leptosols
Sugarcane	sugarcane and alien invasive species	sugarcane and alien invasive species
2-year-old	*Bridelia micrantha*	*Bauhinia spp.*
	Ficus burkei	*Erythrina latissima*
	Syzigium cordartum	*Vachellia natalitia*
4-year-old	*Bridelia micrantha*	*Kigelia Africana*
	Erythrina latissimi	*Millettia grandis*
	Kigelia Africana	*Vachellia natalitia*
6-year-old	*Combretum molle*	*Dalbergia obovata*
	Erythrina latissimi	*Erythrina latissima*
	Senegalia caffra	*Vachellia natalitia*

3.3.2 Soil fauna

Sampling was carried out on Acrisols and Leptosols at 20 m x 20 m plots. Five sampling points were randomly selected on uniform soils for each forest age stand and sugarcane. At each sample point a 25x25x25 cm monolith was driven into the soil using a hammer for sampling soil fauna (Anderson and Ingram, 1993). Considering that soil fauna may be influenced by moisture differences, sampling was carried out in two periods, January 2016 (beginning of the rainy season) and March 2016 (middle of rainy season). Soil from the monolith was hand sorted straight after sampling *in situ* on black plastic bags to collect organisms (Anderson and Ingram, 1993). All collected invertebrates were preserved in 70% ethanol solution for identification in the laboratory. Invertebrates were identified to Order level and counted.

3.3.3 Sampling design

Soil sampling was carried out in Acrisols and Leptosols at 20 x 20 m plots. Five sampling points were randomly selected on uniform soils for each forest stand age and sugarcane. At each sampling point, soil samples from each site were randomly taken at 0-20 and 10-20 cm depth using an auger. Soil was air dried in the milling room and passed through a 2 mm sieve in preparation for analysis.

3.3.4 Soil characterisation

Soils were analysed for extractable Ca, K, Mg and P following method by Farina (1981) and Hunter (1975). Soil pH was measured in a 1:5 ratio (soil: solution) using distilled water (Benton, 1999). Extractable K and P were determined by Ambic-2 method and quantitative analysis was carried out using atomic absorption spectroscopy (Hunter, 1975). Whereas, Ca and Mg were extracted with 1M KCl solution and measured with a spectrophotometer (Farina, 1981). Hydrometer method was used to determine particle size distribution, using soil passed through a 2 mm sieve (Gee et al., 1986).Total C and N for each soil sample (passed through 0.5 mm) were determined by dry combustion using Leco Auto analyser (TruMac CNS/NS (Carbon/Nitrogen/Sulphur)) (Wright and Bailey, 2001). 3.3.5 Statistical analyses

3.3.5. Statistical analyses

3.3.5.1 Calculations

The data allowed the calculation of abundance, diversity and richness. Abundance (number of collected individuals in a sample) data was expressed as number of individuals per square meter. The diversity (number of taxonomic groups and abundance of each taxonomic group) of soil fauna was calculated by Shannon-Weiner diversity index (H′) for each site. The Shannon-Weiner index in calculated as follows:

$$H' = -\sum_{i=1}^{S} p_i \ln p_i$$

H' = biodiversity index, p*i* = ni /N, where ni is the total number of organisms of a particular fauna taxonomic group and N represents the total number of organisms of all taxonomic groups. Calculation of species richness (the number of present taxonomic groups) for each site was based on first-order Jack-knife estimator. The first-order Jack-knife richness was estimated as:

$$S_{\text{jackknife1}} = S_{\text{obs}} + f_1$$

Where S_{obs} is the total number of observed taxa per monolith and treatment (i.e. sugarcane and forest stands) and f_1 is the number of singleton taxa (represented by a single read in a community). According to Walther and Moore (2005) Jack-knife estimators have a better estimator performance than other richness estimators. Soil fauna abundance was calculated as number of individuals per square meter.

3.3.5.2 Data analysis

Soil fauna abundance data was subjected to the software EstimateS 9.1.0 for statistical estimation of species diversity and richness (Colwell, 2013). Soil fauna abundance, species diversity and richness data were subjected to analysis of variance (ANOVA) using Genstat 18th edition, followed by Fisher's post hoc test to separate treatment means. The data was compared to check whether abundance, species diversity and richness varied with sampling period, soil type and reforestation age. Soil fauna abundance data (Nos m^{-2} = number of individuals per square meter) was subjected to principal component analysis (PCA) to differentiate treatments (i.e. 2, 4, 6-year-old forest stands and sugarcane) based on fauna collected in January and March. PCA was used to explore and determine variations in fauna abundance with sampling

period and land-use. Prior to PCA, soil fauna mean abundance data for both soils were standardized on Excel to equalize data variability. Sample scores along the first two principal components in two dimensions were plotted to explain variations. Genstat 18th edition was used for PCA.

3.4. Results

3.4.1 Effects of reforestation on soil properties

Tested soil chemical properties were affected by reforestation age (excluding TN), soil type and soil depth (except Ca, Mg, and C: N ratio) (Table 3.2). There was a significant interaction between reforestation age and soil type for Ca, clay, P and TN (Table 3.2). Soil pH, K, SOC and TN were affected by soil depth. Acrisols had higher Ca and Mg concentration compared to Leptosols (Table 3.3). The concentration of Ca and Mg decreased significantly in the 2-year-old forest stand compared to the control site. In Leptosols, the concentration of Ca and Mg increased significantly in the 6-year-old forest stand compared to the 2- and 4-year-old forest stands. However, the control had the greatest concentrations of Ca and Mg compared with reforested soil. Leptosols had higher K concentration than Acrisols (Table 3.3). The concentration of K increased significantly in the 2-year-old forest stand compared to the control site and decreased with increasing reforestation age in both soils. Acrisols had higher pH with a mean of 6.7 compared to Leptosols with a mean of 5.8.

The 6-year-old forest stand had higher pH level when compared to the control site in both studied soils (Table 3.3). Acrisols had high soil organic carbon content than (mean of 31.4 g kg^{-1}) Leptosols (mean of 24.9 g kg^{-1}. Soil organic carbon declined in the 2-year-old forest stand compared to the control site in both soils (Table 3.3). Acrisols had higher TN content compared to Leptosols. Total nitrogen content in the soil was not affected by reforestation; means did not differ statistically across forest stands of different ages (Table 3.3). Reforestation significantly affected carbon to nitrogen ratio (C: N) (Table 3.3). Carbon to nitrogen ratio was significantly higher in the 4-year-old forest stand compared to the 2- and 6-year old stand for both soils (Table 3.3). The 4-year-old forest stand has the highest C: N ratio compared to other forest stands in Acrisols and the other forest stands did not differ statistically (Table 3.3). In Leptosols, t was ranked as sugarcane > 4-year-old > 2-year-old and 6-year-old forest stand respectively (Table 3.3). Clay percentage varied with reforestation age (Table 3.3).

Table 3.2: Analysis of variance for different soil parameters as affected by forest stand age, soil type and depth after reforestation of abandoned sugarcane land in Buffelsdraai, Durban

Source of variation	DF	Ca	K	Mg	P	pH-H_2O	Clay	C:N	SOC	TN
Forest stand age	3	***	***	***	***	***	***	**	***	ns
Soil type	1	***	***	***	***	***	ns	**	***	***
Soil depth	1	ns	***	ns	**	*	ns	ns	***	*
Forest stand age.Soil type	3	*	ns	ns	***	ns	***	*	ns	*
Forest stand age.Soil depth	3	ns	ns	ns	*	***	ns	ns	ns	ns
Soil type.Soil depth	1	ns	ns	ns	ns	***	ns	ns	ns	ns
Forest stand age.Soil type.Soil depth	3	ns	ns	ns	ns	ns	ns	ns	ns	ns

DF = Degrees of Freedom, Ca = Calcium, K = Potassium, Mg = Magnesium, P = Phosphorus, pH_2O = pH measured in water, Clay = clay percentage, C: N= carbon to nitrogen ratio, SOC = Soil Organic Carbon and TN = Total Nitrogen.

$p < 0.001$ = *** (highly significant), $p < 0.01$ = ** (moderately significant), $p < 0.05$ = * (significant), $p > 0.05$ = ns (not significant).

Table 3.3: The effect of reforestation and soil type on soil properties in the 0-20 cm depth following reforestation of abandoned sugarcane land in Buffelsdraai, Durban

Treatment	pH	P	Ca	K	Mg	Clay	C:N	Organic C	Total N
	H_2O	$mg.L^{-1}$		$cmol_{+}.kg^{-1}$		%		$gk.g^{-1}$	
Acrisols									
Control	6.38 ± 0.12 ^{Ab}	2.60 ± 0.10 ^{Bc}	12.04 ± 1.20 ^{Aa}	0.17 ± 0.03 ^{Bbc}	10.20 ± 0.95 ^{Aa}	43.58 ± 2.03 ^{Ba}	14.59 ± 0.91 ^{Bb}	37.45 ± 2.88 ^{Aa}	2.60 ± 0.20 ^{Aa}
2-year-old	6.97 ± 0.11 ^{Aa}	5.00 ± 0.99 ^{Bbc}	7.00 ± 0.70 ^{Ab}	0.46 ± 0.03 ^{Ba}	7.69 ± 1.02 ^{Ab}	37.32 ± 2.31 ^{Abc}	13.82 ± 1.62 ^{Bb}	25.27 ± 2.80 ^{Ab}	1.87 ± 0.15 ^{Bb}
4-year-old	6.61 ± 0.20 ^{Ab}	8.30 ± 1.77 ^{Ba}	7.30 ± 0.51 ^{Ab}	0.38 ± 0.05 ^{Ba}	5.89 ± 0.75 ^{Ad}	41.54 ± 2.27 ^{Bab}	20.56 ± 1.18 ^{Ba}	36.34 ± 4.98 ^{Ab}	1.80 ± 0.26 ^{Bb}
6-year-old	7.00 ± 0.21 ^{Aa}	7.10 ± 1.10 ^{Bb}	6.68 ± 0.45 ^{Ab}	0.31 ± 0.02 ^{Bab}	7.05 ± 0.70 ^{Abc}	35.36 ± 4.47 ^{Bc}	13.65 ± 1.45 ^{Bb}	26.37 ± 3.73 ^{Bb}	1.90 ± 0.18 ^{Aab}
Leptosols									
Control	5.69 ± 0.09 ^{By}	5.00 ± 1.09 ^{Bz}	7.96 ± 1.28 ^{Bx}	0.51 ± 0.14 ^{Axy}	6.87 ± 1.00 ^{Bx}	56.40 ± 2.69 ^{Ax}	30.76 ± 3.77 ^{Ax}	27.34 ± 2.67 ^{Bx}	0.95 ± 0.15 ^{By}
2-year-old	5.79 ± 0.11 ^{Bxy}	7.50 ± 1.01 ^{By}	2.32 ± 0.41 ^{Bz}	0.63 ± 0.07 ^{Ax}	3.68 ± 1.25 ^{Byz}	31.84 ± 3.34 ^{By}	16.36 ± 3.92 ^{Bz}	14.46 ± 1.54 ^{By}	1.33 ± 0.47 ^{Bxy}
4-year-old	5.65 ± 0.09 ^{By}	10.07 ± 1.35 ^{Ax}	2.20 ± 0.25 ^{Bz}	0.61 ± 0.04 ^{Ax}	2.02 ± 0.17 ^{Bz}	39.43 ± 1.05 ^{Bxy}	22.43 ± 0.93 ^{By}	28.57 ± 2.46 ^{Bx}	1.27 ± 0.07 ^{Bxy}
6-year-old	5.98 ± 0.08 ^{Bx}	8.55 ± 1.25 ^{Bxy}	4.86 ± 0.31 ^{By}	0.45 ± 0.03 ^{Ay}	4.36 ± 0.18 ^{By}	39.21 ± 2.25 ^{Bxy}	16.18 ± 2.50 ^{Bz}	27.64 ± 2.43 ^{Bx}	1.87 ± 0.26 ^{Bx}

pH_2O = pH measured in water, P = Phosphorus, Ca = Calcium, K = Potassium, Mg = Magnesium, Clay = clay percentage, C: N= carbon to nitrogen ratio SOC = Soil Organic Carbon and TN = Total Nitrogen. Data are means ± standard error.
Different upper-case letters in columns represent significant differences between soil types within the respective forest age stands.
Different lower-case letters within the column show significant differences of forest age stands within the same soil type

3.4.2 Total fauna abundances

Abundance for all fauna combined was not affected by reforestation age, but rather by sampling period and soil type (Table 3.4). Sampling period affected soil fauna abundance; with greater abundance of fauna in January, which was 76.0 % higher than in March (Fig. 3.3). Soil fauna abundance was higher in Acrisols compared to Leptosols (Fig. 3.4). Overall the most dominant faunal groups were ants (hymenoptera), comprising of 38.3% of individuals sampled in the study followed by termites (isoptera) then pot worms (enchytraeidae) (Table 3.5).]Termites consisted of 28.9 % of individuals sampled in January and 20% in March (Table 3.5) . Pot worms consisted of 27.1 % of individuals sampled in the beginning of rainy season and 14.5 % in the middle of the rainy season (Table 3.5). Earthworms (megadrilacea) were present in the middle of the rainy season, comprising of 15.7 % of the sampled invertebrates (Table 3.5). Beetles (coleoptera) accounted for 4.7 % of individuals in January and 14.1 % in March (Table 3.5).

Table 3.4: Analysis of variance for soil fauna abundance, diversity and richness as affected by forest stand age, soil type and sampling period following reforestation of abandoned sugarcane land in Buffelsdraai, Durban

Source of variation	DF	Abundance	Diversity	Richness
Forest stand age	3	ns	ns	ns
Soil type	1	*	***	***
Sampling period	1	***	***	ns
Forest stand age x Soil type	3	ns	*	ns
Forest stand age x Sampling period	3	ns	ns	ns
Soil type x Sampling period	1	ns	ns	**
Forest stand age x Soil type x Sampling period	3	ns	*	ns

DF = Degrees of Freedom

$p < 0.001$ = *** (highly significant), $p < 0.01$ = ** (moderately significant), $p < 0.05$ = * (significant), $p > 0.05$ = ns (not significant).

Table 3.5: Total number of individuals collected by monoliths in different forest stand ages (2-, 4- and 6-year-old) and sugarcane (control) under Acrisols and Leptosols during two sampling periods (January and March) in Buffelsdraai, Durban.

Taxonomic group	Control		2-year-old		4-year-old		6-year-old		**Total**
	Acrisols	Leptosols	Acrisols	Leptosols	Acrisols	Leptosols	Acrisols	Leptosols	
					January				
Coleoptera	7	1	15	3	2	2	3	6	39
Enchytraeidae	72	5	16		98	2	34	0	227
Hymenoptera	71	18	14	38	65	52	60	11	329
Isoptera	0	28	7	0	37	2	72	96	242
Megadrilacea	0	0	0	0	0	0	0	0	0
					March				
Coleoptera	5	1	5	8	0	1	2	13	35
Enchytraeidae	6	7	3	0	16	0	4	0	36
Hymenoptera	7	6	10	14	1	5	35	9	87
Isoptera	2	0	6	0	27	4	4	9	52
Megadrilacea	2	0	7	0	21	0	9	0	39
Total	**172**	**66**	**83**	**63**	**267**	**68**	**223**	**144**	**1086**

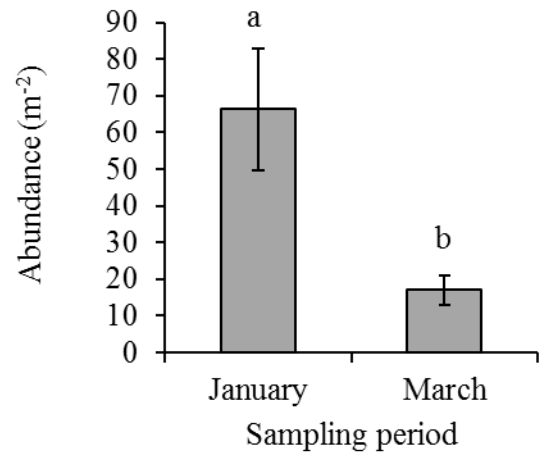

Figure 3.3: Effect of sampling period on abundance of soil macrofaunal groups as shown by number of individuals per square meter (m^{-2}) in Buffelsdraai, Durban. Error bars show standard error and different lower-case letters above bars show statistical difference using Fisher's test.

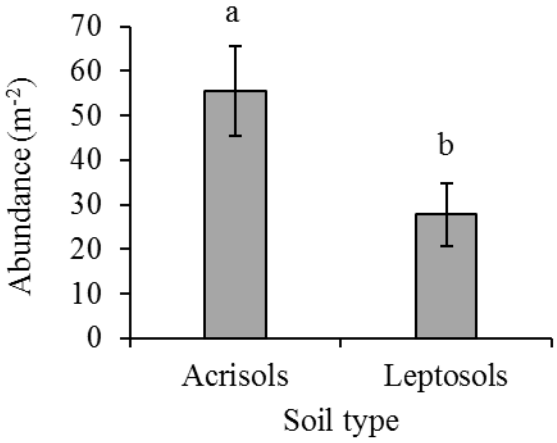

Figure 3.4: Effect of soil type on abundance of soil macrofaunal groups as shown by number of individuals per square meter (m^{-2}) in Buffelsdraai, Durban. Error bars show standard error and different lower-case letters above bars show statistical difference using Fisher's test.

3.4.2.1 Fauna community composition

From the principal component analysis, the first and second components explained 41.5% and 26.7% of the variability of species abundance, respectively, and together they explained 68.12 % of total variability. A biplot of principal component 1(PC-1) and principal component 2 (PC-2) shows scores for both soils across all treatments in both sampling periods (Fig. 3.5). The biplot showed that the 4- and 6-year-old forest stands and sugarcane in January expressed a highly interactive behaviour (PCA scores beyond ±)). In March, the 4- year-old forest stand also had a highly interactive behaviour, their PCA scores were beyond (±) 1. In January, the 2-year-old site was closer to the centre of origin; the site had lower interaction with fauna. Among all the studied sites, a separation of sampling periods was observed (Fig. 3.5). January sampling was on the right-hand side of the diagram and the opposite was true for March sampling (Fig. 3.5). Suggesting that abundance of fauna was mainly influenced by rainfall amount. Four sectors were formed by drawing lines, the 2-, 4- and 6-year-old stands in January fell into one sector. They were more related to higher abundances of ants, pot worms and termites. The second sector contained the control site, which was related to dominance of ants and pot worms during January sampling. The third sector had the control site and 4-year-old forest stand, showing reduction in abundance of all studied taxonomic groups during March sampling. Whereas the last sector contained the 2- and 6-year-old forest stands; associated with increased abundance of beetles and earthworms and highest abundance of ants during March sampling.

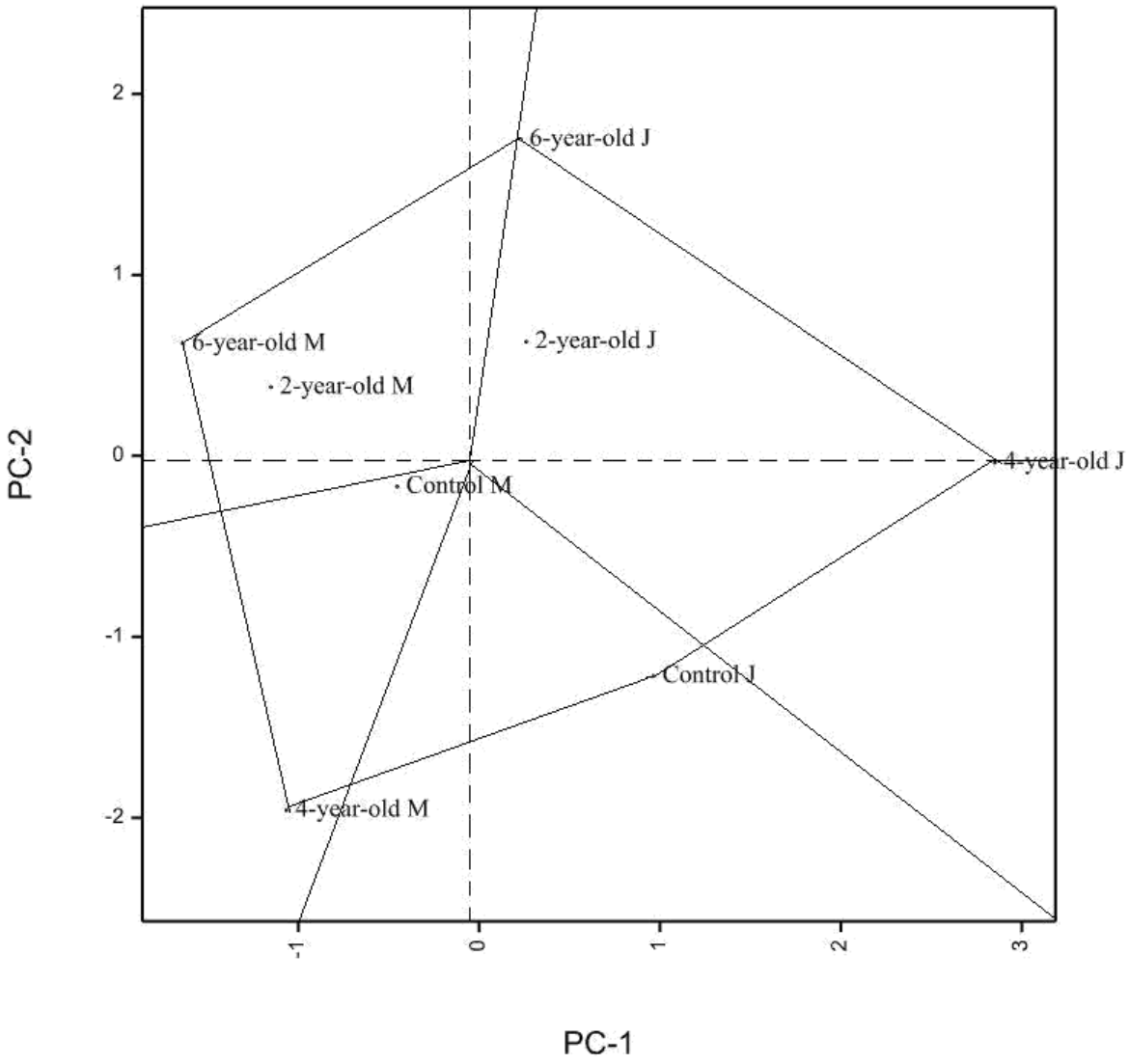

Figure 3.5: Principal component analysis biplot showing the patterns of soil macrofauna abundance in the 2, 4, 6-year-old forest stands and control site (sugarcane) in relation to sampling period (J: January and M: March) in Buffelsdraai, Durban.

3.4.3 Species diversity

The overall fauna diversity had no significant difference across forest stands of different ages and sugarcane (Table 3.4). However, sampling period and soil type significantly affected fauna diversity (Table 3.4). Shannon diversity index was significantly higher in Acrisols compared to Leptosols (Fig. 3.6). It was also higher in March compared to January for both soils (Fig. 3.6).

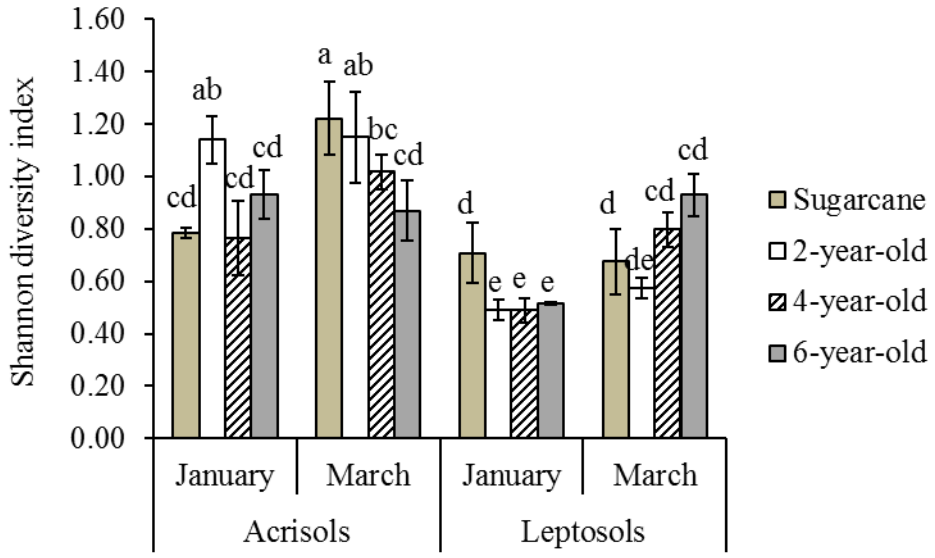

Figure 3.6: Effects of forest stand age, soil type and sampling period on fauna diversity as shown by the Shannon diversity index in Buffelsdraai, Durban. Error bars show standard error and different lower-case letters above bars show statistical difference using Fisher's test.

3.4.4 Soil fauna richness

Soil fauna richness was not affected by reforestation age but by soil type (Table 3.4). Soil fauna richness was significantly higher in Acrisols than Leptosols. There was a significant interaction between soil type and sampling period (Table 3.4). In January sampling, soil fauna richness found in Acrisols and Leptosols did not differ statistically (Fig. 3.7). Acrisols had higher soil fauna richness compared to Leptosols in March (Fig. 3.7).

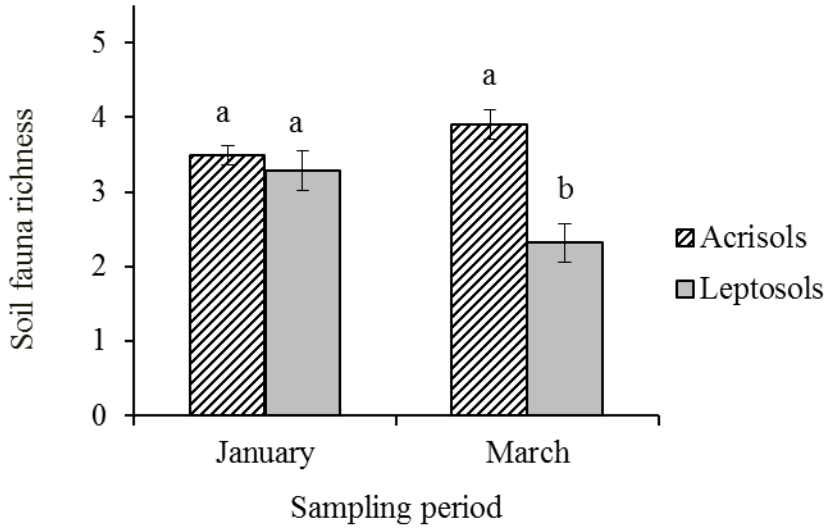

Figure 3.7: Effect of sampling period and soil type on soil fauna richness in Buffelsdraai, Durban. Error bars show standard error and different lower-case letters above bars show statistical difference using Fisher's test.

3.5 Discussion

3.5.1 Soil fauna abundance

It was hypothesized that there would be differences in fauna abundance with reforestation age, but that was not the case. Contrary to the a study by Rosario et al. (2014) which showed that abundance of insect fauna varied with reforestation age, reforestation did not have a significant effect on soil fauna abundance. The lack of differences in fauna abundance in the present study could be attributed to less developed tree canopy cover. Mugwedi et al. (2017) assessed the success of the Buffelsdraai Landfill Site Community Reforestation Project on similar sites as the current study by determining ecological attributes which included vegetation structure and reported that restored sites had less developed tree canopy cover (<50%) . This may have contributed to the lack of differences in soil fauna abundance. Tree canopy cover provides favourable conditions for fauna by reducing solar radiation, thus allowing soil water retention and less temperature fluctuations (Rosario et al., 2014). According to Mugwedi et al. (2017) a fully developed canopy cover can be reached within two decades and it is expected that canopy cover will increase with reforestation age and this may increase soil fauna abundance.

The current study and the study by Rosario et al. (2014) were based on young forests (<6-years-old). However, differences in soil type, climate and tree species could have caused the

difference in findings of the two studies. Tree species used in the study were fast growing, suggesting that soil type and climate could have affected canopy cover, hence fauna abundance. The shallower Leptosols combined with 766 mm of average rainfall per year in the study site may have led to water stress, which may have retarded tree growth, consequently low canopy cover. Compared to the current study the soils in the Amazon region were deeper and the region had higher rainfall (an average rainfall of 1,766 mm year^{-1}), ultimately high soil moisture which improves tree growth and leads to more developed canopy cover (Rosario et al., 2014). Toledo et al. (2011) reported that growth rate variation in Bolivian lowland forests was influenced by climate rather than soil and disturbance, tree growth rates increased with rainfall. Deeper soils and high water availability could have enhanced tree growth rates in the Amazon region, thus allowing the parica (*Schizolobium parahyba Barneby*) trees to have a greater canopy cover. The canopy cover of parica trees may have contributed in higher abundance of fauna, as a result of reduced soil temperature and water fluctuations (Rosario et al., 2014). Nevertheless, the total number of termites was significantly higher in the 6-year-old forest (Table 3.5) stand compared to other forest stands and sugarcane, suggesting that the 6-year-old forest stand is in good repair. Termites were more abundant in the 6-year-old forest stand for both soils and were associated with high soil pH in Leptosols. Termites are known to improve soil pH and soil fertility through decomposition of organic matter and accumulation of nutrient and organic matter on mounds (Jembere et al., 2017). Decomposition of litter releases nutrients, thus improving nutrient turnover and availability in the soil. This can explain the observed higher pH and calcium concentration in the 6-year-old forest stand.

Higher abundance of soil fauna was observed in Acrisols compared to Leptosols (Fig. 3.4), attributed to higher moisture content in Acrisols. The activity and survival of fauna such as earthworms and pot worms is favoured in moist soils, consequently, low soil moisture, such as in the Leptosols, may have negative effects on fauna abundance (Simpson et al., 2012). Unlike Acrisols, Leptosols are conditioned by topography, they are found on steep slopes with shallow depth and this reduces their water holding capacity (Driessen et al., 2000). The principal component analysis showed a separation of sampling periods, with greater fauna abundance at the beginning of the rainy season in January than the middle of the season in March (Fig. 3.5). This was likely because of higher soil moisture as a result of higher rainfall received at the beginning of the rainy season in January than the middle of the season in March (Fig. 3.2). According to Jawaheer et al. (2015) soil moisture affects soil fauna functions including growth and reproduction. This suggests that soil moisture had positive influence on soil fauna

functions, which led to greater fauna abundance at the beginning of the rainy season in January. Studies have shown that seasonality has an influence on soil fauna abundance, rainy seasons are associated with high fauna abundance compared to dry seasons (Muchane et al., 2012, Rosario et al., 2014, Siqueira et al., 2014). A significant decline in fauna abundance was observed during March sampling and this could be attributable to lower soil moisture due to decreased rainfall amount. According to Muchane et al. (2012), increased soil moisture in the rainy season results in increased availability of organic matter due to increased decomposition and greater root biomass. In turn, this might increase fauna abundance since organisms such as endogeic earthworms and soil-feeding termites feed mainly on soil organic matter (Bot and Benites, 2005). Abundance of enchytraeidae (pot worms) was higher in January compared to March. Pot worms have been shown to require moist environments for their survival, their survival could have been affected by soil moisture reduction as a result of rainfall reduction in March (Kamin, 2011).

Unlike other soil organisms, hymenoptera (ants) and isoptera (termites) can survive in dry environments by modifying the soil (through nest construction) to create conditions that are more favourable for their survival (Culliney, 2013). Generally termites are easily desiccated, nests provide ideal microclimate and shelter for both adults and brood, thus improving their survival. This could explain the higher abundance of ants and termites in March compared to other soil fauna groups. It was expected that the abundance of megadrilacea (earthworms) would be high in January as a result of high soil moisture influenced. The presence of earthworms in March was contrary to what was expected. Compared to other fauna groups; they had the least occurrence in the study (Table 3.5). According to Cunningham et al. (2015), changes in earthworms associated with reforestation can only be observed after three decades. In this case, the reforestation is at early stages. As time progresses, changes may occur in size and composition of earthworm populations.

Ants were the most abundant fauna group, followed by termites across sampling periods (Table 3.5).These findings were in accordance with previous studies that showed higher abundance of ants under recovering habitats in the first 5 years of reforestation (Rosario et al., 2014). According to Vasconcellos et al. (2013), ants are the most abundant group of fauna in soils, because they have the ability to utilize a variety of resources and are usually associated with recovering habitats. The high number of ants in Acrisols (Table 3.5) could have led to increased soil pH and concentrations of calcium and magnesium (Table 3.3) observed in these soils. It has been reported that ants improve soil fertility through accumulation of plant material, which

leads to higher nutrient accumulation (Farji-Brener and Werenkraut, 2017, Frouz and Jilková, 2008, Jawaheer et al., 2015).

3.5.2 Soil fauna diversity

The Shannon diversity index showed variation in fauna diversity with sampling period, with greater fauna diversity in March than January (Fig. 3.6). This suggests that the response was mainly associated with community structure differences, meaning that the distribution of taxonomic groups and abundance varied). Decreased dominance of soil fauna groups such as enchytraeidae, hymenoptera and isoptera (Table 3.5) in March resulted in higher community equitability, thus increasing fauna diversity. Soil fauna diversity in Leptosols could have been low as a consequence of low vegetation cover which was observed on the site and low soil moisture compared to Acrisols. Low vegetation cover exposes soil to solar radiation causing evaporation from the soil surface and consequently reduces soil moisture (Rosario et al., 2014), thus affecting soil fauna diversity (Jawaheer et al., 2015).

3.5.3 Soil fauna richness

Soil fauna richness varied with sampling period in Leptosols it was higher in January compared to March (Fig. 3.7). High soil moisture in January as a result of rainfall created favourable environmental conditions for fauna, therefore supporting more faunal groups. Cunha Neto et al. (2012) also reported that in the rainy season, high rainfall led to greater food availability and microenvironments, therefore supporting more faunal groups. The findings of the current study were in agreement with Rosario et al. (2014) , who observed higher species richness in the rainy season compared to the dry season for all reforestation ages assessed in the study. In March, soil fauna richness was lower in Leptosols compared with Acrisols. Acrisols had high soil moisture and understory vegetation of grass, this created a more habitats for fauna and an environment with less soil moisture fluctuation supporting more fauna groups compared with Leptosols (Cunha Neto et al., 2012).

3.6 Conclusion

The study indicated that after 6 years of reforestation, reforested soils were still more similar to soils under sugarcane with respect to fauna abundance, diversity and richness. However, the 6-year-old forest stand gave better abundance of isoptera suggesting that the site is in good recovery. This also suggests that isoptera has high potential to serve as a soil quality indicator.

The hymenoptera and isoptera were the most dominant orders in the study. Soil type and sampling period has significant effects on fauna abundance, diversity and richness. Higher rainfall in January resulted in creation of favourable conditions for fauna, thus increasing abundance. Reduction in rainfall decreased the dominance of enchytraeidae, hymenoptera and isoptera; which led to higher diversity in March. Soil pH and TN content were significantly higher in the 6-year-old forest stand under Leptosols compared to sugarcane and other forest stands. On the other hand, sugarcane showed higher Ca and Mg concentrations compared to reforested soils. The results of this study showed that SOC, TN and nutrient status decreased following reforestation in both soils. This suggests that clearing land upon reforestation has negative effects on soil quality

References

ANDERSON, J. M. & INGRAM, J. 1993. *Tropical Soil Biology and Fertility: A Handbook of Method,* CAB International.

BALVANERA, P., QUIJAS, S., MARTIN-LOPEZ, B., BARRIOS, E., DEE, L., ISBELL, F., DURANCE, I., WHITE, P., BLANCHARD, R. & DE GROOT, R. 2015. The links between biodiversity and ecosystem services. *Handbook of Ecosystems Services.* Earthscan-Routledge.

BARRIOS, E. 2007. Soil biota, ecosystem services and land productivity. *Ecological Economics,* 64**,** 269-285.

BARROS, E., PASHANASI, B., CONSTANTINO, R. & LAVELLE, P. 2002. Effects of land-use system on the soil macrofauna in western Brazilian Amazonia. *Biology and Fertility of Soils,* 35**,** 338-347.

BIANCHI, S. R., MIYAZAWA, M., OLIVEIRA, E. L. D. & PAVAN, M. A. 2008. Relationship between the mass of organic matter and carbon in soil. *Brazilian Archives of Biology and Technology,* 51**,** 263-269.

BOSSUYT, H., SIX, J. & HENDRIX, P. F. 2005. Protection of soil carbon by microaggregates within earthworm casts. *Soil Biology and Biochemistry,* 37**,** 251-258.

BOT, A. & BENITES, J. 2005. *The importance of soil organic matter: key to drought-resistant soil and sustained food production,* Food & Agriculture Org.

CARDOSO, E. J. B. N., VASCONCELLOS, R. L. F., BINI, D., MIYAUCHI, M. Y. H., SANTOS, C. A. D., ALVES, P. R. L., PAULA, A. M. D., NAKATANI, A. S., PEREIRA, J. D. M. & NOGUEIRA, M. A. 2013. Soil health: looking for suitable indicators. What should be considered to assess the effects of use and management on soil health? *Scientia Agricola,* 70**,** 274-289.

COLWELL, R. 2013. EstimateS: Statistical estimation of species richness and shared species from samples. Version 9. Persistent URL <purl.oclc.org/estimates>.

COOPER, T. J. G. 2015. *The effects of land use changes on the distribution of forest dependent bird species in South Africa.* Stellenbosch University.

CULLINEY, T. W. 2013. Role of arthropods in maintaining soil fertility. *Agriculture,* 3**,** 629-659.

CUNHA NETO, F. V. D., CORREIA, M. E. F., PEREIRA, G. H. A., PEREIRA, M. G. & LELES, P. S. D. S. 2012. Soil fauna as an indicator of soil quality in forest stands, pasture and secondary forest. *Revista Brasileira de Ciência do Solo,* 36**,** 1407-1417.

CUNNINGHAM, S. C., MAC NALLY, R., BAKER, P. J., CAVAGNARO, T. R., BERINGER, J., THOMSON, J. R. & THOMPSON, R. M. 2015. Balancing the environmental benefits of reforestation in agricultural regions. *Perspectives in Plant Ecology, Evolution and Systematics,* 17**,** 301-317.

DAJOZ, R. 1998. *Insects and the forest: role and diversity of insects in the forest environment*, Technique et Documentation Lavoisier.

DANGERFIELD, J. 1997. Abundance and diversity of soil macrofauna in Northern Botswana. *Journal of Tropical Ecology,* 13**,** 527-538.

DOUWES, E., ROY, K. E., DIEDERICHS-MANDER, N., MAVUNDLA, K. & ROBERTS, D. 2015. The Buffelsdraai Landfill Site Community Reforestation Project: Leading the way in community ecosystem-based adaptation to climate change. Durban, South Africa.

DORAN, J.W., SAFLEY, M., 1997. Defining and assessing soil health and sustainable productivity. In: Pankhurst, C., Doube, B.M., Gupta, V.V.S.R. (Eds.), Biological Indicators of Soil Health. CAB International, Wallingford, Oxon, UK, pp. 1–28

DRIESSEN, P., DECKERS, J., SPAARGAREN, O. & NACHTERGAELE, F. 2000. *Lecture notes on the major soils of the world*, Food and Agriculture organization (FAO).

ETHEKWINI MUNICIPALITY, 2011. Buffelsdraai Community Reforestation Project. [online]Availableat:http://www.durban.gov.za/City_Services/development_planning_management/environmental_planning_climate_protection/Projects/Pages/Buffelsdraai-Community-Reforestation-Project.aspx [Accessed 21 February 2016]+

FARINA, M. P. W. 1981. The Hunter system of soil analysis. . *Fertilizer Society of South Africa Journal,* 1**,** 39-41.

FARJI-BRENER, A. G. & WERENKRAUT, V. 2017. The effects of ant nests on soil fertility and plant performance: a meta-analysis. *Journal of Animal Ecology,* 86**,** 866-877.

FEY, M. V. 2010. Soils of South Africa. Cambridge University Press, Cape Town.

FROUZ, J. & JILKOVÁ, V. 2008. The effect of ants on soil properties and processes (Hymenoptera: Formicidae). *Myrmecological News,* 11**,** 191-199.

GEE, G. W., BAUDER, J. W. & KLUTE, A. 1986. Particle-size analysis. *Methods of soil analysis. Part 1. Physical and mineralogical methods***,** 383-411.

HUNTER, A. New techniques and equipment for routine soil-plant analytical procedures. 2. Seminario sobre Manejo de Suelos y el Proceso de Desarrollo en America Tropical, Cali (Colombia), 10 Feb 1974, 1975.

JAWAHEER, Z., SINGH, H. & GANESHAN, S. 2015. Effect of Soil Parameters on the Distribution of Soil Fauna from Roadside Trees at Three Elevations in Mauritius. *Entomology, Ornithology & Herpetology,* 4**,** 1.

JEMBERE, A., BERECHA, G. & TOLOSSA, A. R. 2017. Impacts of termites on selected soil physicochemical characteristics in the highlands of Southwest Ethiopia. *Archives of Agronomy and Soil Science***,** 1-9.

JILKOVA, V., FROUZ, J., DOMISCH, T. & FINER, L. 2010. The effect of wood ants (Formica s. str.) on soil chemical and microbiological properties. *Published on DVD*.

KAMIN, T. 2011. Factors that Affect the Make-up of Soil Invertebrate Community. *ESSAI*, 8.

LAVELLE, P., DANGERFIELD, M., FRAGOSO, C., ESCHENBRENNER, V., LÓPEZ-HERNÁNDEZ, D., PASHANASI, B. & BRUSSAARD, L. 1994. The relationship between soil macrofauna and tropical soil fertility. *In:* WOOMER, P. L. & SWIFT, M. J. (eds.) *The biological management of tropical soil fertility.* Chichester Wiley

LAVELLE, P., SPAIN, A., BLANCHART, E., MARTIN, A. & MARTIN, S. 1992. Impact of soil fauna on the properties of soils in the humid tropics. *SSSA Special Publication,* 29**,** 157-157.

LAVELLE, P. & SPAIN, A. V. 2005. *Soil ecology*, Dordrecht; Boston, Kluwer Academic Publishers.

MUCHANE, M. N., KARANJA, D., WAMBUGU, G. M., MUTAHI, J. M., CLET, W., MUGOYA, C. & MUCHAI, M. 2012. Land use practices and their implications on soil macro-fauna in Maasai Mara ecosystem. *International Journal of Biodiversity and Conservation,* 4**,** 500-514.

MUGWEDI, L. F., ROUGET, M., EGOH, B., RAMDHANI, S., SLOTOW, R. & RENTERÍA, J. L. 2017. An Assessment of a Community-Based, Forest Restoration Programme in Durban (eThekwini), South Africa. *Forests,* 8**,** 255.

PETERSEN, C. R., JOVANOVIC, N. Z., LE MAITRE, D. C. & GRENFELL, M. 2017. Effects of land use change on streamflow and stream water quality of a coastal catchment. *Water SA,* 43**,** 139-152.

ROSARIO, V. S. V., BATISTA, T. F. V., PROVENZANO, R., LEMOS, L. J. U., DOS SANTOS, J. D. V. & LUNZ, A. M. 2014. Edaphic insect fauna associated with reforestation with Schizolobium parahyba Barneby in Amazonia. *Amazonian Journal of Agricultural and Environmental Sciences,* 57**,** 373-381.

SIMPSON, J. E., SLADE, E., RIUTTA, T. & TAYLOR, M. E. 2012. Factors affecting soil fauna feeding activity in a fragmented lowland temperate deciduous woodland. *PLoS One,* 7**,** e29616.

SIQUEIRA, G., SILVA, E. & PAZ-FERREIRO, J. 2014. Land use intensification effects in soil arthropod community of an Entisol in Pernambuco State, Brazil. *The Scientific World Journal,* 2014.

SOIL CLASSIFICATION WORKING GROUP 1991. *Soil classification: A taxonomic system for South Africa,* Pretoria, South Africa., Department of Agricultural Development.

TOLEDO, M., POORTER, L., PEÑA-CLAROS, M., ALARCÓN, A., BALCÁZAR, J., LEAÑO, C., LICONA, J. C., LLANQUE, O., VROOMANS, V. & ZUIDEMA, P. 2011. Climate is a stronger driver of tree and forest growth rates than soil and disturbance. *Journal of Ecology,* 99**,** 254-264.

VASCONCELLOS, R. L., SEGAT, J. C., BONFIM, J. A., BARETTA, D. & CARDOSO, E. J. 2013. Soil macrofauna as an indicator of soil quality in an undisturbed riparian forest and recovering sites of different ages. *European Journal of Soil Biology,* 58**,** 105-112.

WALTHER, B. A. & MOORE, J. L. 2005. The concepts of bias, precision and accuracy, and their use in testing the performance of species richness estimators, with a literature review of estimator performance. *Ecography,* 28**,** 815-829.

WARBURTON, M. L., SCHULZE, R. E. & JEWITT, G. P. 2012. Hydrological impacts of land use change in three diverse South African catchments. *Journal of Hydrology,* 414**,** 118-135.

WHALEN, J. K. & SAMPEDRO, L. 2010. *Soil ecology and management,* CABI.

WINSOME, T. 2005. Fauna. *Encyclopedia of Soils in the Environment, Four-Volume Set.*

WITT, B. 1997. Using soil fauna to improve soil health. *Student On-Line Journal,* 2**,** 1-5.

WRIGHT, A. F. & BAILEY, J. S. 2001. Organic carbon, total carbon, and total nitrogen determinations in soils of variable calcium carbonate contents using a Leco CN-2000 dry combustion analyzer. *Communications in Soil Science and Plant Analysis,* 32**,** 3243-3258.

XIANG, Y., CHENG, M., HUANG, Y., AN, S. & DARBOUX, F. 2017. Changes in soil microbial community and its effect on carbon sequestration following afforestation on the Loess Plateau, China. *International Journal of Environmental Research and Public Health,* 14**,** 948.

CHAPTER FOUR: CHANGES IN SOIL ENZYME ACTIVITY AND PHYSICAL PROPERTIES ASSOCIATED WITH REFORESTATION IN BUFFELSDRAAI, DURBAN

Abstract

Reforestation was established in 2008 in the buffer zone of Buffelsdraai landfill site to offset carbon emissions associated with hosting the 2010 FIFA World Cup. The objective of this study was to determine effects of reforestation on soil enzyme activities and physical properties. Soil enzyme activities (dehydrogenase, fluorescein diacetate hydrolase, β-glucosaminidase and β-glucosidase) and physical properties (aggregate stability, bulk density and infiltration) were assessed in a chronosequence of 2-, 4- and 6-years after reforestation of former degraded sugarcane land in Buffelsdraai, Durban. These were compared to the degraded sugarcane land. Soil samples were taken from Acrisols and Leptosols in the 0-20 cm depth. Soil bulk density was higher in the 2-year-old forest stand compared to sugarcane for both soils. Bulk density was higher in Leptosols (1.53 g cm^{-3}) compared to Acrisols (1.35 g cm^{-3}).In Acrisols, bulk density increased with increasing reforestation age. However, in Leptosols bulk density was lower in the 4- and 6-year-old forest stands compared to sugarcane. Aggregate mean weight diameter was higher in the oldest forest stand compared to sugarcane, it was ranked in the order of 6-year- old (3.30 mm)> sugarcane (2.73 mm)> 4-year-old (2.54 mm)> 2-year-old forest stand (2.21 mm). The 6-year-old forest stand had the highest infiltration rate compared to other forest stand and sugarcane, it was ranked in the order of 6-year- old (34.33 mm min^{-1})> 4-year-old (23.31 mm min^{-1})> sugarcane (6.85 mm min^{-1})> 2-year-old forest stand (4.07 mm min^{-1}). Dehydrogenase activity was greater in Acrisols compared to Leptosols. In Acrisols, the 4-year-old forest stand had the highest activity compared to other forest stands and sugarcane. Fluorescein diacetate hydrolase activity was significantly higher in the 6-year-old forest stand compared to sugarcane for both soils. Overall, β-glucosaminidase activity was higher in soils under sugarcane compared to reforested soils. However, β-glucosaminidase activity was higher in the 6-year-old forest stand compared to other forest stands. The activity of β-glucosidase was not affected by land use, rather it decreased with soil depth (0-10 cm = 0.23 µg PNG g^{-1} soil h^{-1} and 10-20 cm =0.13 µg PNG g^{-1} soil h^{-1}). Therefore, more mature forest stands are required to detect changes in β-glucosidase activity following reforestation of sugarcane land. The study reveals an initial decline in mean weight diameter, infiltration, fluorescein diacetate hydrolase activity and β-glucosaminidase activity upon reforestation. Lower soil bulk densities with increasing reforestation age in Acrisols and increasing trends of

mean weight diameter, infiltration, fluorescein diacetate hydrolase activity and β-glucosaminidase activity with reforestation age suggest that reforestation can improve soil quality and the site is in good repair.

4.1 Introduction

In South Africa, particularly on the north and south coasts as well as in the midlands of KwaZulu-Natal province, sugarcane production is one of the major land use types (Dominy et al., 2001, Qongqo and Van Antwerpen, 2000). Sugarcane is also produced in Eastern Cape and Mpumalanga provinces and it generates an income of approximately R5 billion (Media, 2017). Sugarcane is the most cultivated crop in KwaZulu-Natal, followed by maize and dry beans and it contributes 0.5 to 0.7 % of the national gross domestic production (Media, 2017) Sugarcane production is however, associated with soil degradation including soil organic matter (SOM) reduction and increased soil erosion. As a result of organic matter degradation soil biological activity is reduced, thus affecting processes such as nutrient cycling and stabilization of soil aggregate which are important for both microbes and plants, and for regulation of soil erosion processes (Dominy et al., 2001, Qongqo and Van Antwerpen, 2000).

In 2008 the eThekwini Municipality initiated a project of planting indigenous trees in the buffer zone of its Buffelsdraai Landfill Site. The primary purpose of reforestation was to sequester carbon and aid recovery of degraded land and enhance biodiversity (eThekwini Municipality, 2011). The reforestation project involved planting various indigenous tree species including *Acacia Karroo* and *Bridelia micrantha*) on land previously under sugarcane production (eThekwini Municipality, 2011). The project was estimated to offset nearly 42 000 tonsCO_2eq carbon dioxide emissions, which was a quarter of the emissions anticipated to result from hosting the 2010 FIFA world cup. This offset was anticipated to be achieved over a period of 20 years after reforestation (eThekwini Municipality, 2011). Assessing reforestation effects on soil microbial characteristics may serve as an early indicator of carbon sequestration and may guide the city of the possible success or failure of the reforestation project.

Assessing soil microbial properties may serve as an early indicator of change in SOM and recovery of degraded land. Soil microbial biomass (SMB) is considered as a living component of SOM composed of different microorganisms (bacteria and fungi) and it comprises of 1 to 5 % of soil organic carbon (SOC) (Araújo et al., 2014, Brookes, 2001, Cardoso et al., 2013, Kaschuk et al., 2010). Since SMB is the living part of SOM, it responds quickly to environmental change(s) when compared to SOM (Brookes, 2001, Cardoso et al., 2013). Soil

organic carbon is the main component of SOM and changes caused by land use and management in SOM can be detected early through measuring SOC content or SMB, way before apparent changes in SOM (Brookes, 2001, Cardoso et al., 2013). As a result, SMB has been used as an early warning of change in properties of soils under forestry and agriculture (Cardoso et al., 2013, Kaschuk et al., 2010). In general SMB is related to carbon and nutrient cycling. Microbial biomass represents the labile pool of SOM and serves as a sink or source of nutrients (Cardoso et al., 2013). Soil organic carbon serves as a substrate for micro-organisms, as a result, a system with high SOC or easily degradable compounds leads to increased growth and activity of micro-organisms, consequently SMB increases (Jiang-shan et al., 2005). Therefore, the greater the SMB the healthier the soil. According to Doran and Safley (1997) a healthy soil is described as "The continued capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain biological productivity, promote the quality of air and water environments, and maintain plant, animal and human health". Healthy soils are important because they increase soil biodiversity and preserve vital soil ecological functions in the environment (Cardoso et al., 2013). Dominy et al. (2001) reported that long term production of sugarcane resulted in loss of SMB, this was associated with loss of SOM.

Microorganisms serve as a source of enzymes in the soil, which are responsible for cycling elements such as carbon (C), nitrogen (N), phosphorus (P) and sulphur (S) (Alkorta et al., 2003, Cardoso et al., 2013). Enzymes are related to chemical and physical soil properties, microbial community and activity. Enzyme activities change quickly than other soil quality indicators when ecological changes occur within the soil (Araújo et al., 2014, Das and Varma, 2011). Thus, enzyme activities are used as microbiological indicators of soil health and can also be used to measure microbial activity (Das and Varma, 2011, Trasar-Cepeda et al., 2008). In addition, measuring enzyme activities involves simple and cost-effective procedures and they are widely used by researchers to provide early indications of change(s) in the soil (Das and Varma, 2011, Trasar-Cepeda et al., 2008). In this study, 4 soil enzymes that are important in nutrient cycling were selected. The enzymes chosen were β-glucosidase which is involved in carbon cycle (Eivazi and Tabatabai, 1988) and β-glucosaminidase which is important in nitrogen cycling (Parham and Deng, 2000). Dehydrogenase and fluorescein diacetate (FDA) hydrolysis reflect the total activity of microorganisms in the soil (Rajper et al., 2016).

Previous studies have shown that reforestation of land previously under sugarcane has positive influence on microbial activity (da Silva et al., 2012). Research has shown that reforestation has a potential for increasing SOC accumulation. In Turkey, afforestation has been reported to

increase SOC, this was associated with higher input of organic material in the soil compared to bare land (Korkanç, 2014). Shi et al. (2015) reported higher SOC values after afforestation on the Qinghai Plateau in China. Since microbial parameters serve as early indicators there is need to assess them. However, it remains unknown how reforestation influences microbial activity in the early stages of reforestation in Buffelsdraai, Durban. Additionally, there is a general lack of information regarding microbial activity in forestry and restored land in South Africa. It is important to assess changes in microbial activity in KZN since forestry is also an important land use.

4.2 Objectives and hypothesis

The aim of this study was to evaluate effects of reforestation on soil microbial characteristics. The objective of the study was to determine some physical properties (aggregate stability, bulk density and infiltration rate) and enzyme activities (β-glucosidase, β-glucosaminidase, dehydrogenase and fluorescein diacetate hydrolysis). Enzyme activities and physical soil properties were measured and compared in reforested stands (aged 2, 4 and 6-years-old) and degraded land (sugarcane) in Buffelsdraai, Durban. It was hypothesized that soil enzyme activities would increase with reforestation age. An initial decline in soil physical properties was predicted.

4.3 Materials and methods

4.3.1 Study area

The study was carried out at Buffelsdraai landfill site buffer zone situated near Verulam (29.63261° S, 30.98717° E) in KwaZulu-Natal province, South Africa (Please refer to Fig. 3.1). The study site has variable topography with an average slope of 11.91 °, located at an average altitude of 228.07 m. The area is characterized by warm and temperate climate with annual mean temperature of 222.70 °C and annual rainfall of 776 mm. The site has been under sugarcane cultivation for approximately 100 years and prior to sugarcane farming, the land had riverine and scarp forest, thicket and woodland and grassland. As a result of reduction in sugarcane yields, influenced by soil degradation and lower rainfall, the farm was sold to eThekwini Municipality. The site has 8 soil forms and is dominated by duplex and lithic which are translated to Acrisols and Leptosols in the World Reference Base for Soil Resources (Fey, 2010). Leptosols are generally very shallow soils (0-25 cm) (Fey, 2010), they are found on steep slopes formed on Ecca shale. These soils are susceptible to erosion (Fey, 2010), due to

shallow depth infiltration rates are low, thus during rainfall surface runoff erodes material. Unlike Leptosols, Acrisols are deeper, more fertile, and less sensitive to erosion and were found on gentle slopes; as a result of deposition of material eroded on steep slopes. The study area had two land uses, sugarcane and reforested soils. Soils were reforested with a mix of indigenous trees obtained from tree-preneurs (Please refer to Table 3.1). About 677 300 trees of 72 species which included *Acacia robusta, Branchylaena discolour, and Erythrina caffra* have been planted in 602.15 ha of the land as of October 2016. Sites were reforested in 2009-2010 (6-year-old), 2011-2012 (4-year-old) and 2014-2015 (2-year-old). The 2-year-old site was 800 m from sugarcane, whereas the 4-year-old site was 360 m away from sugarcane and the 6-year-old site was approximately 1000 m away from sugarcane. The 2 and 4-year-old forest stands had trees and grass as vegetation. Sugarcane was used as a control site, depicting degraded land, thus allowing the assessment of changes brought by reforestation.

4.3.2 Sampling design

Sampling was carried out in Acrisols and Leptosols at 20m x 20m plots. Three sampling points were randomly selected on uniform soils for each forest age stand and sugarcane. At each sample point soil samples from each site were randomly taken at 0-10 cm and 10-20 cm depth sing an auger for biological analysis and a spade for physical analyses. Soil subsamples for enzyme activities were stored in zip lock plastic bags at 4 °C for a maximum of 14 days. The other portion of field moist soil was sieved (8 mm) for aggregate stability measurement. At the time of sampling, a subset of soil was collected with a metal ring (volume of 220.89 cm^{-3}, 5 cm height and a diameter of 7.5 cm) for determination of bulk density. Bulk density was calculated using the volume of the core used and dry soil weight obtained by drying collected soil at 105 °C for 48 hours. Moisture content for all samples collected was determined by oven-drying 10 g at 105 °C overnight.

4.3.3 Soil characterisation

4.3.3.1 Aggregate stability

Aggregate stability was measured according to wet sieving method by Six et al. (2002). A sample of 80 g of soil passed through 8 mm sieve was place in a 2 mm sieve and soaked in water for five minutes. Then the 2 mm sieve was moved up and down 3 cm from the basin with water for 2 minutes (50 repetitions). After fractionating, the water stable aggregates on the 2 mm sieve were transferred into a pre-weighed aluminium tray and were dried at 60 °C. The soil that passed through the 2 mm sieve was transferred onto the next sieve (0.25 and 0.053 mm) and the same procedure was followed. Oven dry weight of the different aggregates was recorded and used for calculating mean weight diameter (MWD) (Kemper and Rosenau, 1986). Mean weight diameter was calculated using the formula:

$$MWD = \sum_{i=1}^{n} x_i \, w_i$$

Where n = number of separated aggregate size classes,

x_i = mean diameter of the aggregates size fractions and

w_i = weight proportion of the aggregate size on the sieve in relation to total soil dry weight used.

4.3.3.2 Infiltration rate

Soil infiltration was measured according to Hillel (1982) using a double ring infiltrometer, with inner and outer rings of 300 mm and 600 mm diameter, respectively. The rings were hammered to a depth of 30 mm in all studied sites, with 3 infiltration runs per site. Both rings were filled with water to 90 mm mark and the timer was started immediately to record the time for decline in water level below the initial mark in the inner ring. Water level in both rings was kept similar throughout measurements to avoid lateral flow of water. Drop in water level was measured using a ruler (mm). Rings were filled with water to the mark when water level dropped below 60 mm. Water levels before and after filling were noted.

4.3.3.3 Enzyme activities

The activity of dehydrogenase was determined according to Öhlinger (1996). Dehydrogenase activity was assayed by placing 5 g of field moist soil into a 50 ml Erlenmeyer flask and 5 ml of triphenyl tetrazolium chloride (TTC) substrate solution was added. The contents were mixed

and incubated for 16 hours at 25 °C in an incubator. After incubation, 25 ml of acetone was added to extract produced triphenyl formazan. Flasks were then shaken for 2 hours in the dark and filtered. Colour intensity was measured at 546 nm with a UV-1800 Shimadzu spectrophotometer.

Hydrolysis of FDA was based on the method by Green *et al* (2006). Field moist soil (1 g) was placed in a 125 ml Erlenmeyer flask, 50 ml of 60 mM sodium phosphate buffer (pH 7.6) and 0.5 ml of 4.9 mM FDA lipase substrate solution was added. Flasks were incubated for 3 hours at 37 °C and 2 ml of acetone was added to terminate hydrolysis after incubation. Colour intensity was measured at 490 nm with a UV-1800 Shimadzu spectrophotometer.

β-glucosaminidase activity was assayed following the method by (Parham and Deng, 2000). One gram of field moist soil was mixed with 4 ml of 0.1 M acetate buffer (pH 5.5) and 1 ml of substrate solution (10 mM p-nitrophenyl-N-acetyl-β-D- *glucosaminide*). The slurries were incubated for 1 hour at 37 °C and 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH were added to stop the reaction. In controls, substrate was added after terminating the reaction. Colour intensity of the filtrates was measured at 405 nm with a UV-1800 Shimadzu spectrophotometer.

β-glucosidase activity was assayed following the method by Eivazi and Tabatabai (1988), without the addition of toluene. One gram of field moist soil was mixed with 4 ml modified universal buffer and 1 ml of substrate solution (25 mM *p-Nitrophenyl-β-D-glucopyranoside).* The slurries were incubated for 1 hour at 37 °C and 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH were added to stop the reaction. Colour intensity was measured at 400 nm with a UV-1800 Shimadzu spectrophotometer.

4.3.4 Statistical analysis

The values reported for enzyme activities were expressed on oven dry soil basis (at 105°C). Analysis of variance (ANOVA) with three replications was used to compare soil enzyme activities and soil physical properties data among forest stands and sugarcane. Separation of means was done using Fisher's post hoc test. All statistical analysis was done using Genstat Statistical Package, 18th edition.

4.4. Results

4.4.1. Effects of physical properties reforestation on soil

Soil physical properties were affected by reforestation age and soil type. The interaction between reforestation age and soil type was significant for bulk density, infiltration rate and mean weight diameter (Table 4.2). Acrisols had lower bulk density when compared with Leptosols for sugarcane and the 2-year-old forest stand (Fig. 4.2). However, in Leptosols bulk density was lower in the 6-year-old forest stand compare to sugarcane and the 2-year-old forest stand (Fig. 4.2).had an inverse relationship with reforestation age and the opposite was true for Acrisols (Fig. 4.2). Soil bulk density increased significantly in the 2-year-old forest stand compared to the control site in Leptosols (Fig. 4.2).

Table 4.1: Analysis of variance for measured soil physical parameters affected by forest stand age, soil type and soil depth following reforestation of abandoned sugarcane land in Buffelsdraai, Durban

Source of variation	DF	BD	MWD	Infiltration
Forest stand age	3	*	***	***
Soil type	1	***	ns	***
Soil depth	1	**	ns	-
Forest stand age.Soil type	3	***	**	***
Forest stand age.Soil depth	3	ns	ns	-
Soil type.Soil depth	1	ns	ns	-
Forest stand age.Soil type.Soil depth	3	ns	ns	-

$p < 0.001$ = *** (highly significant), $p < 0.01$ = ** (moderately significant), $p < 0.05$ = * (significant), $p > 0.05$ = ns (not significant) - = not determined. DF= degrees of freedom, BD = bulk density ($g.cm^{-3}$), MWD = mean weight diameter (mm) and Infiltration= infiltration rate ($mm.min^{-1}$)

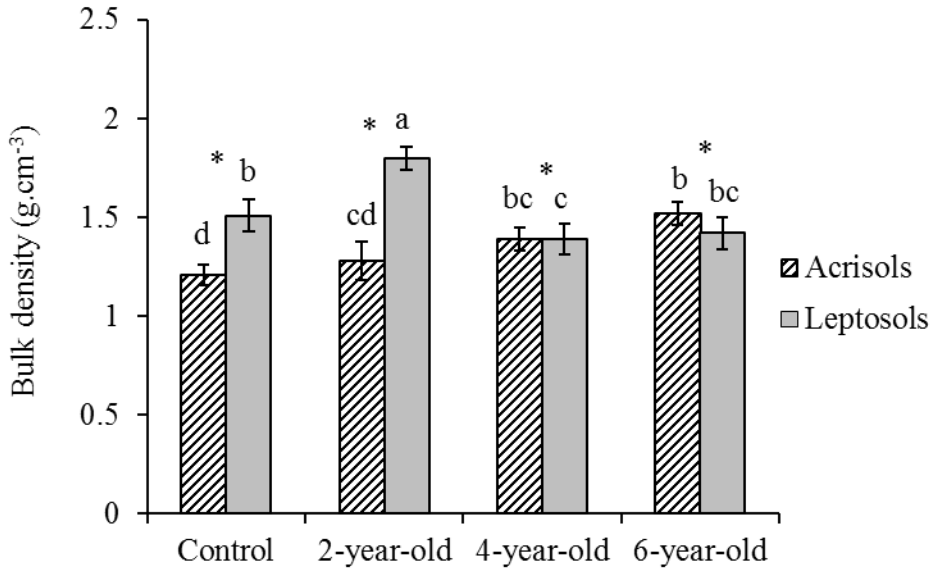

Figure 4.1: Effect of forest stand age and soil type on soil bulk density ($g.cm^{-3}$) in Buffelsdraai, Durban. Error bars show standard error and different lower- letters above bars show statistical differences using Fisher's test. (*between two clustered bars of the same treatment indicates statistical difference between the soils)

Leptosols had significantly higher soil infiltration rate than Acrisols in the 4- and 6-year-old forest stands (Table 4.3). Soil infiltration rate was not determined in the control site under Acrisols due to challenges of high water table. Water level in both inner and outer rings did not decline for more than 2 hours in all three replicates. The infiltration rate for the 2-year-old forest stand in Leptosols was lower in contrast with the control site. The 6-year-old forest stand showed a significantly higher soil infiltration rate compared to other forest stands and the control site in both soils (Table 4.3).

Table 4.2: The mean infiltration rates (mm. min^{-1}) as affected by reforestation age and soil type in Buffelsdraai, Durban

Treatment	Infiltration rate (mm. min^{-1})	
	Acrisols	Leptosols
Control	ND	6.24 ± 0.06^{y}
2-year-old	5.51 ± 0.06^{Ac}	2.63 ± 0.04^{Bz}
4-year-old	19.35 ± 0.15^{Bb}	27.28 ± 0.24^{Ax}
6-year-old	31.95 ± 0.21^{Ba}	36.70 ± 1.57^{Aw}

ND= not determined. Data are means ± standard error. Means followed by the same letter are not significantly different

Different upper-case letters in rows of the same forest age stand across soil types represent significant differences (using Fisher's test) between soil types within the respective forest age stands.

Different lower-case letters within the same column show significant differences (using Fisher's test) of forest age stands within same soil type.

A significant decrease in aggregate mean weight diameter was observed in the 2-year-old forest stand compared to the control in both soils (Fig. 4.3). Thereafter, mean weight diameter increased with increasing reforestation age in both soils. However, the 6-year-old forest stand in Leptosols had the highest mean weight diameter compared to other forest stands and control (Fig. 4.3).

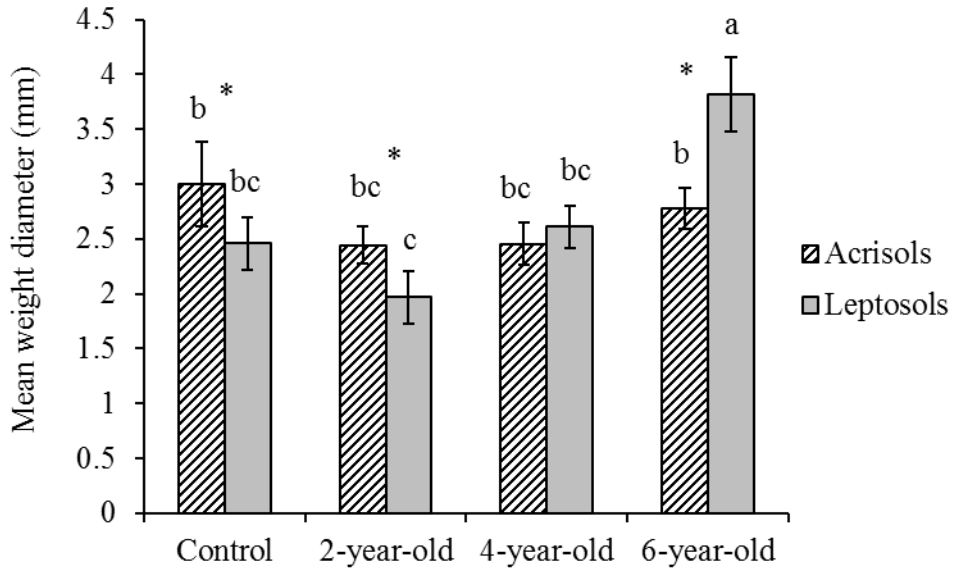

Figure 4.2: Effect of forest stand age and soil type on soil aggregate stability as shown by mean weight diameter (mm) in Buffelsdraai, Durban. Error bars show standard error and different lower- letters above bars show statistical differences using Fisher's test. (*between two clustered bars of the same treatment indicates statistical difference between the soils)

4.4.2 Effects of reforestation on soil enzyme activity

Dehydrogenase

Dehydrogenase activity was lower compared to all measured enzyme activities. Dehydrogenase activity was affected by reforestation age, soil type and soil depth (Table 4.4). There were significant interactions between forest stand age with soil type, and forest stand age with soil depth on dehydrogenase activity (Table 4.4). Dehydrogenase activity was higher in Acrisols compared with Leptosols (Fig. 4.4). Reforestation age significantly influenced dehydrogenase activity in Acrisols. Overall mean dehydrogenase activity was highest in the 4-year-old forest stand followed by 6-year-old forest stand; control and 2-year-old forest stand (Fig 4.4). No significant differences were found in dehydrogenase activity among treatments in Leptosols (Fig 4.4). The6-year-old forest stand in Acrisols had higher dehydrogenase activity in the 0-10 cm depth compared to the 10-20 cm depth (Fig. 4.5).

Table 4.3: Analysis of variance for measured enzymatic activity as affected by forest stand age, soil type and soil depth following reforestation of abandoned sugarcane land

Source of variation	DF	DHA	FDA	Glucosidase	Glucosaminidase
Forest stand age	3	***	***	ns	**
Soil type	1	***	ns	ns	ns
Soil depth	1	*	*	***	ns
Forest stand age.Soil type	3	*	**	***	ns
Forest stand age.Soil depth	3	*	ns	***	ns
Soil type.Soil depth	1	ns	ns	**	ns
Forest stand age.Soil type.Soil depth	3	ns	ns	***	ns

$p < 0.001$ = *** (highly significant), $p < 0.01$ = ** (moderately significant), $p < 0.05$ = * (significant), $p > 0.05$ = ns (not significant)

DHA= dehydrogenase, FDA= fluorescein diacetate hydrolase activity, Glucosidase = β-glucosidase activity and Glucosaminidase = β-glucosaminidase activity

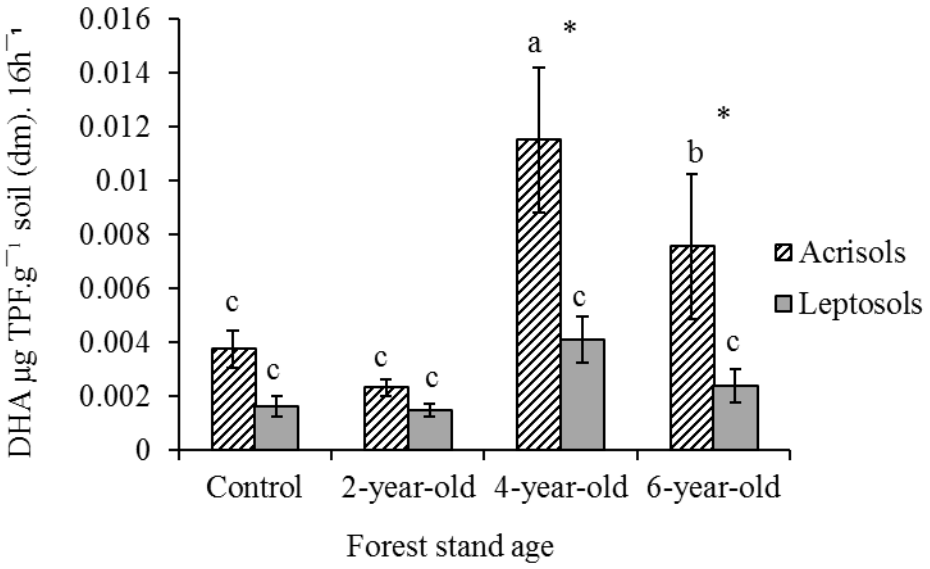

Figure 4.3: Effect of forest stand age and soil type on dehydrogenase activity in Buffelsdraai, Durban. Error bars show standard error and different lower- letters above bars show statistical differences using Fisher's test. (*between two clustered bars of the same treatment indicates statistical difference between the soils)

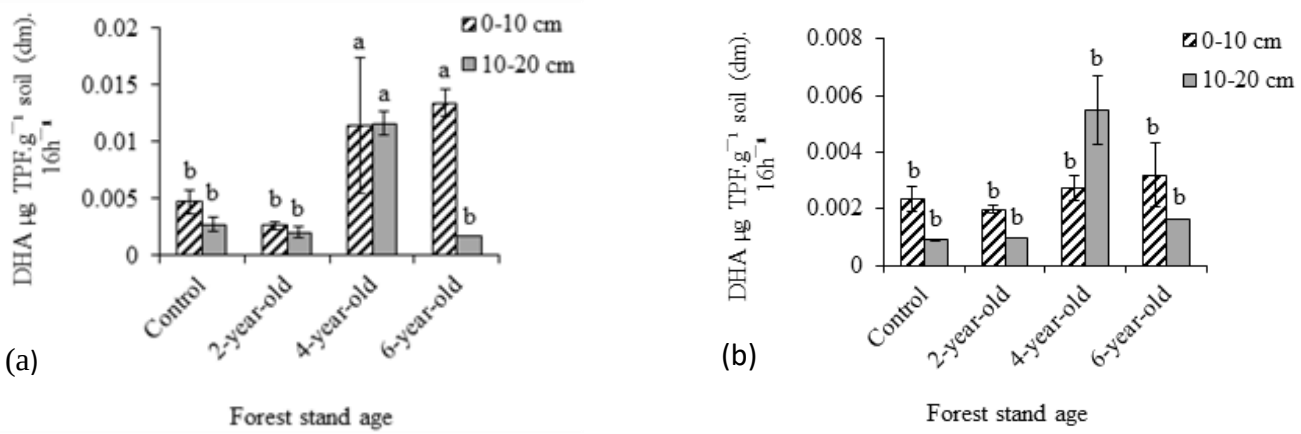

Figure 4.4: Effect of forest stand age and soil depth on dehydrogenase activity in (a) Acrisols and (b) Leptosols in Buffelsdraai, Durban. Error bars show standard error and different lower-letters above bars show statistical differences using Fisher's test. (*between two clustered bars of the same treatment indicates statistical difference between the soil depths)

Fluorescein diacetate (FDA) hydrolase activity

Overall, FDA hydrolase activity was significantly higher when compared to other enzyme activities in this study. Fluorescein diacetate hydrolase activity varied significantly with forest stand age (Table 4.4). The 6-year-old forest stand age had higher FDA hydrolase activity compared to other forest stand ages and the control site in both soils (Fig. 4.6). However, the highest FDA hydrolase activity was observed in the 6-year-old forest stand age in Acrisols (Fig. 4.6). The forest stand age with soil type interaction effect was significant with respect to FDA hydrolase activity (Table 4.4). FDA hydrolase activity was significantly higher in the 0-10 cm depth (7.7 µg FDA g^{-1} soil $3h^{-1}$) compared to the 10-20 cm depth (6.7 µg FDA g^{-1} soil $3h^{-1}$).

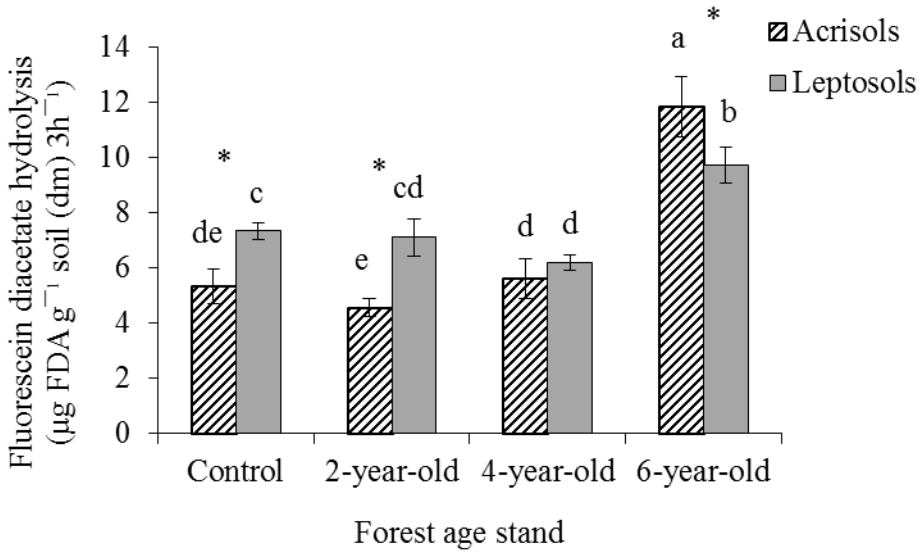

Figure 4.5: Effect of forest stand age and soil type on fluorescein diacetate hydrolase activity in Buffelsdraai, Durban. Error bars show standard error and different lower- letters above bars show statistical differences using Fisher's test. (*between two clustered bars of the same treatment indicates statistical difference between the soils)

β-glucosaminidase activity

Soil type, depth and interactions had no significant effect on β-glucosaminidase activity (Table 4.4). β-glucosaminidase activity was significantly affected by reforestation age (Table 4.4). β-glucosaminidase activity decreased in the 2-year-old forest stand compared to the control site and the 6-year-old forest stand had higher β-glucosaminidase activity when compared with the 2- and 4-year-old forest stands (Fig. 4.7). However, the highest β-glucosaminidase activity was observed in the control site, followed by 6-year-old forest stand; the 4- and 2-year-old forest stands did not differ statistically (Fig. 4.7).

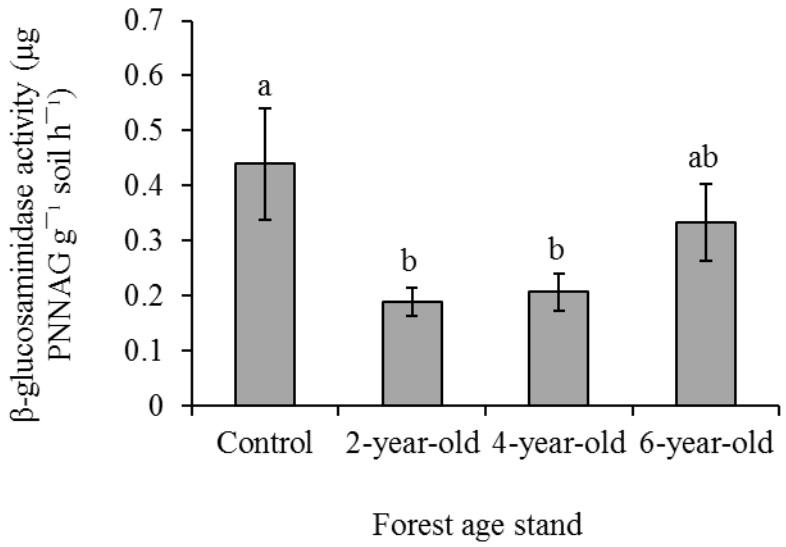

Figure 4.6: Effect of forest stand age and on β-glucosaminidase activity in Buffelsdraai, Durban. Error bars show standard error and different lower- letters above bars show statistical differences using Fisher's test. PNNAG (substrate): p-nitrophenyl-N-acetyl-β-D- *glucosaminide*

β-glucosidase activity

There were no significant differences in β-glucosidase activity with forest stand age and soil type (Table 4.4). Overall, β-glucosidase activity decreased with soil depth (0-10 cm: 0.23 µg PNG g^{-1} soil h^{-1}; 10-20 cm: 0.13 µg PNG g^{-1} soil h^{-1}) (Fig. 4.8). The following interaction effects were significant with respect to β-glucosidase activity: forest stand age with soil type, forest stand age with soil depth, soil type with soil depth and forest stand age with soil type and soil depth (Table 4.4).

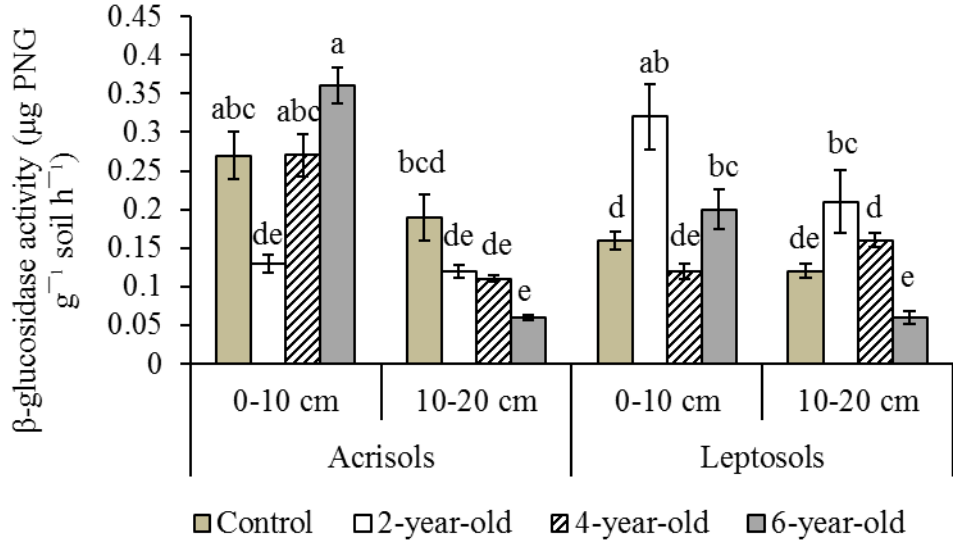

Figure 4.7: Forest stand age, soil type and soil depth interaction effect on β-glucosidase activity in Buffelsdraai, Durban. Error bars show standard error and different lower- letters above bars show statistical differences using Fisher's test. PNG (substrate): *p-Nitrophenyl-β-D-glucopyranoside*

4.5 Discussion

In Acrisols, high activity of dehydrogenase was observed in reforested (especially in the 4- and 6-year old stands) soils than in sugarcane (Fig. 4.4). Differences in dehydrogenase activity between reforested soils and sugarcane might be associated with litter fall and substrate availability in soil. The higher litter fall in reforested soils may have resulted in more and/or variable quality of organic matter, which provides microbes with substrate, consequently improving enzyme production and activity in soil (Wolińska and Stępniewska, 2012). The findings of the current study were in accordance with da Silva et al. (2012) who showed that dehydrogenase activity was higher in reforested sites than conventional sugarcane plantation. Generally, dehydrogenase activity has a strong relationship with organic carbon content (Wolińska and Stępniewska, 2012). It was interesting to note that dehydrogenase activity was higher in Acrisols at the 4-year-old forest stand followed by the 6-year-old forest stand (Fig. 4.4). This could be explained by higher SOC content which increases substrate availability for microbial community in the soil, thus increasing enzyme activity (Kuwano et al., 2014). Dehydrogenase activity decreased in the 10-20 cm depth in the 6-year-old forest stand compared to the 0-10 cm depth in Acrisols (Fig. 4.5). This could be associated with clay, clay percentage was higher in the 10-20 cm (39.6 %) compared to the 0-10 cm depth (36.6) in the 6-year-old forest stand under Acrisols. The higher dehydrogenase activity in Acrisols at the 4-year-old forest stand may be due to the creation of favourable environmental condition by litter from both trees and grass through reduction of microclimate fluctuation. According to Mukumbareza et al. (2015), the quality of organic inputs can be more important for most enzymes. The higher dehydrogenase activity in Acrisols at the 4-year-old forest stand may suggest that the quality of organic inputs on this site could be more important for dehydrogenase activity.

The hydrolysis of FDA was related to C: N ratio. The higher FDA hydrolase activity observed in the 6-year-old forest stand for both soils can be explained by lower C: N ratio of soil (14.9:1). Lower C:N ratio improves litter decomposition (Mukumbareza et al., 2015), this could improve enzyme activity due to release of organic substrates. The soil under sugarcane had the highest β-glucosaminidase activity compared to reforested soils (Fig. 4.7). The findings of this study were in contrary with previous studies by Sotomayor-Ramirez et al. (2009) which showed that after reforestation of sugarcane, β-glucosaminidase activity was higher in reforested sites compared to soils under agriculture. However, the previous study was conducted after 26 years of sugarcane conversion, the results were not expected to be similar since the forest stands

assessed in this study were young (<10 years). Given time, the activity of β-glucosaminidase might be improved in Buffelsdraai, provided that the 6-year-old forest stand had the highest activity compared to other forest stands.

Among the studied enzymes, β-glucosidase was not affected by land use. This may suggest that changes in β-glucosidase activity in reforested soils compared to soils under sugarcane may only be evident in matured forest stands. The activity of enzymes is not only affected by land use and soil properties, adsorption of enzymes to clay may disable or restrict enzyme activity (Acosta-Martínez et al., 2007). According to Sotomayor-Ramirez et al. (2009), if the stabilized fraction of extracellular enzymes is low, their activities will be low. Generally, higher activity of β-glucosidase was observed in the 0-10 cm depth. The findings were in agreement with Sotomayor-Ramirez et al. (2009) who showed that β-glucosidase decreased with soil depth associated with decreased SOC.

Soil aggregate stability as shown by MWD was reduced in the 2-year-old forest stand in contrast to sugarcane for both soils (Figure 4.3). Sugarcane removal upon reforestation could have decreased the degree of soil aggregation. Removing vegetation and residues exposes the soil to raindrop impact and during a rainfall event soil aggregates can be easily detached and eroded by water (Kizilkaya and Dengiz, 2010). Reduction in soil aggregation in the 2-year-old forest stand may have led to SOC reduction (refer to Table 3.3). Results showed that overtime reforestation improved soil aggregate stability. According to Kizilkaya and Dengiz (2010) presence of water stable aggregates is associated with organic matter. Litter input from trees increase organic matter and upon decomposition of organic matter polysaccharides produced bind soil particles , thus improving aggregate stability (Tisdall and Oades, 1982). In addition, the root system of trees can improve aggregate stability by binding soil particles with tree roots and fungal hyphae (Fattet et al., 2011). The 6-year-old forest stand in Leptosols had significantly higher MWD values compared to other forest stands and the control site. The higher activities of fluorescein diacetate hydrolase and β-glucosaminidase activity in the 6-year-old forest stand compared to other forest stands could be associated with improved soil aggregate stability. According to Trivedi et al. (2017) microbial functions including enzyme production are associated with aggregate stabilization since it affects soil carbon turnover. Aggregate stabilization is associated with the formation of macro-aggregates and it has been reported that macro-aggregates accumulate SOC which improves soil enzyme activity (Wang et al., 2017). Naturally, carbon in macro-aggregates is labile, as a result substrate availability to microbes is increased, hence increased enzyme production and activity (Trivedi et al., 2017).

Furthermore, aggregate stabilization increases availability of microbial habitats, thus supporting more microbial communities, consequently increasing enzyme production and activity (Trivedi et al., 2017). The loss of vegetation cover, soil organic matter and reduction of soil aggregation upon reforestation may have resulted in increased bulk density observed in the 2-year-old forest stand compared to the control site especially in the Leptosols. In Acrisols, bulk density increased with reforestation age. Vopravil et al. (2014) study showed that bulk density did not decrease after 7 – 10 years of reforestation of previously arable land. The effect of cultivation on soil may persist even after many years following reforestation, thus affecting soil characteristics (Cunningham et al., 2015, Podrazsky et al., 2015). This suggests that improvement of soil bulk density following reforestation may not be evident on recently reforested sites. There was a relationship between aggregate stability and bulk density. The 6-year-old forest stand under Acrisols had a lower bulk density (Fig. 4.2) compared to the 6-year-old forest stand and the opposite was true with respect to aggregate mean weight diameter (Fig. 4.3). Aggregate stabilization influences bulk density by creation of macro-aggregates which enhances soil porosity, thus decreasing bulk density (Vopravil et al., 2014).

The study showed an increase in soil infiltration rate overtime with reforestation (Table 4.3). A review of afforestation effects on infiltration in the tropics showed that reforestation increased infiltration up to three times compared to arable land (Ilstedt et al., 2007). The higher infiltration rate observed in reforested soil compared to sugarcane could be attributed to improved soil aggregation as shown by higher MWD values (Haghighi et al., 2010). Soil aggregation in forest soils lead to the formation of more macro-pores, which increase soil infiltration rate (Cunningham et al., 2015, Mapa, 1995). Soil infiltration rate was not determined in the control site under Acrisols. Provided that the soil was clayey (>40 % clay) and closer to saturation zone, the capillary pores spaces could have been filled, thus restricting infiltration (Gray and Norum, 1967). Soil pores that are already filled with water cannot absorb more, thus increasing runoff potential. Increasing trends in the values of FDA hydrolase activity, β-glucosaminidase activity and infiltration with reforestation age suggest that reforestation improves soil health.

4.6 Conclusion

Based on the findings, reforestation had an advantage over sugarcane treatment with respect to aggregate mean weight diameter (especially in Leptosols) and infiltration rate in both soils. The creation of macro-pores as a result of aggregate stabilization enhanced infiltration rate. The increase in aggregate size in the 6-year-old forest stand (especially for Leptosols) reduced bulk density. The activity of FDA hydrolase was higher in the 6-year-old forest stand compared to sugarcane for both soils, suggesting that reforestation improves soil biological activity. However, the highest activity of FDA hydrolase was observed in Acrisols, due to higher SOC compared to Leptosols. Results suggested that organic inputs in the 4-year-old stand of Acrisols may be important for dehydrogenase activity.β-glucosaminidase activity decreased in the 2-year-old forest stand compared to sugarcane and remained lower even after 6 years of reforestation when compared to sugarcane. More mature forest stands are required to detect changes in β-glucosidase activity following reforestation of sugarcane land.

References

ABUNYEWA, A., AGYARKO, K. & ASIEDU, E. 2015. Effects of reforestation on degraded soils in the moist semi deciduous zone of West Africa. Journal of Science and Technology (Ghana), 35, 1-9.

ACOSTA-MARTINEZ, V., CRUZ, L., SOTOMAYOR-RAMIREZ, D. & PÉREZ-ALEGRÍA, L. 2007. Enzyme activities as affected by soil properties and land use in a tropical watershed. Applied Soil Ecology, 35, 35-45.

ACOSTA-MARTÍNEZ, V., CRUZ, L., SOTOMAYOR-RAMÍREZ, D. & PÉREZ-ALEGRÍA, L. 2007. Enzyme activities as affected by soil properties and land use in a tropical watershed. Applied Soil Ecology, 35, 35-45.

ADAM, G. & DUNCAN, H. 2001. Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils. Soil Biology and Biochemistry, 33, 943-951.

ALKORTA, I., AIZPURUA, A., RIGA, P., ALBIZU, I., AMEZAGA, I. & GARBISU, C. 2003. Soil enzyme activities as biological indicators of soil health. Reviews on environmental health, 18, 65-73.

ARAÚJO, A. S. F., BORGES, C. D., TSAI, S. M., CESARZ, S. & EISENHAUER, N. 2014. Soil bacterial diversity in degraded and restored lands of Northeast Brazil. Antonie van Leeuwenhoek, 106, 891-899.

BENTON, J. J. J. 1999. Soil analysis handbook of reference methods, CRC Press.

BROOKES, P. 2001. The soil microbial biomass: concept, measurement and applications in soil ecosystem research. Microbes and Environments, 16, 131-140.

CARDOSO, E. J. B. N., VASCONCELLOS, R. L. F., BINI, D., MIYAUCHI, M. Y. H., SANTOS, C. A. D., ALVES, P. R. L., PAULA, A. M. D., NAKATANI, A. S., PEREIRA, J. D. M. & NOGUEIRA, M. A. 2013. Soil health: looking for suitable indicators. What should be considered to assess the effects of use and management on soil health? Scientia Agricola, 70, 274-289

CHAUDHARI, P. R., AHIRE, D. V., AHIRE, V. D., CHKRAVARTY, M. & MAITY, S. 2013. Soil bulk density as related to soil texture, organic matter content and available total nutrients of Coimbatore soil. International Journal of Scientific and Research Publications, 3, 1-8.

CUNNINGHAM, S. C., MAC NALLY, R., BAKER, P. J., CAVAGNARO, T. R., BERINGER, J., THOMSON, J. R. & THOMPSON, R. M. 2015. Balancing the environmental benefits of reforestation in agricultural regions. Perspectives in Plant Ecology, Evolution and Systematics, 17, 301-317.

DA SILVA, D. K. A., DE OLIVEIRA FREITAS, N., DE SOUZA, R. G., DA SILVA, F. S. B., DE ARAUJO, A. S. F. & MAIA, L. C. 2012. Soil microbial biomass and activity under natural and regenerated forests and conventional sugarcane plantations in Brazil. Geoderma, 189, 257-261.

DAS, S. K. & VARMA, A. 2011. Role of enzymes in maintaining soil health. In: SHUKLA, G. & VARMA, A. (eds.) Soil enzymology. Berlin: Springer.

DOMINY, C., HAYNES, R. & VAN ANTWERPEN, R. Long-term effects of sugarcane production on soil quality in the South Coast and Midlands areas of KwaZulu-Natal. Proc S Afr Sug Technol Ass, 2001. Citeseer, 222-227.

EIVAZI, F. & TABATABAI, M. 1988. Glucosidases and galactosidases in soils. Soil Biology and Biochemistry, 20, 601-606.

eThekwini Municipality, 2011. "Buffelsdraai Community Reforestation Project." Retrieved 19 May2017,http://www.durban.gov.za/City_Services/development_planning_management/environmental_planning_climate_protection/Projects/Pages/Buffelsdraai-Community-Reforestation-Project.aspx

FARINA, M. P. W. 1981. The Hunter system of soil analysis. . Fertilizer Society of South Africa Journal, 1, 39-41.

FEY, M. 2010. Soils of South Africa. Cambridge University Press, Cape Town.

GEE, G. W., BAUDER, J. W. & KLUTE, A. 1986. Particle-size analysis. Methods of soil analysis. Part 1. Physical and mineralogical methods, 383-411.

GRAY, D. M. & NORUM, D. The effect of soil moisture on infiltration as related to runoff and recharge. Proceedings of hydrology symposium, 1967.

GREEN, V., STOTT, D. & DIACK, M. 2006. Assay for fluorescein diacetate hydrolytic activity: optimization for soil samples. Soil Biology and Biochemistry, 38, 693-701.

HAGHIGHI, F., GORJI, M. & SHORAFA, M. 2010. A study of the effects of land use changes on soil physical properties and organic matter. Land Degradation & Development, 21, 496-502.

HILLEL, D. J. 1982. Applications of soil physics, New York, Academic Press.

HUNTER, A. New techniques and equipment for routine soil-plant analytical procedures. 2. Seminario sobre Manejo de Suelos y el Proceso de Desarrollo en America Tropical, Cali (Colombia), 10 Feb 1974, 1975.

ILSTEDT, U., MALMER, A., VERBEETEN, E. & MURDIYARSO, D. 2007. The effect of afforestation on water infiltration in the tropics: a systematic review and meta-analysis. Forest Ecology and Management, 251, 45-51.

JIANG-SHAN, Z., JIAN-FEN, G., GUANG-SHUI, C. & WEI, Q. 2005. Soil microbial biomass and its controls. Journal of Forestry Research, 16, 327-330.

KASCHUK, G., ALBERTON, O. & HUNGRIA, M. 2010. Three decades of soil microbial biomass studies in Brazilian ecosystems: lessons learned about soil quality and indications for improving sustainability. Soil Biology and Biochemistry, 42, 1-13.

KEMPER, W. D. & ROSENAU, R. C. 1986. Aggregate stability and size distribution. In: KLUTE, A. (ed.) Methods of Soil Analysis. Madison W: Soil Science Society of American Journal.

KIZILKAYA, R. & DENGIZ, O. 2010. Variation of land use and land cover effects on some soil physico-chemical characteristics and soil enzyme activity. Zemdirbyste-Agriculture, 97, 15-24.

KORKANÇ, S. Y. 2014. Effects of afforestation on soil organic carbon and other soil properties. CATENA, 123, 62-69.

KUWANO, B. H., KNOB, A., FAGOTTI, D. S. L., MELÉM JÚNIOR, N. J., GODOY, L., DIEHL, R. C., KRAWULSKI, C. C., ANDRADE FILHO, G., ZANGARO FILHO, W. & TAVARES-FILHO, J. 2014. Soil quality indicators in a Rhodic Kandiudult under different uses in Northern Paraná, Brazil. Revista Brasileira de Ciência do Solo, 38, 50-59.

MAPA, R. B. 1995. Effect of reforestation using Tectona grandis on infiltration and soil water retention. Forest Ecology and Management, 77, 119-125.

MEDIA, S. 2017. KwaZulu-Natal Top Business | KwaZulu-Natal Agriculture [Online]. Available: http://kzntopbusiness.co.za/site/agriculture [Accessed 25 November 2017.

MUKUMBAREZA, C., CHIDUZA, C. & MUCHAONYERWA, P. 2015. Effects of oats and grazing vetch cover crops and fertilisation on microbial biomass and activity after five years of rotation with maize. South African Journal of Plant and Soil, 32, 189-197.

ÖHLINGER, R. 1996. Dehydrogenase activity with the substrate TTC In: ÖHLINGER, R., SCHINNER, F., KANDELER, E. & MARGESIN, R. (eds.) Methods in Soil Biology. Berlin; New York: Springer.

PARHAM, J. & DENG, S. 2000. Detection, quantification and characterization of β-glucosaminidase activity in soil. Soil Biology and Biochemistry, 32, 1183-1190.

PODRAZSKY, V., HOLUBÍK, O., VOPRAVIL, J., KHEL, T., MOSER, W. & PRKNOVA, H. 2015. Effects of afforestation on soil structure formation in two climatic regions of the Czech Republic.

QONGQO, L. & VAN ANTWERPEN, R. Effect of long-term sugarcane production on physical and chemical properties of soils in KwaZulu-Natal. Proc S Afr Sug Technol Ass, 2000. Citeseer, 114-121.

RAJPER, A. M., UDAWATTA, R. P., KREMER, R. J., LIN, C.-H. & JOSE, S. 2016. Effects of probiotics on soil microbial activity, biomass and enzymatic activity under cover crops in field and greenhouse studies. Agroforestry Systems, 90, 811-827.

SHI, S.-W., HAN, P.-F., ZHANG, P., DING, F. & MA, C.-L. 2015. The impact of afforestation on soil organic carbon sequestration on the Qinghai Plateau, China. PloS one, 10, e0116591.

SIX, J., CALLEWAERT, P., LENDERS, S., DE GRYZE, S., MORRIS, S., GREGORICH, E., PAUL, E. & PAUSTIAN, K. 2002. Measuring and understanding carbon storage in afforested soils by physical fractionation. Soil science society of America journal, 66, 1981-1987.

SOIL CLASSIFICATION WORKING GROUP .1991. Soil classification: A taxonomic system for South Africa, Pretoria, South Africa., Department of Agricultural Development.

SOTOMAYOR-RAMIREZ, D., ESPINOZA, Y. & ACOSTA-MARTINEZ, V. 2009. Land use effects on microbial biomass C, β-glucosidaseand β-glucosaminidase activities, and availability, storage,and age of organic C in soil. Biology and Fertility of Soils, 45, 487-497.

SRIVASTAVA, P. K. 2010. Microbial Activity and Nutrient Status in Oak and Pine Oriented ForestSoil of Mid Altitude Central Himalaya. Geneconserve, 9, 1-10.

TABATABAI, M. 1994. Soil enzymes. In: WEAVER, R. W., ANGLE, J. S. & BOTTOMELY, P. S. (eds.) Methods of Soil Analysis, part 2: Microbiological and biochemical properties. Madison: Soil Science Society of America.

TRASAR-CEPEDA, C., LEIRÓS, M. & GIL-SOTRES, F. 2008. Hydrolytic enzyme activities in agricultural and forest soils. Some implications for their use as indicators of soil quality. Soil Biology and Biochemistry, 40, 2146-2155.

TRINSOUTROT, I., RECOUS, S., BENTZ, B., LINERES, M., CHENEBY, D. & NICOLARDOT, B. 2000. Biochemical quality of crop residues and carbon and nitrogen mineralization kinetics under nonlimiting nitrogen conditions.

TRIVEDI, P., DELGADO-BAQUERIZO, M., JEFFRIES, T. C., TRIVEDI, C., ANDERSON, I. C., LAI, K., MCNEE, M., FLOWER, K., PAL SINGH, B. & MINKEY, D. 2017. Soil aggregation and associated microbial communities modify the impact of agricultural management on carbon content. Environmental microbiology, 19, 3070-3086.

VOPRAVIL, J., PODRÁZSKÝ, V., KHEL, T., HOLUBÍK, O. & VACEK, S. 2014. Effect of afforestation of agricultural soils and tree species composition on soil physical characteristics changes. Ekologia, 33, 67-80.

WANG, Y., HU, N., GE, T., KUZYAKOV, Y., WANG, Z.-L., LI, Z., TANG, Z., CHEN, Y., WU, C. & LOU, Y. 2017. Soil aggregation regulates distributions of carbon, microbial community and enzyme activities after 23-year manure amendment. Applied Soil Ecology, 111, 65-72.

WOLIŃSKA, A. & STĘPNIEWSKA, Z. 2012. Dehydrogenase activity in the soil environment, INTECH Open Access Publisher.

WRIGHT, A. F. & BAILEY, J. S. 2001. Organic carbon, total carbon, and total nitrogen determinations in soils of variable calcium carbonate contents using a Leco CN-2000 dry combustion analyzer. Communications in Soil Science and Plant Analysis, 32, 3243-3258.

CHAPTER FIVE: GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 General discussion

Reforestation is being promoted worldwide for sequestering and reducing greenhouse gas emissions, and it has been reported that it has a potential of improving soil quality. However, it remains unknown how reforestation affects soil invertebrate community, enzyme activity and physico-chemical properties in Africa, including South Africa. The main objective of this research was to assess the changes in soil fauna abundance, diversity and diversity, enzyme activities and physico-chemical properties in a chronosequence (2-, 4- and 6-year-old) forest stands compared to sugarcane in contrasting soils. Previous studies have shown that soil enzyme activities and physical soil properties serve as good indicators of change associated with land use change and management. This was also the case in the current study where reforestation improved aggregate stability especially for the 6-year-old forest stand in Leptosols, which led to increased infiltration rate, as a result of macro-pores created by the presence of stable aggregates. The results showed that 6 years of reforestation allowed a recovery of fluorescein diacetate hydrolase activity.

The first data chapter assessed the effects of reforestation on soil fauna abundance, diversity and richness. It was hypothesized that reforestation would increase soil abundance, diversity and richness, however, that was not the case. There were no significant differences among forest stands of different ages with respect to soil fauna abundance, diversity and richness. This was probably because canopy cover had not developed fully. Even though there were no significant differences in fauna abundance, the fauna may have influenced soil structure.

Soil fauna abundance and richness were higher in the beginning of the rainy season compared to the middle of the rainy season as a result of higher rainfall, suggesting that moisture is important for the survival of fauna. This was in agreement with Rosario et al. (2014) , who showed that the rainy season favoured abundance of fauna. Soil fauna diversity decreased in the middle of the rainy season as a result of community equitability. Compared to Acrisols, Leptosols had lower soil fauna abundance, diversity and richness as a result of lower vegetation cover and soil moisture. The second data chapter assessed the effects of reforestation on soil enzyme activities and physical properties. Improvements in infiltration rate were observed after 6 years of reforestation. Other than aggregate stabilization, soil infiltration rate could have

been influenced by fauna. The feeding and casting activities of fauna leads to creation of galleries which enhances infiltration rate (de Bruyn and Conacher, 1990). Activity of dehydrogenase and fluorescein diacetate hydrolase were higher in reforested soils compared to sugarcane. This could be attributable to aggregate stability since it's associated with improved aeration and microhabitats, conducive for microbial growth and activity. β-glucosaminidase activity increased with increasing reforestation age, suggesting that β-glucosaminidase activity will be higher in reforested stands than sugarcane as the forest matures. Sotomayor-Ramirez et al. (2009) showed that after 26 years of sugarcane conversion, β-glucosaminidase and β-glucosidase activity were higher in reforested sites than agricultural soil. This was because of enhanced soil organic carbon accumulation under reforested sites which increased substrate availability for microbes, thus improving microbial activity and growth. The activities of β-glucosaminidase and β-glucosidase can be expected to increase with forest succession. All the reforested sites did not show significant differences with respect to β-glucosidase activity, suggesting that more mature forest nay be required to detect changes in β-glucosidase activity.

5.2 General conclusion

The study assessed the effects of reforestation on soil chemical and physical properties, invertebrate community and enzyme activities in Buffelsdraai. No significant differences were found between forest stands of different ages and sugarcane for soil fauna abundance, diversity and richness. Results indicated that soil fauna abundance, diversity and richness varied with sampling period as a result of rainfall. Calcium and Magnesium had higher values under sugarcane compared to reforested soil. However, soil pH and total nitrogen had higher values in the 6-year-old forest stand compared to sugarcane in Leptosols. Suggesting that reforestation could restore soil chemical properties. Reduced bulk density, enhanced aggregate mean weight diameter (especially in Leptosols on the 6-year-old forest stand) and increased infiltration rate in both soils suggest that reforestation can improve soil physical properties immediately since these changes occurred within 6 years of reforestation. Results showed that reforested soil had higher microbial activity as shown by FDA hydrolase. This suggest that reforested areas are recovering soil quality.

5.3 Recommendations

More research needs to be done focusing on factors that affect soil fauna and enzyme activities such as plant litter quality (i.e. lignin content, nutrient and polyphenol concentration). Collection of soil samples at different seasons is required to capture soil microbial activity dynamics with seasons. It is recommended that research be done when forests are older to capture changes in soil properties with time. It is recommended that soil physical properties (mainly infiltration rate and aggregate stability) be used as indicators for long term monitoring and evaluation.

References

ROSARIO, V. S. V., BATISTA, T. F. V., PROVENZANO, R., LEMOS, L. J. U., DOSANTOS, J. D. V AND LUNZ, A. M. 2014. Edaphic insect fauna associated with reforestation with Schizolobium parahyba Barneby in Amazonia. *Amazonian Journal of Agricultural and Environmental Sciences,* 57**,** 373-381.

SOTOMAYOR-RAMIREZ, D., ESPINOZA, Y. & ACOSTA-MARTINEZ, V. 2009. Land use effects on microbial biomass C, β-glucosidaseand β-glucosaminidase activities, and availability, storage,and age of organic C in soil. Biology and Fertility of Soils, 45, 487-497.