The effect of water treatment residues on soil microbial and related chemical properties

by

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ABSTRACT

Water treatment residue (WTR), a by-product of the water treatment process, consists primarily of precipitated hydroxides of the coagulants used in the water treatment process, along with sand, silt, clay, humic compounds, and dissolved organic matter. It is usually disposed of by landfill, a technology with numerous problems that include dwindling landfill capacity, extensive dewatering requirements for the WTRs, high costs of transportation, and potential liability for landfill clean-up. Therefore, land disposal (or land treatment) presents a popular alternative disposal method based on the principle that the physical, chemical, and microbial properties of the soil can be used to assimilate applied waste without inducing any negative effects on soil quality.

The objective of this study was to investigate the effects of land disposal of the WTR generated by Umgeni Water, a local water treatment authority, on soil quality. These effects were investigated using depth samples from soil profiles of Westleigh and Hutton soil forms at field trials located at Ukulinga Research Farm, near Pietermartizburg and Brookdale Farm, Howick, KwaZulu-Natal, South Africa, respectively. Four rates of WTR (0, 80, 320, and 1280Mg ha^{-1} incorporated into the soil) were investigated at both trials, in addition to mulched treatments at rates of 320 and 1280Mg ha^{-1} at Brookdale only. Sampling of plots was carried out in September 2001 and May 2002, and all treatments were investigated under fallow and grassed cultivation. Laboratory measurements used to assess soil quality included pH, electrical conductivity (EC), organic carbon (OC), and microbial activity using fluorescein diacetate (FDA) hydrolysis.

At both trials in September 2001 WTR-amended plots displayed higher pH in the 0-200mm soil in comparison to the controls, whereas by May 2002 pH had returned to the condition of the controls. Addition of WTR at Ukulinga resulted in higher OC in September 2001, but in May 2002 this was similar to the controls. However, at Brookdale OC was unaffected by WTR. At Ukulinga and Brookdale

the effect of WTR on EC was variable, and microbial activity in the soil profile was unaffected by WTR addition.

Observations at Ukulinga and Brookdale reflected long term changes (3 and 5 years, respectively) to soil quality following WTR addition. To examine the initial changes in soil quality a laboratory experiment was set up using the field trial soils. Research objectives were also extended to include WTRs from Rand Water (Johannesburg), Midvaal Water Company (Stilfontein), Amatola Water (East London), and two samples from the Faure Water Treatment Plant (near Cape Town). The second Faure sample (Faure^2) was collected when blue green algal problems were experienced at the plant. The measurements used to investigate these short term effects on soil quality were soil pH, EC, and microbial activity as indicated by respiration rate.

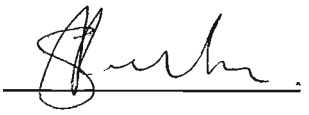
Each of the WTRs added to the Hutton and Westleigh soils increased soil pH by varying increments, and the higher the WTR application rate, the higher was the pH recorded. With the exception of the Rand and Umgeni WTRs that clearly increased soil EC, the effect of the other WTRs on EC was variable. The Faure^1 and Amatola WTRs appeared to have no effect on microbial activity, whereas the Umgeni, Rand, Midvaal, and Faure^2 WTRs stimulated microbial activity by Day 2 following the addition of WTR, but this had declined by Day 14. As for pH, higher microbial activity was recorded at higher WTR application rates.

Changes in microbial community structure of the Hutton soil only, following the addition of WTR were examined using denaturing gradient gel electrophoresis (DGGE) analysis. Community profiles of the different WTRs proved to be markedly different. However, WTR-amended soil retained banding patterns consistent with the control soil indicating that dominant populations in the Hutton soil had been retained.

The field trials indicated that long term effects of land disposal of WTR were not detrimental to the measured indicators of soil quality namely, pH, EC, OC, and microbial activity. The laboratory assessments of the short term response of the Hutton and Westleigh soil forms to WTR addition suggested that the tested variables were altered by WTR, but not significantly changed to the detriment of soil quality. Microbial community analysis indicated that the community structure of the Hutton soil was not significantly altered by WTR amendments. Present findings provide no evidence to suggest that land disposal of WTR is detrimental to soil quality. It is therefore regarded as a feasible disposal option although there are some aspects that should be investigated further. These include investigations into rhizosphere/microbial interactions and the feasibility of growing cash crops.

Declaration

I hereby certify that this research is the result of my own investigation, except as acknowledged herein, and that it has not been submitted for a higher degree in any other university.



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CHAPTER 1

Introduction

1.1 The origin of water treatment residue and associated waste disposal options

During water treatment processes, precipitants, coagulants and disinfectants are added to remove dissolved and suspended solids from the raw water. The chemicals used for this purpose include various organic polymers, liming agents, aluminium sulphate (alum), ferric salts and chlorine. Once flocculated, the solid fraction that settles under gravity is then regarded as water treatment sludge (Elliot *et al.,* 1990).

Although the term "water treatment sludge" has historically been used, the term "water treatment residue (WTR)" will be used in this thesis, in order to make a clear distinction between WTR and wastewater treatment sludge (WTS). This is considered necessary since the chemical, physical and biological composition of WTR and wastewater sludge is vastly different. A WTR consists primarily of the hydroxides of the precipitated coagulant as well as sand, silt, clay, humic compounds and dissolved organic matter, while sewage sludge is dominantly organic and often associated with high heavy metal concentrations and pathogens.

In many parts of the world including South Africa, WTS and WTR have conventionally been disposed of by landfilling (Department of Water Affairs and Forestry, 1998). However, with the advent of stricter waste disposal regulations and increasing costs of landfill, land disposal has been proposed as a suitable disposal alternative (Elliot and Dempsey, 1991). Land disposal presents an appealing alternative to conventional disposal since it suggests that wastes can be assimilated, without inducing negative effects on soil quality. Indeed, there

have also been reports suggesting that land disposal of WTR may improve soil quality (Roy and Couillard, 1998).

Land disposal of WTS is not a new concept and has received much attention in the literature (Chander and Brookes, 1993). Richards *et al.* (1998) cited the importance of the reuse of nutrients and organic matter in WTS via land application. In addition, the long-term and short-term effects of land disposal of urban sludge residues (Roy and Couillard, 1998) and sewage sludges (Chander *et al.,* 1995) have been investigated. However, more recent research efforts have focussed on the potential of WTR for land disposal. Although research into microbial changes resulting from land application of WTR has been sparse, there is much literature on the soil conditioning properties of WTR and its phosphorus sorbing potential (Rengasamy *et al.*, 1980, Bugbee and Frink, 1985; Heil and Barbarick, 1989; Jonasson, 1996; Cox *et al.*, 1997).

1.2 Project background

Given the strict waste disposal regulations, a primary objective of any given water treatment works is the efficient management of wastes. In 1997 Umgeni Water, the bulk supplier of water in KwaZulu-Natal, commenced operation of a central water treatment works namely Midmar Water Treatment Works near Howick in KwaZulu-Natal. Due to the anticipated high volume of WTR likely to be generated, waste disposal via landfill was determined to be economically non-viable. Umgeni Water thus purchased the 300ha Brookdale Farm, near the water treatment works, to dispose of the WTR onto land, and required the development of operating guidelines.

A project was therefore initiated in 1998 to look at the long-term effects on soil quality of land disposal of WTR generated from the Midmar Water Treatment Works. Essentially there are three components of soil quality, namely the physical, chemical and microbiological characteristics, and each aspect was

addressed by individual researchers (Water Research Commission, 2003). The research reported herein is concerned with the effects of WTR on soil microbial and associated chemical properties.

1.3 Aims and objectives

Because the effects of WTR on soil microbial parameters have been little studied, the research protocol followed in this study adopted a broad scale approach. The research efforts were concentrated on examining selected soil chemical and microbial parameters at the sites of two field experiments that had been established to measure long-term changes resulting from WTR application. The first of these was established in October 1998 and the second in March 2000. The data gathered from the field trials would then serve to:

- evaluate the effect of WTR on microbial activity with depth and at the different application rates of WTR; and
- evaluate the effect of WTR on selected soil chemical parameters, namely, soil organic carbon (OC), pH and electrical conductivity (EC) with depth and at the different applications rates of WTR.

Although the data obtained from the field experiments are vitally important, it allowed for the assessment of the long-term effects of only the WTR from the Midmar Water Treatments Works. Furthermore, due to the large number of samples that needed to be processed, sampling of the field trials could only be carried out twice i.e., during September 2001 (summer) and May 2002 (winter). Hence the field samples allowed for a 'snapshot' of the tested conditions at the given sampling time.

To test the short-term microbial response of soils to a range of WTRs, two laboratory experiments were set up to measure microbial respiration. The experiments used the two field trial soils, amended with WTR from selected

water treatment works namely, Faure Water Treatment Plant (Cape Town), Rand Water (Johannesburg), Amatola Water (East London), Midvaal Water Company (Stilfontein) and Umgeni Water (Pietermaritzburg). To complement the respiration data and provide a more detailed picture of soil quality changes, soil pH and EC were also measured. The aims of the respiration experiments were as follows:

- to assess the short-term changes in soil respiration, which is also an indication of microbial activity, pH, and EC as a result of WTR addition;
- to draw comparisons between the different WTRs and their effects on soil respiration, pH, and EC; and
- to compare, where possible, respiration data with microbial activity data from the field trials.

Lastly, microbial community analysis using denaturing gradient gel electrophoresis (DGGE) was carried out. Due to the cost of this analysis, only the soil from the field trial established in 1998 was tested. This soil was selected for DGGE analysis since data from field samples showed higher microbial activity than in the soil from the second field site. By amending the soil with a range of WTRs, and providing suitable soil moisture and temperature conditions, microbial growth was facilitated. The primary aim of DGGE was to compare the microbial communities established on the different treatments to investigate whether the different WTRs had stimulated different microbial populations.

This research thus provides a broad scale perspective of the soil quality of WTR-amended soils, as well as laying the foundation for further work in this field, and should allow for the identification of future research areas of importance.

CHAPTER 2

Land disposal of water treatment residue and microbial characteristics of soil quality

2.1 An overview of landfill and land disposal as waste disposal options

In many parts of the world, landfill is the most important method of waste disposal. Landfill constitutes a closed system where wastes are confined to a specified area by the presence of a barrier (Simon and Tedesco, 1987). However, they are a source of pollution due to the evolution of gases and are often associated with groundwater pollution due to poor site selection and/or poor landfill design (Jarmain *et al.,* 1994). There are also physical problems associated with landfills such as flies, odour, unsightliness and windblown litter (Ball *et al.,* 1994).

In South Africa, the reason for the previous popularity of landfills was that, with environmental concerns being of low priority, landfill constituted a convenient method of waste disposal. Due to apartheid policies, poor communities were often situated close to industrial areas and waste disposal sites. However, post-apartheid South Africa has seen the closure of numerous landfill sites for both social and environmental reasons (Department of Water Affairs and Forestry, 2003).

International agreements since 1994 have put increasing pressure on government to improve environmental policy (Department of Water Affairs and Forestry, 2003). As South Africans become more environmentally aware of the potential hazards of landfills, together with stricter legislation, alternatives for waste disposal are being sought. One such alternative is land disposal or land application of waste; a methodology also known as land treatment.

Overcash and Pal (1979) described land treatment as "the intimate mixing or dispersion of wastes into the upper zone of the soil-plant system, with the objective of microbial stabilization, adsorption, immobilization, selective dispersion or crop recovery, leading to an environmentally acceptable assimilation of the waste". Therefore, land disposal constitutes an open system offering potential for waste treatment, as opposed to simply waste disposal such as is associated with landfill.

Land disposal is suitable for many types of wastes, including treated sewage, liquid animal waste and wastewaters from fruit and vegetable processing plants, animal processing plants, dairies, and fibre products industries (Bouwer and Chaney, 1974). However, this technology is not without hazards and associated environmental risks include reduced soil aeration and reduction of infiltration due to the clogging of soil pores with applied suspended solids. Applied wastes may also cause a build-up of salts leading to salinity problems. There are also concerns regarding heavy metal contamination and associated groundwater pollution (Logan, 1992).

Despite these concerns, researchers have stressed the potential of land disposal since it offers a cost-effective alternative to landfill. Additionally, land disposal has gained in popularity since it offers the added benefit of utilizing part or all of the applied waste. One particular waste type that has been recommended for land disposal is water treatment residue (Elliot *et al.*, 1990).

2.2 The water treatment process and generation of water treatment residue

Although there is an abundance of water on Earth, less than one percent is available and suitable for human use (Department of Water Affairs and Forestry, 2003). The need for effective water treatment is thus apparent. Although the methods employed by various water treatment works may differ, the underlying

water treatment principles remain the same. Figure 2.1 provides a description of the typical procedures used in the conventional water purification process. However, based on the raw water quality, pre-treatment processes may be necessary to facilitate the conventional water treatment processes (Denysschen *et al.,* 1985).

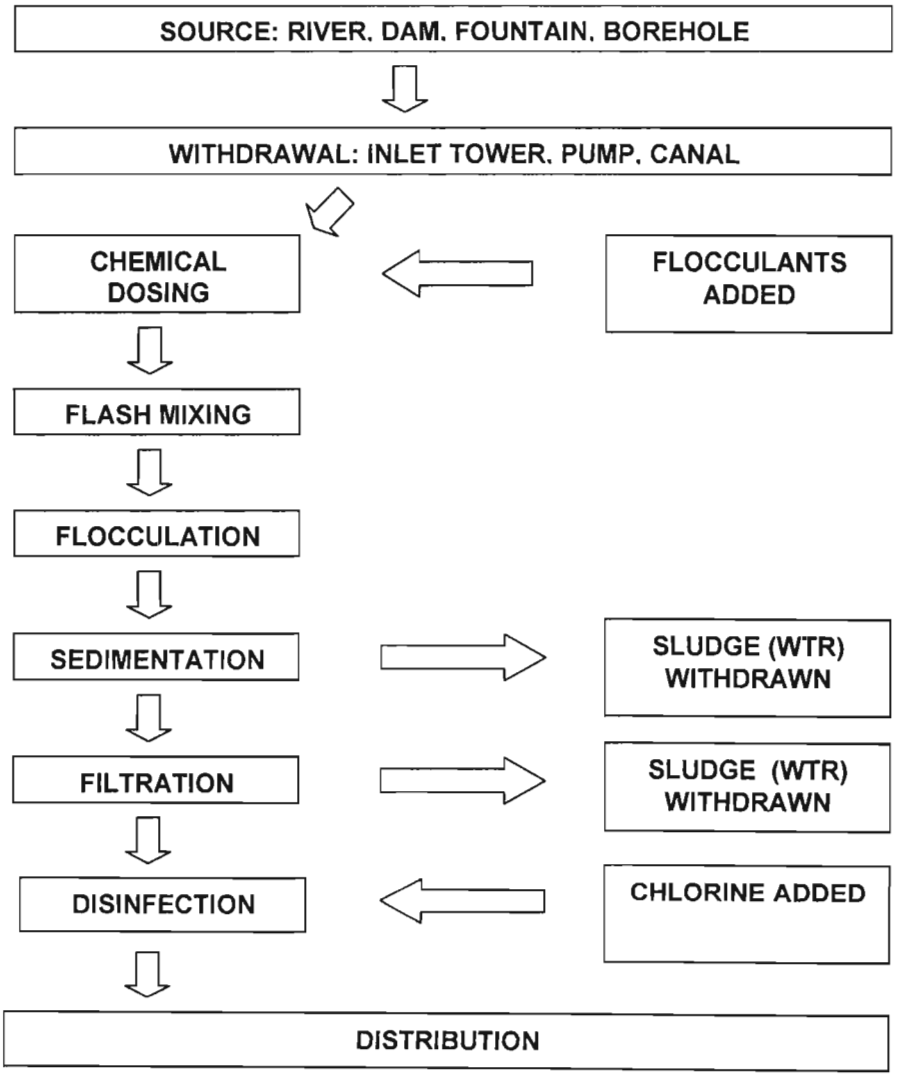


Figure 2.1. Schematic illustration of a conventional water treatment process (adapted from Denysschen *et al.,* 1985).

Pretreatment processes include primary screening of the inflow of water from dams and rivers to remove solid objects. This is followed by the addition of chlorine to kill off microorganisms and the addition of powdered activated charcoal to improve taste and odour of the final product (Rencken, 1997). Additionally, natural waters may be turbid due to the presence of colloidal clay particles, which need to be aggregated to facilitate removal. Thus, the first step in conventional water treatment involves dosing with chemicals such as aluminium sulphate, ferric chloride and polyelectrolytes. This is followed by rapid mixing or 'flash mixing', which disperses the chemicals (Denysschen *et al.*, 1985), and which is necessary to facilitate coagulation (particle destabilization) and flocculation. These are interdependent processes because particle destabilization cannot be achieved unless the added chemicals are well distributed, and floc formation requires maximum opportunity for collision of dispersed particles (Tumeo, 1993).

The flocculated fraction then sinks to the bottom of the tank in a process known as sedimentation, and is removed either intermittently or continuously. The flocculated fraction is then transferred to drying beds and the resultant product, referred to as water treatment residue (WTR), consists primarily of sand, silt, clay, and humic compounds bound to the flocculent chemicals. However, sedimentation does not remove finer flocs, thus the need for filtration steps. In South Africa, filtration is used at every water treatment works and employs either rapid gravity filtration or slow sand filtration (Haarhoff, 1997).

The sole purpose of the final disinfection is to destroy any remaining pathogenic microorganisms and prevent water-borne diseases. Although chlorine is the most popular disinfectant, its role in the water purification process has been seriously threatened since the discovery of the presence of carcinogenic organo-halogenated compounds in chlorinated drinking water. However, chlorination still remains a popular disinfectant in South Africa and considerable research has been carried out to prevent the formation of these compounds (Pretorius, 1997).

Lastly, water testing is carried out to ensure the safety of drinking water which is then ready for distribution and consumption (Umgeni Water, 2003).

2.3 Land disposal of water treatment residue in South Africa: A legal perspective

The responsibility for regulating the final disposal of waste to a landfill site is assigned to the Department of Water Affairs and Forestry (DWAF), in terms of Section 20 of the Environment Conservation Act, 1989 (Act 73 of 1989) (ECA). In terms of Section 20, no person may dispose of waste unless under the authority of a permit issued in terms of the ECA (Department of Water Affairs and Forestry, 2003).

Water treatment residue is a waste and, in terms of the National Water Act (Act 36 of 1998), waste is classified as follows, "...'waste' includes any solid material or material that is suspended, dissolved or transported into a water resource in such volume, composition or manner as to cause, or to be reasonably likely to cause, the water resource to be polluted". This implies that for regulation of the substance, a water licence would be needed. This licence would be guided by the Minimum Requirement Series for Waste Disposal, which informs users in terms of waste disposal standards, waste classification and monitoring (Department of Water Affairs and Forestry, 1998).

However, a great setback in conducting the research that follows lies in the fact that the law classifies WTR as water treatment sludge. Although the research presented in this report uses the term WTR, South African regulations must also draw this distinction to allow for more effective handling and disposal of WTR. Should this distinction be made, the problem that then surfaces is that the Minimum Requirement Series for Waste Disposal would have to first include a classification of all WTRs produced in South Africa, and subsequently set standards for disposal.

The tasks of classifying WTRs and the setting of standards for disposal are complicated, since the composition of WTRs vary. This variation is not only due to the difference in water treatment procedures followed at the various water treatment works but also as a result of seasonal differences in dosages of the various chemicals used at any single treatment plant.

2.4 An overview of land disposal as a waste disposal option for water treatment residue

Water treatment residues are predominantly inorganic, with dewatered WTR displaying physical and chemical characteristics similar to that of a fine structured soil (Zupancic, 1996). Although WTR trace metal concentrations are lower than in sewage sludge, they are higher than the levels found in most soils (Elliot *et al.,* 1990). Pathogenic problems commonly associated with wastewater and sewage sludge do not pose a problem when handling WTR, since it is generated from the water treatment process which includes disinfection steps (Basta, 2000).

A WTR is commonly characterized based on the coagulants used in the water treatment process, for example, alum, ferrous sulphate, ferric chloride, polymers, lime, etc. In South Africa most surface waters can be easily treated with hydrolyzing iron (Fe) or aluminium (Al) salts (Hodgkinson and Rencken, 1992). There are concerns regarding the land application of alum WTR. Since soils contain substantial levels of Al, there is concern regarding the total Al content of WTR and the possibility of phytotoxicity (Ahmed *et al.,* 1997). However, the conditions governing Al solubility, and not the total amount of Al in soil, determines the amount of Al available to plants (Elliot *at al.,* 1990).

A study by Bugbee and Frink (1985) showed that alum WTR improved the physical properties of moderately acidic potting soil and acted as a liming material. There were no indications of Al toxicity. Further, Elliot and Singer (1988) showed that addition of an iron WTR decreased crop concentrations of Al.

Provided that soil acidity is properly controlled to keep pH at or above approximately pH 6, problems of Al toxicity can be avoided (Ahmed *et al.,* 1997).

Skene *et al.* (1995) amended sandy soil with alum WTR and a polymeric WTR. Broad bean plants (*Vicia faba)*, known for their sensitivity to aluminium toxicity, were grown on the WTR-amended soils. Improved growth was noted, more especially in the polymeric WTR treatment, and was attributed to the presence of nitrogen, phosphorus and potassium (constituents of the polymer). Elliot and Singer (1988) showed improved growth of tomato (*Lycopersicon esculentum*) grown in a ferric WTR-amended soil. They stressed the beneficial physical properties of WTR, such as improved water retention, while also stipulating the need for fertilization.

Fertilization of WTR-amended soils is essential when WTR contains iron and/or aluminium hydroxide solids that are strong adsorbents of P, a limiting nutrient for plant growth (Elliot *et al.*, 1990). Bugbee and Frink (1985) showed reduced yields of lettuce (*Lactuca sativa*) that were attributed to phosphorus deficiency. However, Heil and Barbarick (1989) found that rates of 10 and 20 Mg ha^{-1} of both alum and iron WTR produced greater yields of sorghum-sudangrass (*Sorghum sudanense*), without inducing P deficiency. Significantly higher rates of WTR were used in this study. Literature has shown that water treatment residues are far more innocuous than wastewater sludges (WTS). In light of this it was felt that the maximum permissible rates applicable to the WTS (80 Mg ha^{-1} $annum^{-1}$ in South Africa) could probably be safely exceeded.

The key to utilizing WTR is efficient management. The P-sorbing potential of WTR does not necessarily have negative implications, since it can be used with crops that have a low P requirement, or it can be simply managed by extra fertilization. Water treatment residue may also be used to remove P from wastewater at sewage treatment plants (Elliot *et al.*, 1990).

2.5 Microbial aspects of soil quality

Soil quality is an emerging concept that attempts to integrate descriptive and analytical measurements of the physical, chemical and biological components of the soil (Kennedy and Papendick, 1995). It has been defined as "the capacity of a soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health" (Doran *et al.,* 1996). Soil quality encompasses not only crop productivity and environmental protection, but also food safety, and animal and human health. However, there is much confusion regarding what constitutes 'good' soil quality due, in essence, to the variety of land uses that exist (Kennedy and Papendick, 1995).

Soil quality is frequently measured by indicators which are selected to measure changes in soil function that result from management changes. These indicators should be sensitive to changes over time spans from growing seasons to a few years (Sparling, 1997). Although physical and chemical indicators of soil quality have been identified, the biological component of soil quality has often been ignored due to the perceived difficulty of analysis or the interpretation thereof. However, soil microorganisms are vital to soil quality, and are potentially one of the most sensitive biological markers available (Turco *et al.,* 1994). A representative species is often selected as an indicator, although many individual organisms or groups of organisms have been used as indicators (Kennedy and Papendick, 1995).

A wide range of microorganisms naturally reside in soil and perform a wide range of functions which are essential for a 'healthy' soil (Sparling, 1997). Soils contain eubacteria, archaebacteria, fungi, microalgae, protozoa, nematodes, and other microscopic invertebrates. Additionally, the numbers of organisms and their collective biomass may vary within and between soils (Stotzky and Burns, 1982). It is estimated that less than one percent of all bacterial species is known yet the

role of microorganisms on soil quality is extensive. Soil microorganisms are responsible for organic matter decomposition, humus formation and nutrient cycling. They are also essential for the formation of good soil structure due to the presence of mucigel from bacteria and fungal hyphae that bind soil particles, improve water infiltration and assist in maintaining adequate soil aeration (Kennedy and Papendick, 1995).

The role of microorganisms in mediating soil processes, and their relatively high rate of turnover, suggests that the microbial fraction can be a sensitive indicator and an early predictor of changing soil organic matter processes (Sparling, 1997). A soil of high quality is thought of as being biologically active and containing a wide cross section of microorganisms. However, the exact role that the biological fraction plays in maintaining soil quality is unclear and this is due to the great magnitude of microorganisms and the complexity of microbial interactions (Turco *et al.,* 1994).

There is a wide range of tools available, such as estimations of microbial biomass, enzyme activity, microbial respiration, and nutrient cycling, to assess the microbial component of soil quality. Despite the multitude of methods available, researchers have not agreed on a common set of biological indicators and this presents a great problem in understanding the microbial component of soil quality.

Extensive research has been conducted on microbial biomass as an indicator of soil quality. However, it has also been stated that it is impossible to measure the ecological significance of a microorganism in a given environment simply by measuring the number present (Brookes *et al.,* 1987). Thus, many methods have also been developed to obtain information regarding microbial activity. More recent advances have recognised that estimation of microbial diversity may provide more useful information because it allows for the study of organisms which cannot be cultured.

The discussion that follows presents an account of the most common methods used to evaluate soil biological status, and reviews the applications of microbial activity and diversity measurements to soil ecological studies. The applications of microbial biomass estimation are discussed initially since it is the most researched microbial indicator of soil quality, and is the method to which more recent research is often compared. This is followed by a discussion of some of the more popular methods of determining microbial activity and community structure, with emphasis on the measurement of respiration, fluorescein diacetate (FDA) hydrolysis, and denaturing gradient gel electrophoresis (DGGE) which are used in this thesis.

2.5.1 Microbial biomass as an indicator of soil quality

Soil microbial biomass is the living component of the soil organic matter. It excludes soil animals and plant roots, and is made up of bacteria, fungi, actinomycetes, algae, protozoa and some nematodes, and is estimated to contribute about a quarter of the total biomass on Earth (Pankhurst *et al.*, 1995). As a result it has been extensively studied to investigate its role as an indicator of soil quality. Direct methods, which make it possible to measure microbial biomass microscopically, and indirect methods based on analysis of microbial constituents such as carbon and nitrogen, have both been used (Brookes *et al.*, 1987).

The size and activity of the microbial biomass is regulated by the soil organic matter quantity and quality and has been related to climatic conditions (Insam, 1990), soil moisture content (Van Veen *et al.*, 1985; Doran *et al.*, 1990; Van Gestel *et al.*, 1996), soil temperature (Joergensen *et al.*, 1990), soil pH (Jenkinson and Powlson, 1976; Roper and Gupta, 1995), soil structure and texture (Jocteur-Monrozier *et al.*, 1992; Ladd, 1992), and soil and crop management practices (Ocio *et al.*, 1991; Mueller *et al.*, 1992; Ritz *et al.*, 1992; Srivastava and Lal, 1994).

Microbial biomass is a labile source of carbon, nitrogen, phosphorus, and sulphur (McGill *et al.,* 1986). It also serves as a source and sink for mineral nutrients and organic substrates in the short-term, and acts as a catalyst to convert plant nutrients from stable organic forms to available mineral forms over longer periods. Anderson and Domsch (1980) reported that the microbial biomass of soils contains substantial quantities of both C and plant nutrients, whereby the nutrients temporarily held in the biomass largely contribute to the pool of available plant nutrients in soils. For example, the microbial biomass content in agricultural soils under temperate conditions in Germany ranged from 0.27 to 4.8% of the total soil C and the average quantities of N, P, K and Ca were about 108, 83, 70 and 11kg ha^{-1}, respectively.

Microbial biomass contributes to the maintenance of soil fertility and soil quality in both natural and managed terrestrial ecosystems in that it controls major key functions in soil (Turco *et al.,* 1994; Elliott *et al.,* 1996). In addition, microorganisms form symbiotic associations with roots, act as biological agents against plant pathogens, contribute towards soil aggregation and participate in soil formation (Dalal, 1998).

Authors have considered that changes in the microbial biomass are an early indication of changes in soil organic matter (Powlson *et al.,* 1987). For example, under temperate conditions Powlson *et al.* (1987) demonstrated in long-term straw-amended field experiments over 18 years, that the relative increases in biomass-C (37-45%) and biomass-N (46-50%) were much greater than those in total soil organic C (5%) or N (10%). Similar results were reported by Saffigna *et al.* (1989) for a sub-tropical Australian Vertisol cropped with sorghum under different tillage and residue management practices. In addition, the application of farmyard manure (15-90t ha^{-1} $year^{-1}$) under sub-tropical and semi-arid conditions in India was also found to increase the microbial biomass without appreciably increasing soil organic C levels (Goyal *et al.,* 1993).

Furthermore, microbes contribute to the formation of soil structure in that they help to aggregate the soil by polysaccharide production (Kennedy and Papendick, 1995). Soil organic matter consists of 25% carbohydrates and about 40% polysaccharides as the main fractions. The polysaccharides are predominantly of microbial origin and are very important in forming stable micro-aggregates in the soil with clay minerals, multivalent cations and humic substances because they are not readily decomposed as compared to plant polysaccharides (Anderson and Domsch, 1989). The microbial biomass also releases and contains enzymes which are responsible for nutrient cycling (Saffigna *et al.,* 1989; Carter, 1991; Ocio *et al.*, 1991; Srivastava and Singh, 1991).

2.5.2 Microbial activity as an indicator of soil quality

While microbial biomass estimation provides a measure of intact, active and dormant soil microorganisms, microbial activity estimation includes all biochemical reactions catalyzed by microorganisms. Thus microbial activity measurement can also be referred to as the active microbial biomass present in soil.

Microbial activity has been considered as an appropriate index of soil fertility (Kennedy and Papendick, 1995). Total microbial activity is also a good general measure of organic matter turnover in natural habitats since generally more than 90% of the energy flow passes through microbial decomposers (Schnurer and Rosswall, 1982).

Microbial activity may be assessed in a number of ways that indicate the status of either the total community or specific members of a community (Kennedy and Papendick, 1995). However, one must take care to distinguish between the actual activity of microbial organisms which exists under field conditions, and the

potential activity which shows how organisms respond to optimal incubation conditions (Gray, 1990).

Various methods have been developed to compare microbial activity between soils, or between different horizons of the same soil, or to assess the effects of climate and soil treatments on microbial activity. With the exception of plant rhizospheres, mineral soils are generally considered nutritionally poor locales for microbial growth. Therefore, a high proportion of the soil microbes are inactive or dormant, and increasingly attention has been given to quantify both active and inactive components and to take into consideration organisms with a long generation time (Parkinson and Coleman, 1991).

2.5.2.1 Soil enzyme activities

Enzyme activities are an important index of the biological activity of a soil because they are involved in the dynamics of soil nutrient cycling and energy transfer. The enzymatic activity of soil reflects the intensity and direction of biochemical processes in the soil matrix. Hence enzyme activity indicates the biological capacity of a soil to carry out biochemical processes important to maintaining soil fertility (Burns, 1989; Alef, 1995; Alvarez *et al.,* 1995). Soil fertility depends not only on nutrient status and availability but also on the turnover of N, P and other nutrients (Angers *et al.,* 1992).

Enzyme assays provide a simple, direct method for collecting functional data on microbial communities. Dehydrogenase, phosphatase, arginine and arly-sulfatase are some of the enzymes that may be helpful to assess the quality of the soil system (Kennedy and Papendick, 1995).

However, there are potential problems that may limit the application of enzymic technology or the interpretation of assay data. Methodological diversity is unavoidable given the diversity of experimental systems and research objectives.

In addition, samples themselves are heterogeneous in nature. Assay results may also be affected by the adsorption of substrates or products, the presence of competing substrates, or by enzyme inhibitors. A further consideration is the problem of correlating enzyme activity with ecological process (Alvarez *et al.*, 1995).

Although there are numerous enzymic activities that can be measured, dehydrogenase activity of soil is commonly used since it is thought to reflect the total range of biological oxidative activities of the soil microflora (Alef, 1995). Active dehydrogenases are thought to be present in soils as part of intact cells and are generally determined by the reduction of 2,3,5-triphenyl tetrazolium chloride to tryphenyl formazan. This product is then extracted and measured spectrophotometrically (Nannipieri, 1994).

Dehydrogenase is an intra-cellular enzyme (Dick, 1997) and could be used to measure the total oxidative activities of the microbial soil population (Turco *et al.*, 1994). Thus, it is reported as an excellent tool for measuring soil microbial activity. However, dehydrogenase activity does not consistently correlate with bacterial numbers and this can be attributed to the presence of dormant microbes. Further, correlation with respiration rates is not possible due to the presence of oxygenases that catalyze the direct oxidation of the substrate with molecular oxygen. This limits triphenyl uptake by microbial cells, and allows for the accumulation of inhibitory concentrations of formazan (Nannipieri, 1994).

Another popular method for estimating total microbial activity is the use of fluorescein diacetate (3',6'-diacetylfluorescein [FDA]). This is a fluorogen which is transformed to the fluorescent compound fluorescein when it is attacked by enzymes such as proteases, lipases, and esterases (Gray, 1990). Since these different enzymes are able to hydrolyze FDA, the method is considered to reflect overall microbial activity. Fluorescein diacetate has been used to determine amounts of active fungi and bacteria and to locate acetylesterases in living protist

cells. A good correlation has been found to exist between respiration rate and FDA hydrolysis. The product of this enzymic conversion, namely fluorescein, can be visualized within cells by fluorescence microscopy, or can be quantified by fluorometry or spectrophotometry (Schnurer and Rosswall, 1982).

2.5.2.2 Microbial respiration

The measurement of carbon dioxide evolution has a wider application than oxygen uptake since carbon dioxide is evolved by both aerobic and anaerobic processes. The measurement of microbial respiration without the addition of organic substrates is termed basal respiration, whereas substrate-induced respiration (SIR) describes the measurement of respiration following the amendment of the soil, usually with glucose solution. Basal respiration and SIR provide measures of actual and potential soil heterotrophic activity, respectively, and the latter can be used to estimate microbial biomass (Martens, 1995).

Additionally, selective inhibitors may be included to separate the bacterial and fungal contributions to microbial respiration (Dalal, 1998). However, potential problems of incomplete antibiotic specificity, inhibitor deactivation by soils or residues, antibiotic degradation by non-target populations, development of resistant forms, and changes in microbial competition may be experienced (Beare *et al.,* 1990).

Pascual *et al.* (1997) used the measurement of respiration to analyze the microbial changes of an arid soil in response to the addition of sewage sludge and municipal solid waste. The study showed a general increase in microbial biomass and a corresponding increase in microbial respiration due to the presence of available organic materials in the wastes. Joergensen *et al.* (1996) examined differences in microbial respiration of various mixtures of arable soil and compost derived from municipal organic refuse (garden litter, food remains, etc.), while Ritz *et al.* (1997) determined the respiration response of planted soils

to animal manure application. Researchers have also used the measurement of microbial respiration to compare the microbial quality of soils under different management systems (Keift, 1994; Haynes, 1999; Haynes and Tregurtha, 1999; Haynes and Williams, 1999).

Respiration rates have frequently been used to determine the biological activity in soil in relation to seasonal change and changes in physical and chemical properties of soil. Generally the measurements are used to gain insight into how mineral nutrients and organic matter can be more efficiently managed (Nannipieri, 1994). In addition, measurement of soil respiration after mixing samples with an organic compound has often been used to determine if the materials are biodegradable (Anderson, 1982).

Soil respiration is also a well-established parameter to monitor organic matter decomposition, but is highly variable depending on substrate availability, moisture and temperature. For valid comparisons to be made between soils, respiration must be measured under controlled laboratory conditions. When conditions of moisture and temperature are not limiting, the rate of carbon dioxide efflux can provide an indication of whether the soil is conducive to decomposition processes (Sparling, 1997).

The amount of respiration activity may be attributed to differences in soil moisture, soil temperature, soil texture, and the amount and quality of carbon returned to the system. Small-scale heterogeneity may be driven by the interaction of plant-derived substrates, such as roots and decaying plant particles, and intra-aggregate habitat differences due to clay content, pore size, and aeration (Anderson, 1982).

However, in interpreting the results, it must be recognized that the respiration of animals and plant roots in soil may produce extremely complicated results from gas exchange measurements (Nannipieri, 1994). Further, for respiration studies

soil is often sieved, with the disadvantage that soil structure is destroyed. However, field measurements are difficult due to seasonal changes which affect both the soil and associated respiration. Thus, care should be taken when interpreting results, and generalizations should not be made unless supported by field observations (Anderson, 1982).

The measurement of microbial respiration has the following advantages: (i) it is relatively simple and rapid; (ii) it identifies a physiologically active component of the microbial biomass; and (iii) when used with selective inhibitors, it allows for the separation of prokaryotic and eukaryotic contributors to the total physiological response (Beare *et al.,* 1991).

The limitations of the method include: (i) the pattern of soil microbial response to glucose differs between soils; (ii) only glucose responsive soil microbial biomass is measured; (iii) microbial biomass is not measured; (iv) microbial N, P and S cannot be measured; and (v) for soils recently amended with organic materials, the conversion factor used for soil biomass C is not valid (Dalal, 1998).

2.5.3 Soil microbial community analysis

In the past, studies on the diversity of natural microbial populations have been restricted to culturable microorganisms (Heuer and Smalla, 1997). Studies of soil microbial properties have commonly been conducted at process level, where biomass, respiration rates and enzyme activities have been measured (Hill *et al.,* 2000). The discussion that follows focuses on denaturing gradient gel electrophoresis and its potential application to the study of microbial ecology, while highlighting some of the other popular culture-independent methods of community analysis.

Comparisons of the percentage culturable bacteria with total cell counts from different habitats generally show great discrepancies. One of the reasons for this

may be the interdependency of different organisms. Another reason is certainly the lack of knowledge of the 'real' conditions under which most bacteria grow in the natural environment (Muyzer and Ramsingh, 1995).

To obtain a better understanding of the role of microbial diversity in the maintenance of ecosystems, other approaches, which complement traditional microbiological procedures, are needed. The direct extraction of total DNA in soil has indicated high microbial diversity. In fact, DNA analysis has shown up to 200 times greater diversity than indicated from bacterial isolation studies (Heuer and Smalla, 1997). Thus, the extraction of microbial nucleic acids from soil samples is considered to represent an emerging strategy for studying both the ecological fate and the diversity of soil microorganisms (Gelsomino *et al.,* 1999).

2.5.3.1 Denaturing gradient gel electrophoresis

A recent approach to the study of structural diversity of microbial communities is the analysis of polymerase chain reaction (PCR) - amplified DNA fragments by denaturing gradient gel electrophoresis (DGGE) (Heuer and Smalla, 1997). In DGGE separation of PCR-amplified gene fragments is based on the variation in targeted nucleotide sequences (Head *et al.,* 1998). DNA and RNA-based techniques allow a thorough investigation of soil microbial community structure. This is because nucleic acids derived from non-culturable or as yet uncultured microorganisms, which may represent up to 99% of naturally occurring cells in the soil environment, can be included in the analysis (Gelsomino *et al.,* 1999).

PCR-amplified products are considered important to DGGE since it allows for the amplification of nucleic acid sequences that often occur in low abundance in a complex mixture of whole cell extracts. The most popular DNA polymerase used in PCR is *Taq* polymerase, which is extracted from the organism *Thermus aquaticus*. Although PCR presents an immensely useful tool, it is not without limitations (Heuer and Smalla, 1997) and one problem with PCR-dependent

characterization of microbial populations is that it does not distinguish between metabolically active microbial populations and those populations that are inactive or dormant (Suzuki and Giovannoni, 1996).

It is assumed that the number and intensity of bands yielded by the PCR-DGGE technique reflects the relative dominance of the bacteria. However, several problems can distort this interpretation. Firstly, the different DNA extraction methods may yield different levels of inhibitors which are reflected in the banding patterns. A single band may also have two types of organisms due to different sequences having similar melting behaviour. It is also possible for one organism to produce more than one band due to the presence of several copies of the rrN operon with slightly differing sequences (Kozdroj and van Elsas, 2000).

Once optimized, DGGE is a relatively quick and easy way to analyze genetic diversity within a microbial community. Electrophoretic profiles can readily give observable indications of community structure. Probing of resolved bands with oligonucleotides and/or sequencing of excised bands are additional procedures that yield additional information on microbial diversity (Head *et al.,* 1998).

Although there is no documented study known to the author that investigates the DGGE profiles of WTR-amended soils, DGGE has been recognized as a powerful tool for analyzing soil microorganisms. For example, Muyzer and Ramsingh (1995) indicated that DGGE was well suited to investigate both temporal and spatial distributions of bacterial populations, and this is the main reason for its popularity in microbial ecology studies. It may also be used to investigate the fate of microbial communities or certain indicator microorganisms after environmental disturbance (Heuer and Smalla, 1997). Additionally, Jeffrey *et al.* (1996) and Paul (1996) have suggested that functional genes can be used as molecular markers to indicate microbial activity. Wawer *et al.* (1997) extended the application of DGGE to determine the differential expression of the [NiFe]

hydrogenase gene by different *Desulfovibrio* populations in experimental bioreactors.

The DGGE technology has renewed interest in the genetic fingerprinting of microbial communities, and has resulted in the development of other genetic fingerprinting methods. For example Xia *et al.* (1995) used randomly amplified polymorphic DNA fingerprinting (Williams *et al.,* 1990) to follow the response of different microbial communities to the application of 2,4-dichlorophenoxyacetic acid (2,4-D). Amplified ribosomal DNA restriction analysis has also been used to monitor community shifts after environmental perturbation such as copper contamination (Smith *et al.,* 1997).

In a study to determine the bacterial community structure of sixteen different soils Gelsomino *et al.* (1999) showed strikingly different DGGE profiles between the soils. The study also showed that similar soil types contained similar dominant bacteria and the PCR-DGGE method used in the analysis displayed highly reproducible results. Duineveld *et al.* (1998) compared bacterial diversity in the bulk soil and rhizosphere of *Chrysanthemum* plants and reported great variability in bacterial types found over time and with different samples of *Chrysanthemum* rhizosphere soil. In contrast, Kasahara and Hattori (1992) found that samples of grassland soil taken at different times displayed similar profiles.

2.5.3.2 Phospholipid fatty acid analysis

The presence of unique fatty acids may be indicative of specific groups of organisms since, in bacterial taxonomy, specific fatty acid methyl esters have been used for species identification. Consequently, phospholipid fatty acids (PLFAs) are potentially useful signature molecules due to their presence in all living organisms. In microorganisms, phospholipids are found in cell membranes only. This is important since cell membranes are rapidly degraded and the component PLFAs are rapidly metabolized following cell death. Consequently,

PLFA analysis has been used as a culture-independent method of assessing the structure of soil microbial communities and changes in soil quality (Hill *et al.*, 2000).

Studies by Tunlid *et al.* (1989) were able to demonstrate differences in microbial communities associated with *Rhizoctonia* damping-off. Also, Bossio *et al.* (1998) showed that organically managed soils had significantly different PLFA profiles from those that were conventionally managed.

Despite the usefulness of PLFA analysis, there are limitations e.g. (i) signature molecules for all organisms are not known, and there are cases where specific fatty acids cannot be linked to specific microorganisms or a group of microorganisms; (ii) variation in the signature molecules presents problems; and (iii) the types of PLFAs produced by bacteria and fungi differ with growth stage and environmental stress (Hill *et al.*, 2000).

2.5.3.3 Fluorescent *in situ* hybridization

Fluorescent *in situ* hybridization (FISH) is a process by which whole cells are fixed, their 16S and 23S rRNA is hybridized with fluorescent-labelled taxon-specific oligonucleotide probes, and then the labelled cells are viewed by scanning confocal laser microscopy. It has been used primarily with prokaryotic communities and allows for the direct isolation and quantification of specific and general taxonomic groups of microorganisms in their natural habitat (Hill *et al.*, 2000).

The advantage of FISH over immunofluorescent techniques is that it is more sensitive because non-specific binding to soil particles is avoided. In addition, FISH can detect microorganisms across all phylogenetic levels, and FISH probes can be generated without the need to isolate the microorganism. Scanning confocal laser microscopy is also more sensitive than epifluorescence

microscopy and allows for different taxonomic groups to be viewed simultaneously. A more accurate quantification of cells is possible using FISH as compared to rough estimates obtained from dot-blot assays (Hill *et al.,* 2000).

2.5.3.4 Phylogenetic analysis

A number of phylogenetic methods have been used in the study of microbial ecology. Although rDNA and rRNA are commonly used as characters in phylogenetic analysis, the list of characters that can be used is extensive (Woese, 1987). Phylogenetic studies are important in identifying similarities between organisms, leading to an understanding of the physiology and ecology of, as yet, non-culturable species. However, phylogenetic analyses have one great drawback. The fact that an analysis based on a single type of molecule results in a close relationship between taxa, does not necessarily mean that another equally suitable molecule will support these results. Therefore, microbial phylogenetics should be interpreted with caution when used in soil microbial community analysis (Hill *et al.,* 2000).

CHAPTER 3

Materials and methods

3.1 The field experiments

Two field experiments were established in the Midlands of KwaZulu-Natal using the Umgeni WTR - one at Ukulinga Research Farm (8km from Pietermaritzburg) and the other at Brookdale Farm (in Howick), the site for land disposal of the Umgeni WTR. Both were designed to provide a long-term evaluation of the potential for land disposal of the Umgeni WTR. These particular sites were chosen due to the different soil properties at Brookdale and Ukulinga. The trial at Brookdale Farm was conducted on a deep, well-drained Hutton soil, and at Ukulinga on a shallow, poorly drained Westleigh soil (Soil Classification Working Group, 1991) (Table 3.1).

Table 3.1. Some properties of the Hutton and Westleigh soil forms prior to application of water treatment residue at Brookdale Farm and Ukulinga Research Farm, respectively (after Moodley, 2001).

Property		Soil form*	
		Hutton	**Westleigh**
Soil texture		Clay	Silty clay loam
Particle size distribution (%)	Coarse sand	7	9
	Medium sand	2	2
	Fine sand	5	2
	Coarse silt	16	27
	Fine silt	18	27
	Clay	52	33
Particle density (Mg m^{-3})		2.60	2.68
Cation exchange capacity ($cmol_c$ kg^{-1})		8.58	9.77
Organic carbon (%)		3.35	2.17
pH (water)		5.21	5.91
pH (KCl)		4.22	4.90

* Soil Classification Working Group (1991)

3.1.1 Water treatment residue preparation and pathogen content

Wet WTR with a solids content of about 30% was collected from Midmar Water Treatment Works in March 1998 and February 1999 for application at Brookdale and Ukulinga, respectively. However, prior to land application at Ukulinga and Brookdale, the WTR was stored for eight and six months, respectively, to allow for drying. The drying period resulted in the breakdown of the WTR into smaller aggregates of approximately 50-80mm in diameter, which were applied to the field experiments (Moodley, 2001). Chemical characterization of WTR is given in Appendix 3.1. The WTR was analyzed at Umgeni Water (Pietermaritzburg) for the presence of pathogenic microorganisms. It tested negative for the presence of both *Salmonella* species and *Escherichia coli*. .

3.1.2 Trial site description

The Ukulinga experimental site had been maintained with *Eragrostis curvula* three years prior to setting up the trial, and the Brookdale experimental site had been under arable crop production for the previous forty years. The trials at Ukulinga Research Farm and Brookdale Farm comprised of 28 and 64 experimental plots, respectively. Plots were 6m x 4m in size, separated by a 2m buffer strip. At Brookdale Farm there were four contours, each consisting of sixteen experimental plots. Two contours were maintained fallow while the other two contours were grassed, initially with ryegrass (*Lolium perenne*) and subsequently with Dovey tall fescue (*Festuca arundinacea*). The trial layouts at Ukulinga Research Farm and Brookdale Farm are outlined in Appendix 3.2 and 3.3, respectively. Both trials were periodically sprayed with a glyphosphate-based herbicide to control weeds and other vegetation.

At both experimental sites, plot treatments were duplicated under both fallow and grassed condition. The field experiments were designed to evaluate the soil conditioning properties of WTR. Therefore, the treatments evaluated included

gypsum, dolomitic lime and anionic polyacrylamide, which are known soil conditioners, as well as WTR. However, the research presented in this thesis is concerned with the effects of WTR on soil microbiological and related chemical properties, and only the WTR-amended treatments and control plots were considered.

Literature has shown that water treatment residues are far more innocuous than wastewater sludges (WTS), and in light of this it was felt that the maximum permissible rates applicable to the WTS (80 Mg ha-^{1} annum-^{1} in South Africa) could probably be safely exceeded. At both Ukulinga Research Farm and Brookdale Farm the WTR was disced into the 0-200mm soil depth. At Ukulinga Research Farm, WTR was applied at rates of 0, 80, 320, and 1280Mg ha^{-1}, while at Brookdale Farm, WTR was applied at rates of 0, 40, 80, 160, 320, 640 and 1280Mg ha^{-1}. Additionally, at Brookdale a mulched set of treatments at rates of 320, 640 and 1280Mg ha^{-1} was included (Moodley, 2001).

3.1.3 Selection of treatments, sampling and preparation of soils for laboratory analyses

Sampling of grassed and fallow treatments was carried out in September 2001 and May 2002 (to provide an indication of summer and winter conditions). At Ukulinga Research Farm WTR treatments at rates of 0, 80, 320 and 1280Mg ha^{-1} were sampled. For the sake of consistency and in order to reduce the number of samples that needed processing, only these WTR treatments were also sampled at Brookdale Farm. Additionally, at Brookdale the mulched treatments at rates of 320 and 1280Mg ha^{-1} were sampled.

Using a bucket auger, triplicate soil samples were taken from each treatment and analyses were carried out on individual soil samples i.e., no compositing (n=6). At Ukulinga Research Farm, soil was sampled at 100mm intervals to a depth of 500mm (maximum depth of soil), while at Brookdale Farm samples were taken at

100mm intervals to a depth of 400mm, and subsequently at 200mm intervals to a depth of 1200mm.

The field moist samples were divided into two sub-samples. For biological analyses, one sub-sample of field moist soil was sieved (<2mm), placed into plastic bags and stored at 4°C for up to four months. For chemical analyses, the other sub-sample was air-dried and stored in sealed containers at room temperature.

3.1.4 Statistical analyses

Statistical analysis was carried out using Genstat® Release 6.1 (Lawes Agricultural Trust, Rothamsted Experiment Station). Analysis of variance (ANOVA) was carried out with data treated as a split-split-split plot design. Separation of means was based on the principle of least significant difference (LSD) at the 5% level of significance.

For each measured variable (OC, pH, EC and microbial activity) the ANOVA indicates two main effects i.e., WTR and depth. The WTR effect is determined by exclusion of the depth effect, while the depth effect was determined by exclusion of the treatment effect. For example, when looking at the effect of WTR on a measured variable (OC, pH, EC or microbial activity), data pertaining to each WTR application rate are grouped to obtain a single mean value for each WTR application rate. When ANOVA indicates a significant main effect, the graphs that are plotted are based on the mean data obtained by the above-mentioned grouping of data.

While a graphical representation of the main effects provides a useful indication of general trends, one should acknowledge that an interplay of factors is more likely to affect the measured variables. Thus, the interaction effects provide more useful information. As with main effects, where ANOVA showed significant

interaction effects the mean data were plotted to provide a clearer indication of the trend.

3.2 Organic carbon

Air-dried soil was milled (<500 µm) and 0.5g weighed into 500ml Erlenmeyer flasks. Potassium dichromate was added (10mL), and mixed for 1 minute. Thereafter, 20mL concentrated sulphuric acid was added and swirled in the flask for 1 minute. Samples were allowed to stand for 20 minutes, following which 170mL distilled water, 10mL of an 85% phosphoric acid solution and 0.5g sodium fluoride were added. Ferroin indicator (5 drops) was added and samples were titrated with Mohrs solution to the brownish-black end point (Blakemore *et al.*, 1972).

3.3 Electrical conductivity and pH

Electrical conductivity was measured in a 1:2.5 soil:water extract using a CDM 83 conductivity meter. pH was then measured in the same extract using a PHM 210 standard pH meter (Meter Lab).

3.4 Microbial activity

Microbial activity was estimated using a modified fluorescein diacetate (FDA) hydrolysis method described by Perucci (1992). Twenty four hours before analysis, samples were removed from cold storage (4°C) and stored at room temperature. A 2.5g moist soil sample was weighed into a 100mL Erlenmeyer flask. 400µL of a 20 000 ppm solution of FDA, made up with acetone, and 20mL of 0.6M sodium phosphate buffer (pH 7.6) were added.

Samples were shaken in a controlled environment shaker incubator (IncoShake-Labotec) set at 30°C and 150rpm for one hour. Following incubation, 20mL

acetone were added to stop the reaction. Samples were filtered through Whatman No. 1 filter paper. The filtrate was diluted ten fold with distilled water and absorbence readings carried out at 490nm using a Cary 1E UV-Visible spectrophotometer (Varian).

A standard curve was constructed using 0, 1.75, 2.5, 3.75 and 5 ppm fluorescein in 20mL of phosphate buffer and 20mL acetone. Data were calculated as µMol product (fluorescein) formed g^{-1} oven dry soil hr^{-1}.

3.5 Microbial respiration

Respiration was determined by placing 30g of soil/WTR mixture in a 50mL beaker along with a vial containing 10mL of 0.05M NaOH in a 1L air-tight jar, and incubating the sealed jar in the dark at 25°C for 24 hours (Anderson, 1982). The CO_2-C evolved was determined by back titration with HCl. Modifications to the method included the use of 0.05M HCl as opposed to a 0.1M solution and the incubation of samples for 24 hours as opposed to 10 days.

3.5.1 Preparation of water treatment residue

Dried WTR from Umgeni Water (KwaZulu-Natal), Faure Water Treatment Plant (Cape Town), Rand Water (Johannesburg), Amatola Water (East London) and Midvaal Water Company (Stilfontein) were milled and sieved to pass a 2mm mesh. Information pertaining to the WTRs is given in Tables 3.2 and 3.3.

Table 3.2. Chemicals and flocculants used in the production of the water treatment residues studied.

Waterworks	Source of abstraction	Lime	Bentonite	Polyelectrolyte	$FeCl_3$	$Al_2(SO_4)_3$
Umgeni Water	Midmar Dam	yes	yes	yes	-	-
Rand Water	Vaal Dam	yes	-	yes	yes	-
Faure Water Treatment Plant	Theewaterskloof and Palmiet	yes	-	-	-	-
Amatola Water	Nahoon Dam	yes	-	yes	-	-
Midvaal Water Company	Middle Vaal River	yes	-	yes	yes	yes

Table 3.3. pH, electrical conductivity and organic carbon values of the water treatment residues studied.

Waterworks	pH (water)	Electrical conductivity ($\mu S\ cm^{-1}$)	Organic carbon (%)	Microbial respiration** ($\mu g\ CO_2\text{-}C\ g^{-1}\ day^{-1}$)
Umgeni Water	7.7	597	2.8	54
Rand Water	8.5	258	0.8	212
Faure[1] Water Treatment Plant	6.6	82.7	7.4	160
Faure[2] Water Treatment Plant	7.4	419	10.3	251
Amatola Water	8.1	171	1.9	68
Midvaal Water Company	7.8	247	1.6	198

*Where 1 = WTR used in Experiment 1; and 2 = WTR used in Experiment 2
** = measured on WTRs moistened to 20% moisture following two days of incubation at 25°C

3.5.2 Soil preparation and experimental design

Bulk samples (to a depth of 200mm) of the Westleigh and Hutton soil forms were collected from fallow locations within 10m of the field experimental sites. On the day of sampling, soils were sieved (<15mm) to remove stones and debris and re-moistened to 20% soil moisture. Soils were weighed into the respective treatment pots of 2L volume, sealed and incubated at 25°C for 5 days to allow the soil to equilibrate. However, there was sufficient headspace to prevent anaerobic conditions from developing. Following the equilibration period, WTR was added to the soils to achieve four loading rates (Table 3.4).

Table 3.4. Preparation of treatments for the respiration experiments.

WTR loading rates (% m/m)	Field equivalent rates (Mg ha^{-1})	Soil (g)	WTR (g)
0	0	1000	0
5	100	950	50
15	300	850	150
25	500	750	250

The experiment was conducted in two phases as the WTRs became available as follows:

EXPERIMENT 1

Rand, Umgeni and Faure[1] WTR at rates of 5, 15 and 25% (m/m) were added to the Hutton and Westleigh soils after the 5-day equilibration period, mixed well, re-moistened (20% moisture), sealed and returned to an incubation temperature of 25°C. Six control pots consisting of unamended Hutton and Westleigh soil were prepared, while other treatments were prepared in duplicate.

EXPERIMENT 2

Due to the lack of quantity of WTR from Amatola Water and Midvaal Water, only rates of 5 and 15% were included in the experiment. Although there was ample Faure[2] WTR to allow for the 25% WTR treatment, this was omitted to allow for statistical analysis of the experiment. All treatment pots were prepared in duplicate as detailed in Experiment 1.

Subsequently, each treatment pot was sampled on Day 2, 5, 10 and 14, following the addition of WTR to the soil. The samples (30g) removed for respiration analysis were oven-dried, composited and milled (<2mm) for measurement of pH and EC (section 3.3), following the measurement of respiration.

3.5.3 Statistical analysis

Statistical analysis was carried out using Genstat® Release 6.1 (Lawes Agricultural Trust, Rothamsted Experiment Station). Analysis of variance (ANOVA) was carried out and separation of means was based on the principle of least significant difference (LSD) at the 5% level of significance. For both experiments ANOVA was carried out by the creation of three controls. Control 1 grouped all Hutton (unamended) treatments, Control 2 grouped all Westleigh (unamended) treatments, and Control 3 grouped all WTR amended treatments.

3.6 Microbial community analysis

Water treatment residue was prepared as outlined in section 3.5.1, and soil was collected and prepared as outlined in section 3.5.2.

Due to the cost of this analysis and the higher microbial activity displayed by the Hutton soil in the field experiment, it was chosen for this laboratory experiment. Treatments tested consisted of unamended Hutton soil, unamended WTRs (Table 3.3), and Hutton soil amended with WTRs at 15% (m/m) (Table 3.4).

Microbial community analysis was carried out using denaturing gradient gel electrophoresis (DGGE), following 5 days of incubation at 25°C. This procedure first requires that DNA be extracted from test samples and amplified using polymerase chain reaction (PCR). The preparation of solutions used is given in Appendix 3.4.

3.6.1 DNA extraction

Direct extraction of DNA was carried out on 1g samples using the Ultra Clean Soil DNA Extraction Kit (Mo-Bio Laboratories, Inc). The methods used were as per product specified protocol, and extracted DNA was stored at -20°C to prevent it from degrading.

3.6.2 Detection of extracted DNA

A 1.2% (m/v) agarose gel was prepared by adding 0.3g of electrophoresis grade agarose (Whitehead Scientific Ltd.) to 25mL of Tris(hydroxymethyl)aminoethane (TAE) buffer (1x). The mixture was dissolved by heating in a microwave (Tedelex) for 40 seconds. When the solution had cooled to ± 45°C, 0.5µL of concentrated ethidium bromide (0.5µg/mL) was added. The molten gel was then poured into a gel caster (Hoefer Scientific Instruments), a comb was inserted at one end to form sample wells, and was left to set for 30 minutes at room temperature. The gel was then placed in a horizontal gel unit (Hoefer Scientific Instruments) and immersed in 1x TAE buffer (± 200mL). 5µL of sample DNA and 3µL of loading buffer (Promega) were loaded into each well. A 1kbp molecular weight marker was run in tandem with the DNA samples. Electrophoresis was carried out at 90V for 55 minutes using a Bio-Rad power pack. The presence of DNA was verified by viewing the gel under a UV transilluminator.

3.6.3 Polymerase chain reaction

Absorbency measurements at A_{260} and A_{280} to calculate the A_{260}/A_{280} purity ratio of the DNA samples were determined (Crecchio and Stotzky, 1998) with a GeneQuant pro DNA/RNA calculator (Biochrom Ltd).

Polymerase chain reaction (PCR) was carried out as indicated in the PCR Core Kit (Roche). The reagents used in the PCR to amplify the 16S rDNA component of the genomic DNA are listed in Table 3.5. Primers were synthesized and supplied by Integrated DNA Technologies, Inc. The PCR was conducted in an automated thermal cycler (Perkin-Elmer Gene Amp PCR System 2400, Norwalk, USA). The sequence of the forward (P_f) and reverse (P_r) primers were as follows (Watanabe *et al.*, 2001):

P_f: 5' - CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC TAC GGG AGG CAG CAG - 3'

P_r: 5' – ATT ACC GCG GCT GCT GG – 3'

A positive control consisting of a reference culture of *Bacillus subtilis,* and a negative control consisting of the PCR premix, were also prepared. A 40 nucleotide GC clamp was incorporated into the forward primer at its 5' end. The PCR amplification sequence used included the following steps: (i) initial denaturation at 92°C for 4 minutes; (ii) 35 cycles of denaturation (at 94°C for 60 seconds), annealing (at 55°C for 1 minute), elongation (at 72°C for 1 minute); (iii) final elongation at 72°C for 5 minutes; and (iv) cooling and storage at 4°C.

Table 3.5. Reagents used for PCR amplification of 16S rDNA.

Reagent per reaction tube	Volume (µL)
0.01M Primer (forward)	1
0.01M Primer (reverse)	1
10mM dNTPs (Promega)	1
10mg mL^{-1} Bovine serum albumin (Boehringer Mannheim)	1
Taq buffer	5
Milli-Q water	39
Taq polymerase	1
Target DNA	1
Total of reaction mixture	50

Amplified PCR products were assessed by agarose gel electrophoresis followed by direct visualization of DNA using UV transillumination (Section 3.6.2). A 100bp marker (Promega) was also run to confirm the approximate size of the amplified products.

3.6.4 Denaturing gradient gel electrophoresis

Extracted DNA was of a low concentration and fell below the detection limits of the GeneQuant pro DNA/RNA calculator. Quantification of the DNA was therefore not possible. Consequently, DGGE was run by using constant volumes DNA from each of the treatments and allowed for the qualitative analysis of data.

The DGGE electrophoresis unit (Bio-Rad) was set up as per the manufacturer's instruction.

A 30% denaturing gradient (low) and a 70% gel gradient (high) were prepared and placed in the respective 'low' and 'high' denaturant wells of the gradient mixer. 100µL ammonium persulfate (APS) and 10µL N, N, N', N' –

tetramethylethylenediamine (TEMED) (Sigma, electrophoresis grade) were added to each concentration well. Prior to the addition of the TEMED, a 'bottom' gel was prepared, the purpose of which was to prevent leaking during the gel casting process. After addition of the TEMED, a gradient delivery system was used to deliver solutions to the gel sandwich. After delivery, a 'top' gel or stacking gel was prepared using 5mL of the 0% denaturing gel solution mixed with 50µL of gel dye. The stacking gel formed the top 20mm of each gel. A comb was then inserted for well formation and gels were left to polymerize for two hours before the comb was removed. Thereafter the wells were rinsed with running buffer.

The control unit was then placed on top of the tank and turned on. The system was allowed to reach 60°C and pre-electrophoresed for 15 minutes at 70 volts. Gelsaver tips (Whitehead Scientific Ltd) were used to load 5µL of loading buffer and 20µL of sample into the wells of the 7% (m/v) polyacrylamide gel. The system was run for 16 hours at 70 volts. The power supply was then turned off and the gel was removed and stained with silver nitrate (Appendix 3.5).

The stained image was placed on a light box and captured with a Sony Mavica digital camera. The image was converted to a TIFF file and analysed using the BioRad Quantity 1D analysis software. Statistical analysis using the Dice Coefficient method was used to generate a similarity matrix of the treatment lanes.

CHAPTER 4

Microbial activity and related chemical properties of the soils from the Ukulinga and Brookdale Farm field trials

4.1 Results

The results have been discussed in two categories i.e. main effects and interaction effects. A main effect is obtained by the exclusion of all treatment effects (Section 3.1.4). The interaction effects examine the relationships between the tested variables, namely, depth, water treatment residue (WTR), and status (i.e. whether the land is under fallow or grassed treatment).

4.1.1 Organic carbon

4.1.1.1 Main effects

At both Brookdale and Ukulinga Farms, the change in organic carbon (OC) with depth was significant in September 2001 and May 2002. Further, at Ukulinga Farm the change in OC with the different application rates of WTR was significant at both sampling times. However, at Brookdale Farm changes in OC with the different application rates of WTR were non-significant at September 2001, but significant at May 2002 (Appendix 4.1).

At both trials there was a decline in OC with increasing soil depth. Ukulinga OC values ranged between 1 and 2.5% at both sampling times (Figure 4.1a), while at Brookdale the range was between 0.6 and 3.2% (Figure 4.1b).

The mean quantity of OC recorded at Ukulinga and Brookdale Farms at both sampling times is given in Appendix 4.2 and 4.3, respectively. Where the ANOVA indicated significant effects, separation of means using LSD's allowed

for closer examination of the effects of WTR and depth on OC. At both trials differences in OC content were more pronounced with depth than with WTR application.

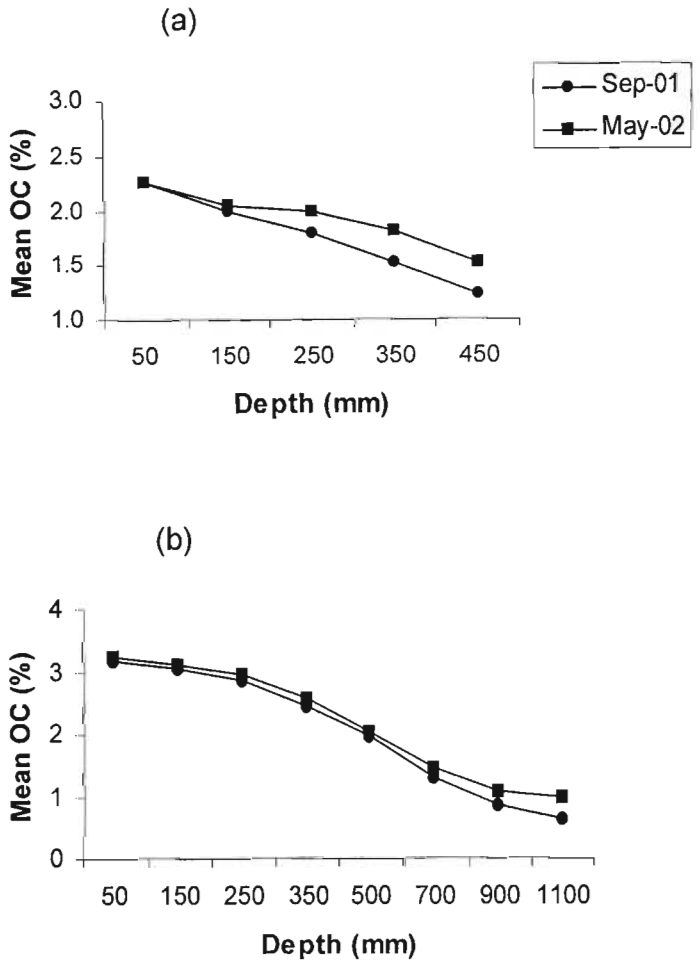


Figure 4.1. The trend in mean organic carbon (OC) in the soil profile at (a) Ukulinga Research Farm and (b) Brookdale Farm sampled in September 2001 and May 2002.

The relationship between OC and the different application rates of WTR at Ukulinga and Brookdale is shown in Figures 4.2a and b, respectively. The September 2001 sampling at Ukulinga displayed higher OC content (between 0.4 and 0.5%) on WTR-amended treatments in comparison to the control. At this

sampling time the OC content of the WTR-amended treatments was statistically different from the controls, but not statistically different from each other. The Ukulinga May 2002 samples showed significant but only small differences in OC between the different treatments. At Brookdale Farm, although significant differences in OC were observed across the different treatments in September 2001, both sampling times revealed small differences in OC (<0.3%) across the different treatments and between sampling times.

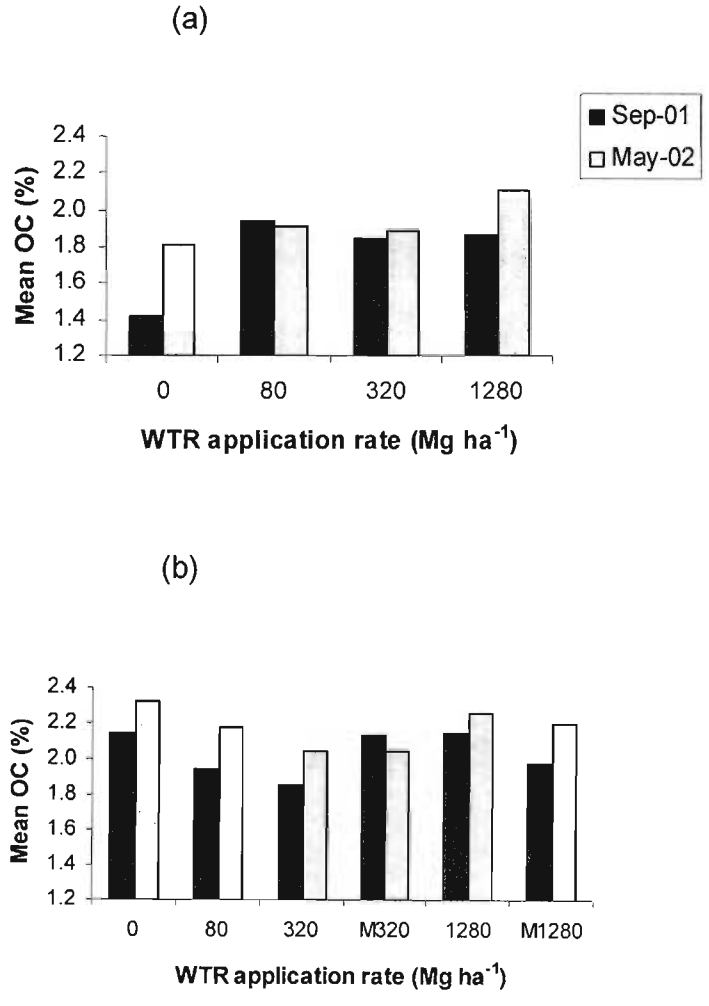


Figure 4.2. The trend in mean organic carbon (OC) at (a) Ukulinga Research Farm and (b) Brookdale Farm at the different application rates of WTR sampled in September 2001 and May 2002.

4.1.1.2 Interaction effects

At Ukulinga the (status x depth) interaction (Figure 4.3a) was significant in September 2001 and May 2002 (Appendix 4.1). The OC content of experimental plots under fallow treatment was marginally but consistently higher than the grassed treatments throughout the soil profile at both sampling times. Additionally, the OC ranges recorded in September 2001 and May 2002 were very similar. The (status x depth) interaction at Brookdale Farm was non-significant at both sampling times (Appendix 4.1) and so almost no differences in OC between fallow and grassed treatments are shown in Figure 4.3b.

At Brookdale the (WTR x depth) interaction was significant at both sampling times, while at Ukulinga the interaction was significant in September 2001 only (Appendix 4.1). However, at both trials the significance of this interaction is more likely due to the significance of the depth effect since the trend in OC across all WTR application rates was very similar (Figures 4.2 a and b). At Ukulinga the May 2002 sampling revealed similar OC trends across all rates of WTR, while in September 2001 the WTR-amended treatments displayed higher OC than the control in the 100-500 mm soil samples (Figure 4.4). At Brookdale the plot of this interaction revealed very similar trends in OC across all WTR application rates at September 2001 and May 2002. Additionally, the range of OC at the two sampling times is very similar (Figure 4.5).

Additionally, while Ukulinga samples showed significant (status x WTR) and (status x WTR x depth) interactions for the September 2001 sampling, these interactions were non-significant at Brookdale (Appendix 4.1). However, it is probable that the significance of these interactions resulted mainly from the WTR and depth effects and so they are not discussed further.

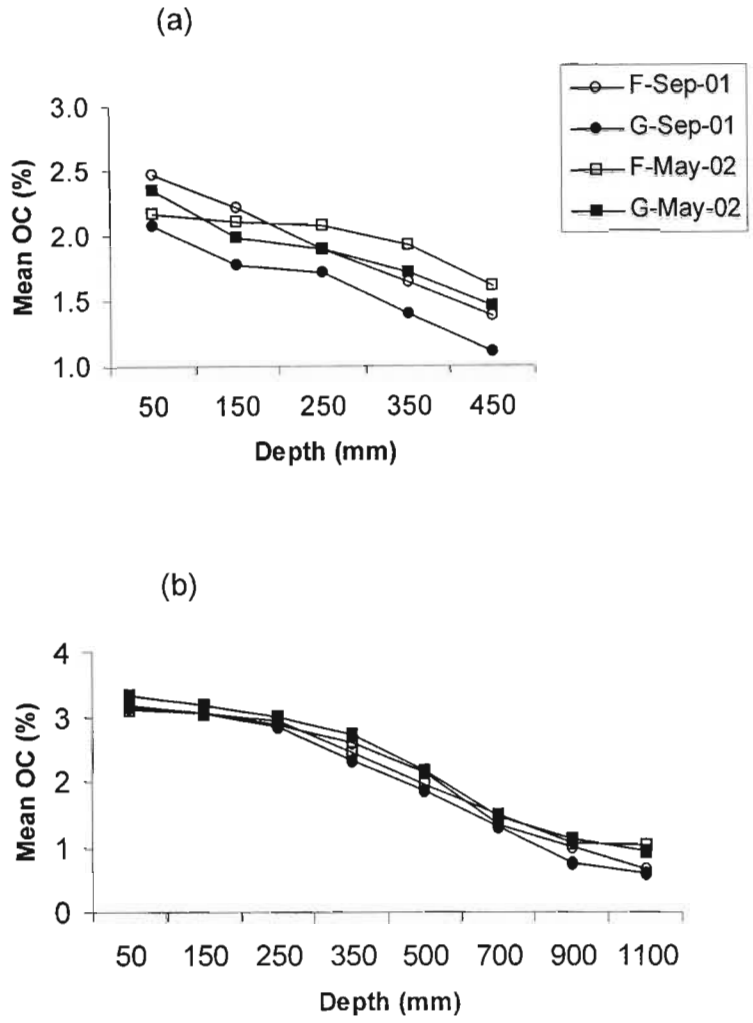


Figure 4.3. The trend in mean organic carbon (OC) at (a) Ukulinga Research Farm and (b) Brookdale Farm under fallow (F) and grassed (G) conditions sampled in September 2001 and May 2002.

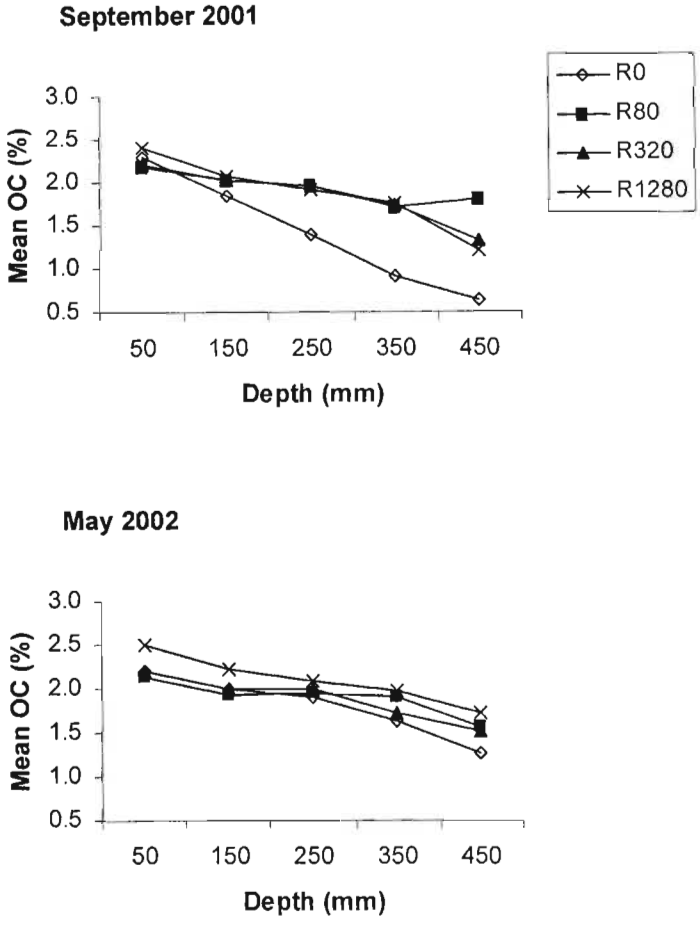


Figure 4.4. The trend in mean organic carbon (OC) in the soil profile at the different application rates of water treatment residue (R) at Ukulinga Research Farm sampled in September 2001 and May 2002.

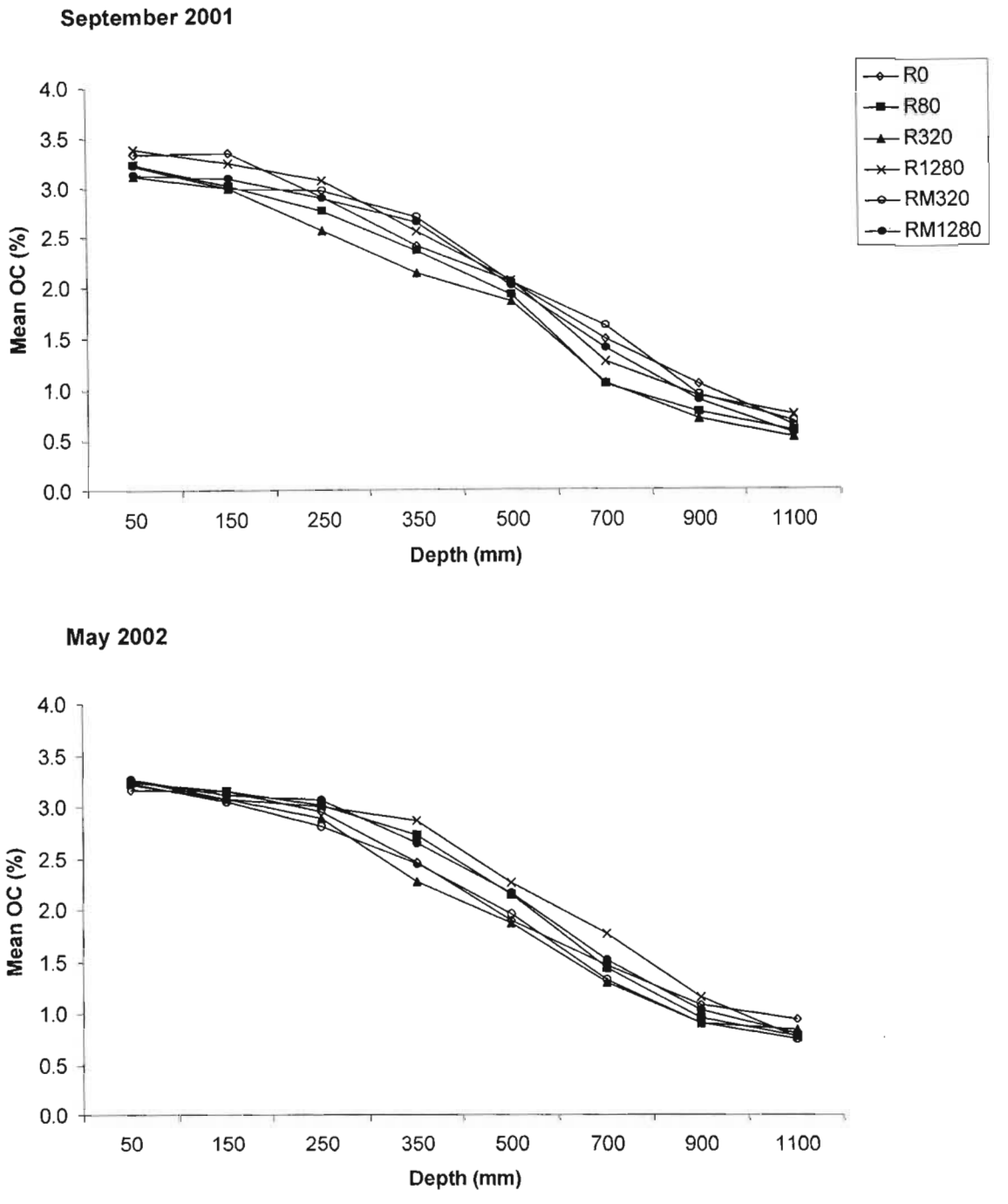


Figure 4.5. The trend in mean organic carbon (OC) in the soil profile at the different application rates of water treatment residue (R) at Brookdale Farm sampled in September 2001 and May 2002 (M = mulch).

4.1.2 pH

4.1.2.1 Main effects

At Brookdale the different application rates of WTR did not significantly impact on soil pH at either sampling time. At Ukulinga the change in pH resulting from the different application rates of WTR was significant in May 2002, only (Appendix 4.4). Analysis of the Ukulinga May 2002 samples revealed that the pH of the WTR-amended soils was statistically different from the controls (Appendix 4.5). Although the change in pH at the different application rates of WTR is statistically significant, these changes were not in excess of 1 pH unit.

At Ukulinga and Brookdale Farms, the change in pH with depth was significant at both sampling times (Appendix 4.4). At Ukulinga Farm, the range and trend of pH with depth in September 2001 and May 2002 were very similar (Figure 4.6a). However, at Brookdale Farm the surface soil samples displayed higher pH in September 2001 than in May 2002 (Figure 4.6b). At Brookdale the mean pH throughout the soil profile ranged between 5 and 6 at both September 2001 and May 2002, and the range of pH recorded is given in Appendix 4.6. Comparatively, mean pH at Ukulinga was higher than at Brookdale, ranging between 6 and 7 at both sampling times.

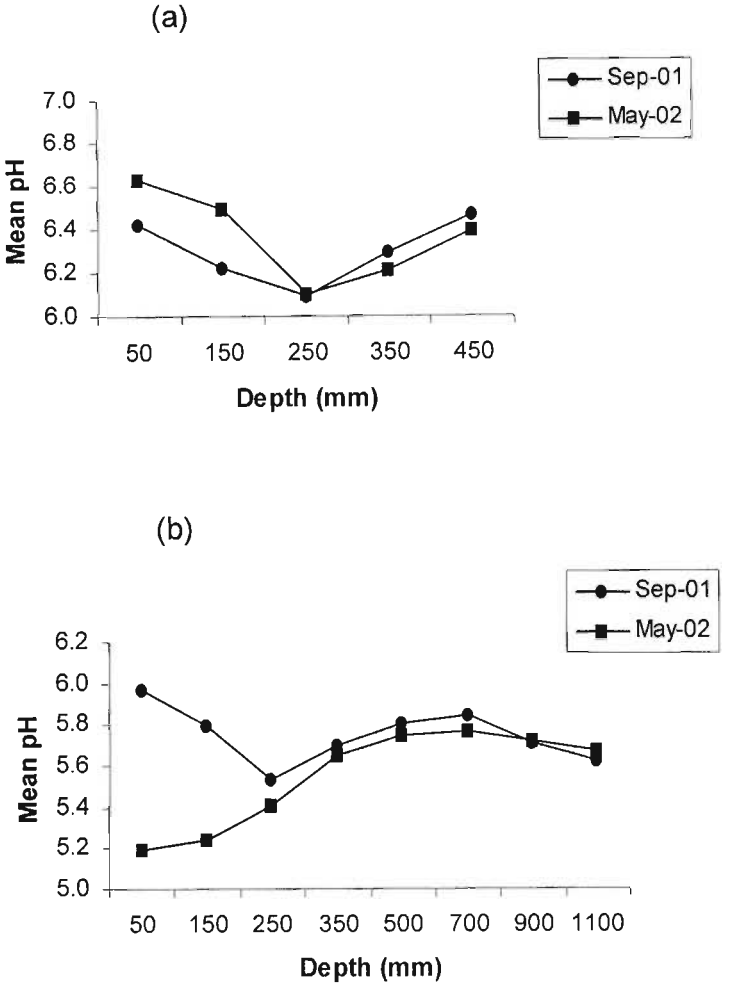


Figure 4.6. The trend in mean pH in the soil profile at (a) Ukulinga Research Farm and (b) Brookdale Farm sampled in September 2001 and May 2002.

4.1.2.2 Interaction effects

At Ukulinga and Brookdale Farms the (WTR x depth) interaction was significant at both sampling times (Appendix 4.4). At both trials WTR addition resulted in an increase in pH in the top 200mm of the soil into which the WTR was incorporated. Below this depth the pH of the WTR-amended treatments was similar to that of the controls.

At Ukulinga there was an increase in pH with increasing application rates of WTR that was more pronounced in the September 2001 samples (Figure 4.7). The same trend was observed on mulched and non-mulched treatments at Brookdale Farm. While the September 2001 samples displayed pH increases of up to approximately 2 units on the R1280 treatment (Figure 4.8), the May 2002 samples showed an increase in pH on the mulched treatments only (Figure 4.9). However, these increases in pH were smaller in May 2002 than in September 2001. This is a likely explanation for the large pH difference (in the 0-200mm depth) noted in Figure 4.6b.

At Ukulinga Research Farm the (status x depth) interaction (Figure 4.10a) was significant at both sampling times (Appendix 4.4). At both sampling times the pH of the grassed treatments was higher than the fallow treatments. While the pH of grassed plots remained fairly constant throughout the soil profile, under fallow there was a decrease in pH from the surface to a depth of 200-300mm. With greater depth the pH increased to approximately the values of the surface soil. Additionally, the range of pH at both sampling times was very similar.

At Brookdale Farm the (status x depth) interaction (Figure 4.10b) was significant in September 2001 and non-significant in May 2002 (Appendix 4.4). As with the Ukulinga data, the May grassed treatments displayed higher pH than the fallow treatments, and displayed more constant pH throughout the soil profile. However, there was no apparent pH trend in September 2001 and observed differences in pH were small.

At Ukulinga the (status x WTR x depth) interaction (Figure 4.11) was significant in September 2001 and non-significant in May 2002, and displayed a more pronounced increase in pH in the surface soil under fallow conditions. At Brookdale Farm the (status x WTR x depth) interaction was non-significant at both sampling times and so these data are not graphically represented (Appendix 4.4).

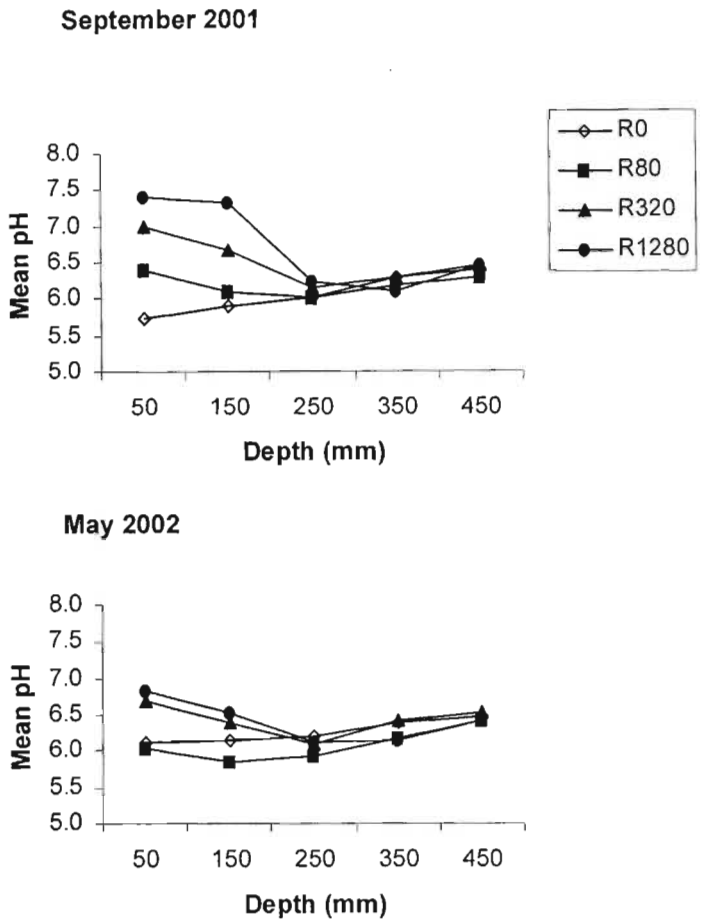


Figure 4.7. The trend in mean pH in the soil profile at different application rates of water treatment residue (R) at Ukulinga Farm sampled in September 2001 and May 2002.

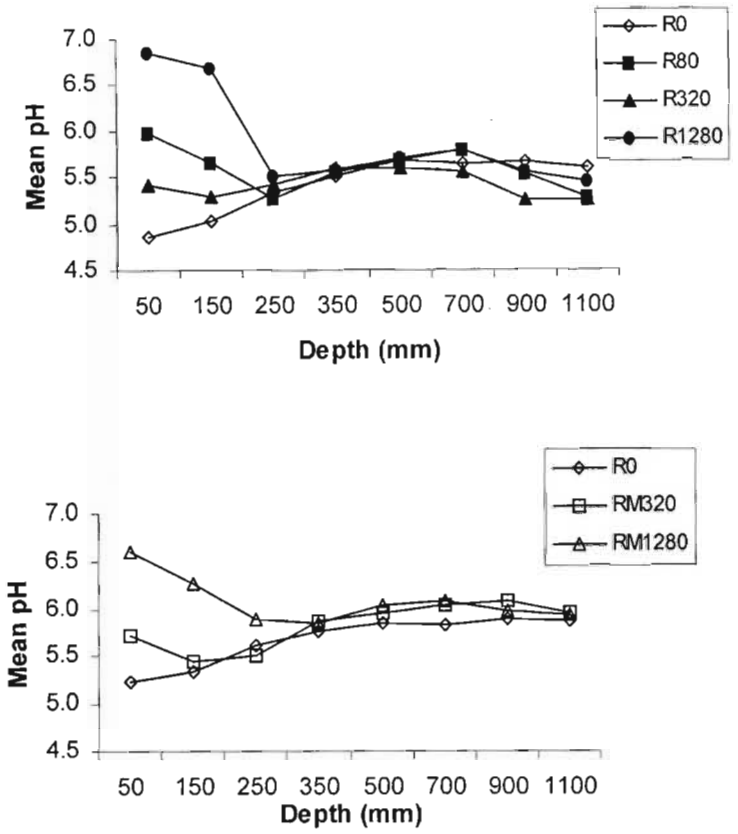


Figure 4.8. The trend in mean pH in the soil profile at different application rates of water treatment residue (R) at Brookdale Farm sampled in September 2001 (M = mulch).

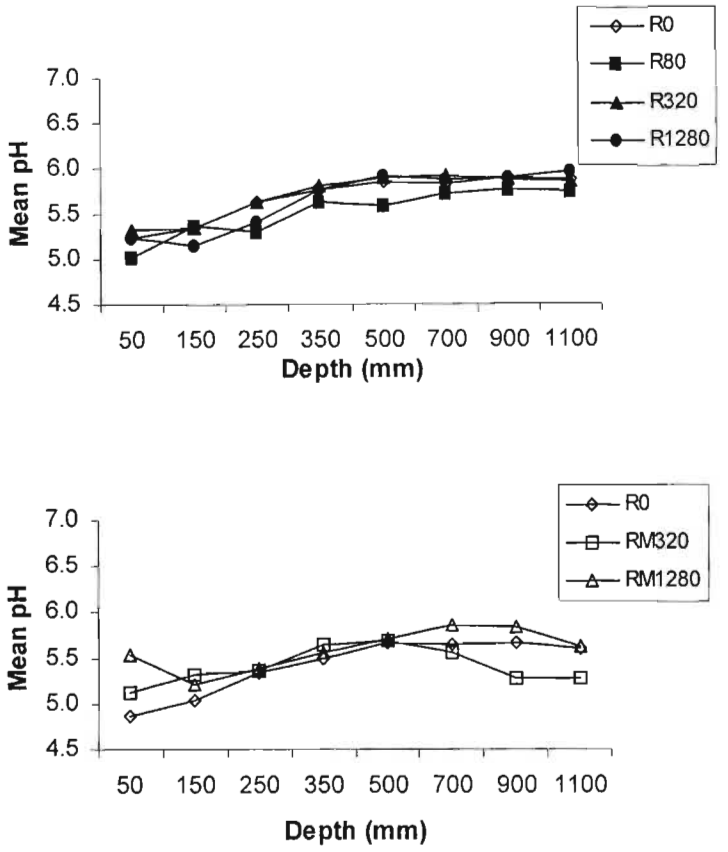


Figure 4.9. The trend in mean pH in the soil profile at different application rates of water treatment residue (R) at Brookdale Farm sampled in May 2002 (M = mulch).

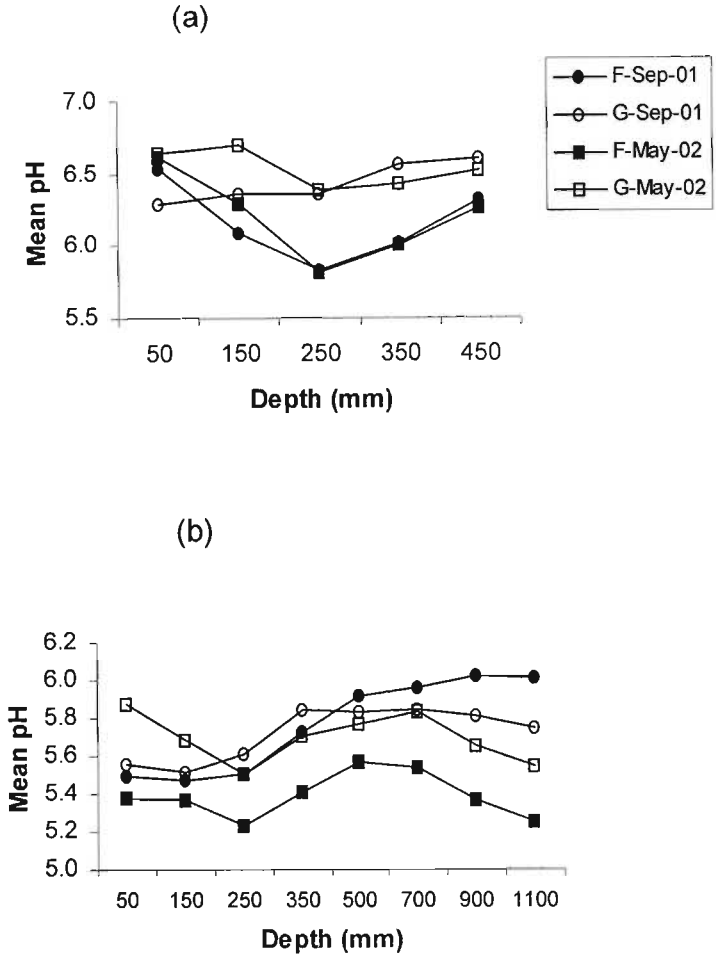


Figure 4.10. The trend in mean pH at (a) Ukulinga Research Farm and (b) Brookdale Farm under fallow (F) and grassed (G) conditions sampled in September 2001 and May 2002.

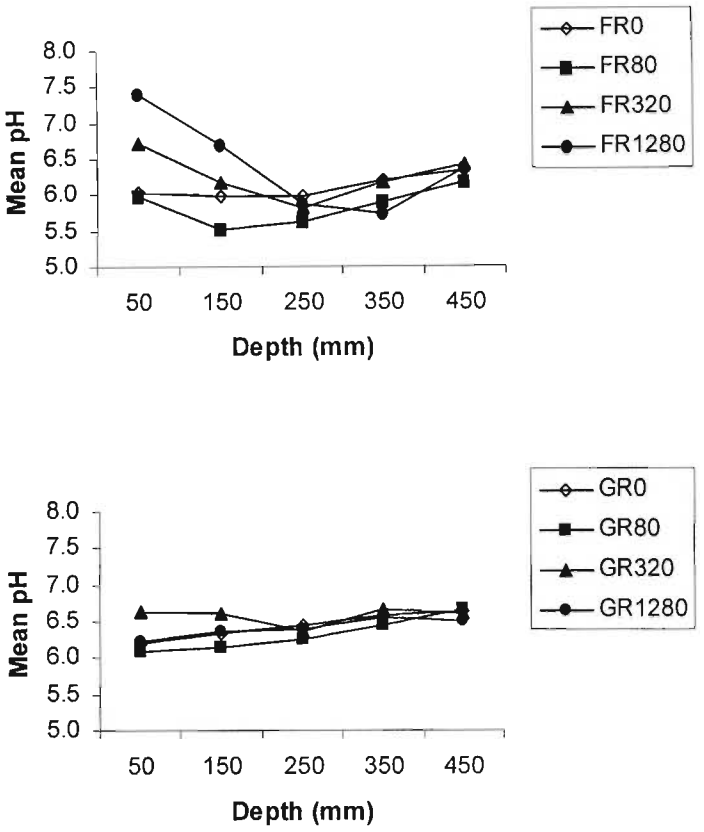


Figure 4.11. The trend in mean pH under fallow (F) and grassed (G) conditions at Ukulinga Research Farm at the different water treatment residue (R) application rates sampled in September 2001.

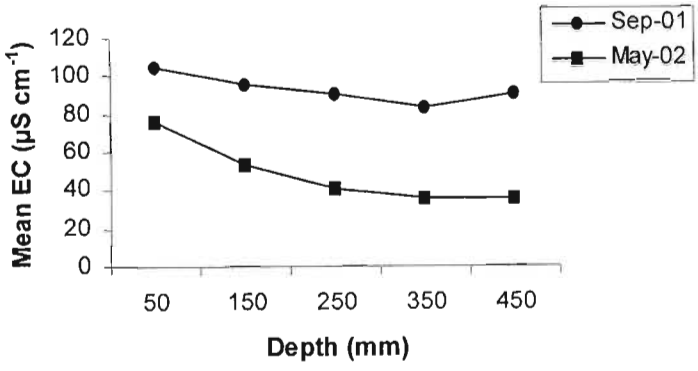
4.1.3 Electrical conductivity

4.1.3.1 Main effects

At Ukulinga and Brookdale the change in EC with depth was significant in September 2001 and May 2002 (Appendix 4.7). At Ukulinga EC appeared to decrease with increasing depth (Figure 4.12a), while at Brookdale (Figure 4.12b) EC appeared reasonably constant with a slight noted from the 500 to 1200mm depth. Both trials displayed higher EC in September 2001 than in May 2002. At Ukulinga the overall mean EC across all treatments ranged between 80 and 105µS cm^{-1} in September 2001 and between 35 and 80µS cm^{-1} in May 2002 (Appendix 4.8). At Brookdale it ranged between 65 and 105µS cm^{-1} in September 2001 and between 45 and 85µS cm^{-1} in May 2002 (Appendix 4.9).

At Ukulinga the change in EC resulting from the different application rates of WTR was only significant in September 2001, whereas at Brookdale it was significant at both sampling times (Appendix 4.7). At Ukulinga, the EC of the R80 and R320 treatments sampled in September 2001 and May 2002 was very similar. However, the September 2001 control (R0) and R1280 treatments displayed higher EC than in May 2002 (Figure 4.13a). Although at Brookdale Farm statistically significant differences were noted between treatments, these differences were generally small. Additionally, the EC of September 2001 and May 2002 samples was very similar, with the most pronounced differences noted on the mulched treatments (Figure 4.13b).

(a)



(b)

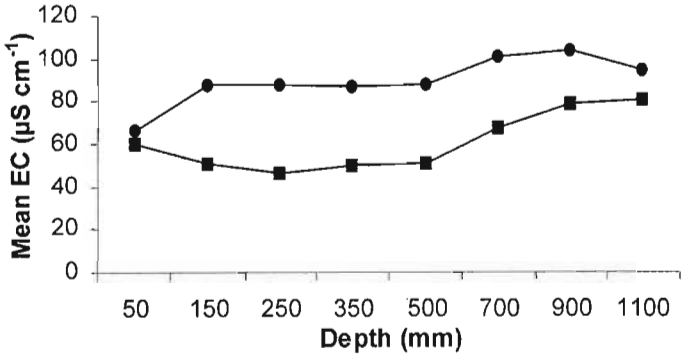


Figure 4.12. The trend in mean electrical conductivity (EC) in the soil profile at (a) Ukulinga Research Farm and (b) Brookdale Farm sampled in September 2001 and May 2002.

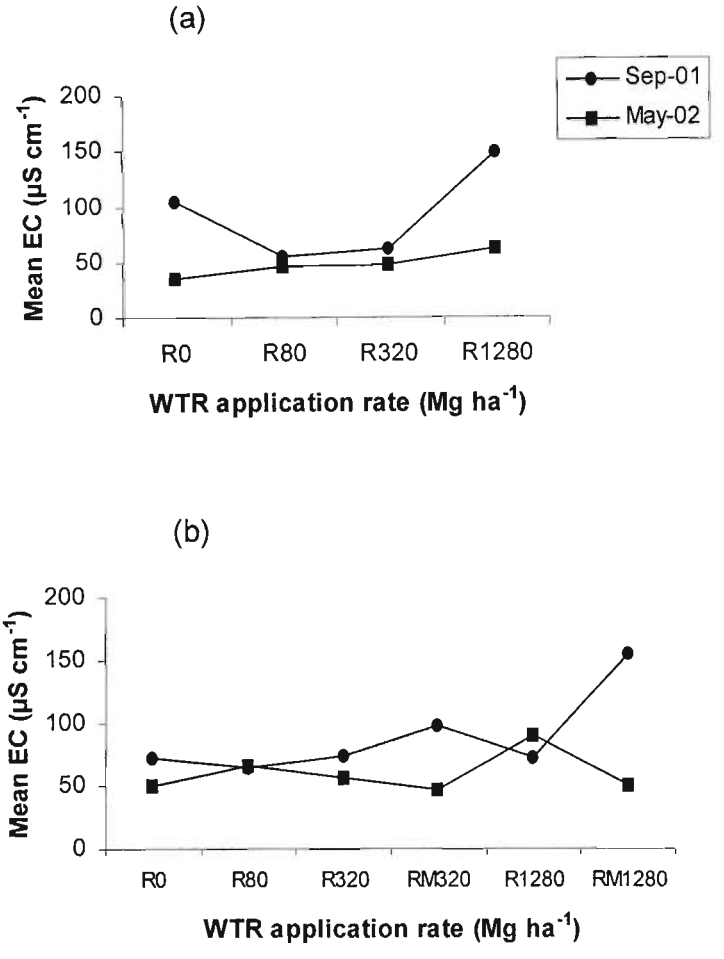


Figure 4.13. The trend in mean electrical conductivity (EC) at (a) Ukulinga Research Farm and (b) Brookdale Farm at different application rates of water treatment residue (R) sampled in September 2001 and May 2002 (M = mulch).

4.1.3.2 Interaction effects

At Ukulinga, the (WTR x depth) interaction was significant in September 2001 and non-significant in May 2002. Comparatively, at Brookdale Farm this interaction was significant at both sampling times (Appendix 4.7). At Ukulinga the May 2002 samples displayed similar EC across all treatments. In comparison, the September 2001 sampling displayed highest EC on the R1280 treatment while the R80 and R320 plots displayed lower EC than the control (R0) (Figure 4.14). However, at Brookdale Farm the September 2001 mulched samples displayed higher EC than the non-mulched treatments. However, the May 2002 treatments displayed similar EC throughout the soil profile and the range of EC was lower than the September 2001 samples (Figure 4.15).

At Ukulinga the (status x depth) interaction (Figure 4.16a) was only significant in May 2002 (Appendix 4.7). At Brookdale Farm this interaction (Figure 4.16b) was only significant in September 2001. Both graphs revealed higher EC on the fallow treatments than on the grassed treatments. However, even when the (status x depth) interaction was significant, differences in mean EC between the fallow and grassed treatments were marginal.

At both Ukulinga (Figure 4.17) and Brookdale (Figure 4.18), the (status x WTR x depth) interaction was significant in September 2001 only (Appendix 4.7).

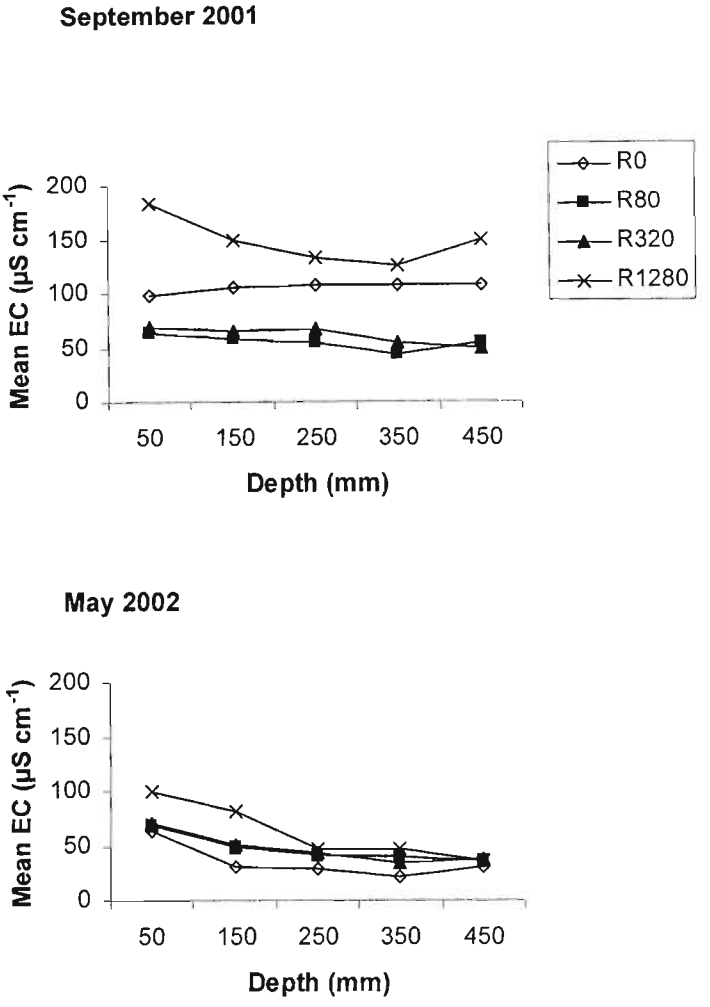


Figure 4.14. The trend in mean electrical conductivity (EC) in the soil profile at different application rates of water treatment residue (R) at Ukulinga Research Farm sampled in September 2001 and May 2002.

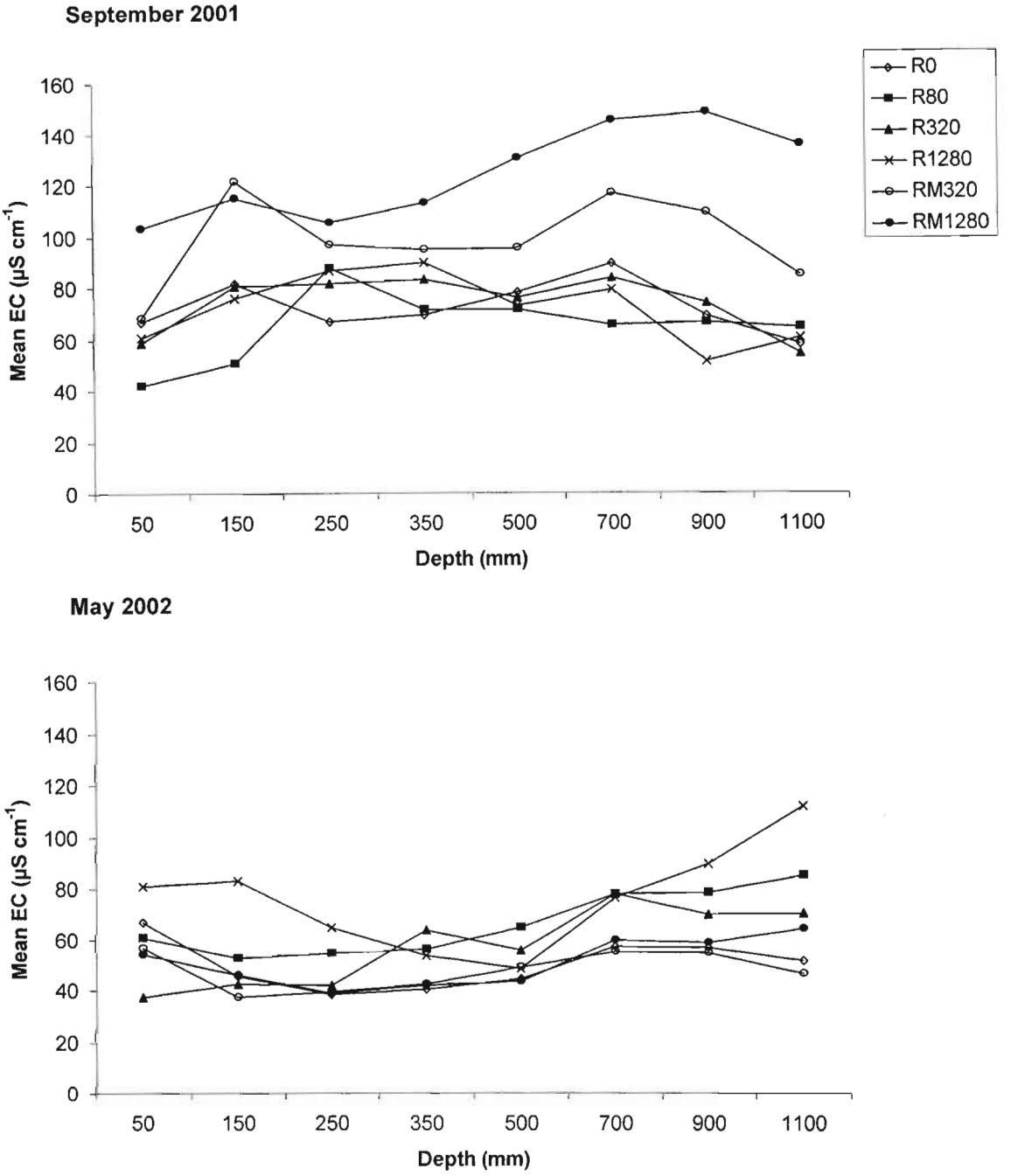
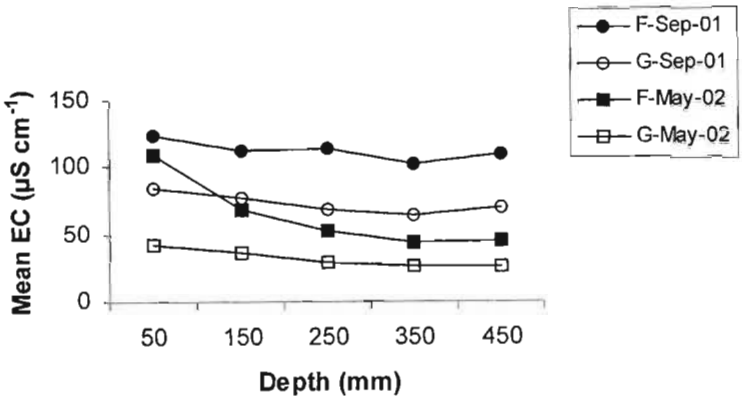


Figure 4.15. The trend in mean electrical conductivity (EC) in the soil profile at the different application rates of water treatment residue (R) at Brookdale Farm sampled in September 2001 and May 2002 (M = mulch).

(a)



(b)

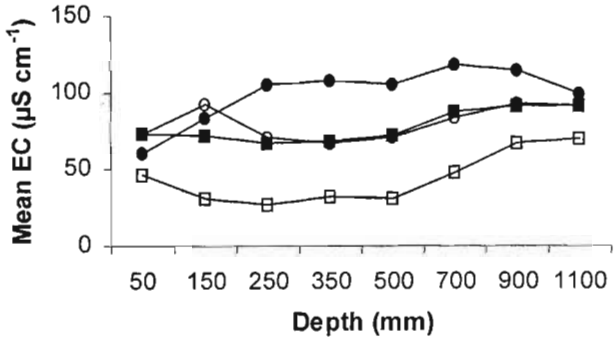


Figure 4.16. The trend in mean electrical conductivity (EC) at (a) Ukulinga Research Farm and (b) Brookdale Farm under fallow (F) and grassed (G) conditions sampled in September 2001 and May 2002.

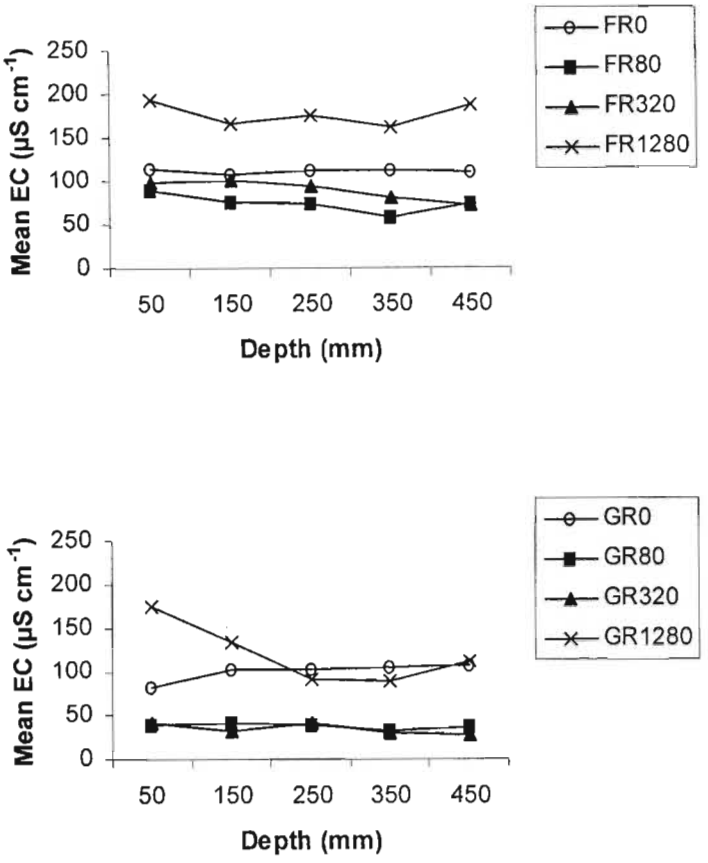


Figure 4.17. The trend in mean electrical conductivity (EC) under fallow (F) and grassed (G) conditions at Ukulinga Research Farm at the different water treatment residue (R) application rates sampled in September 2001 (M = mulch).

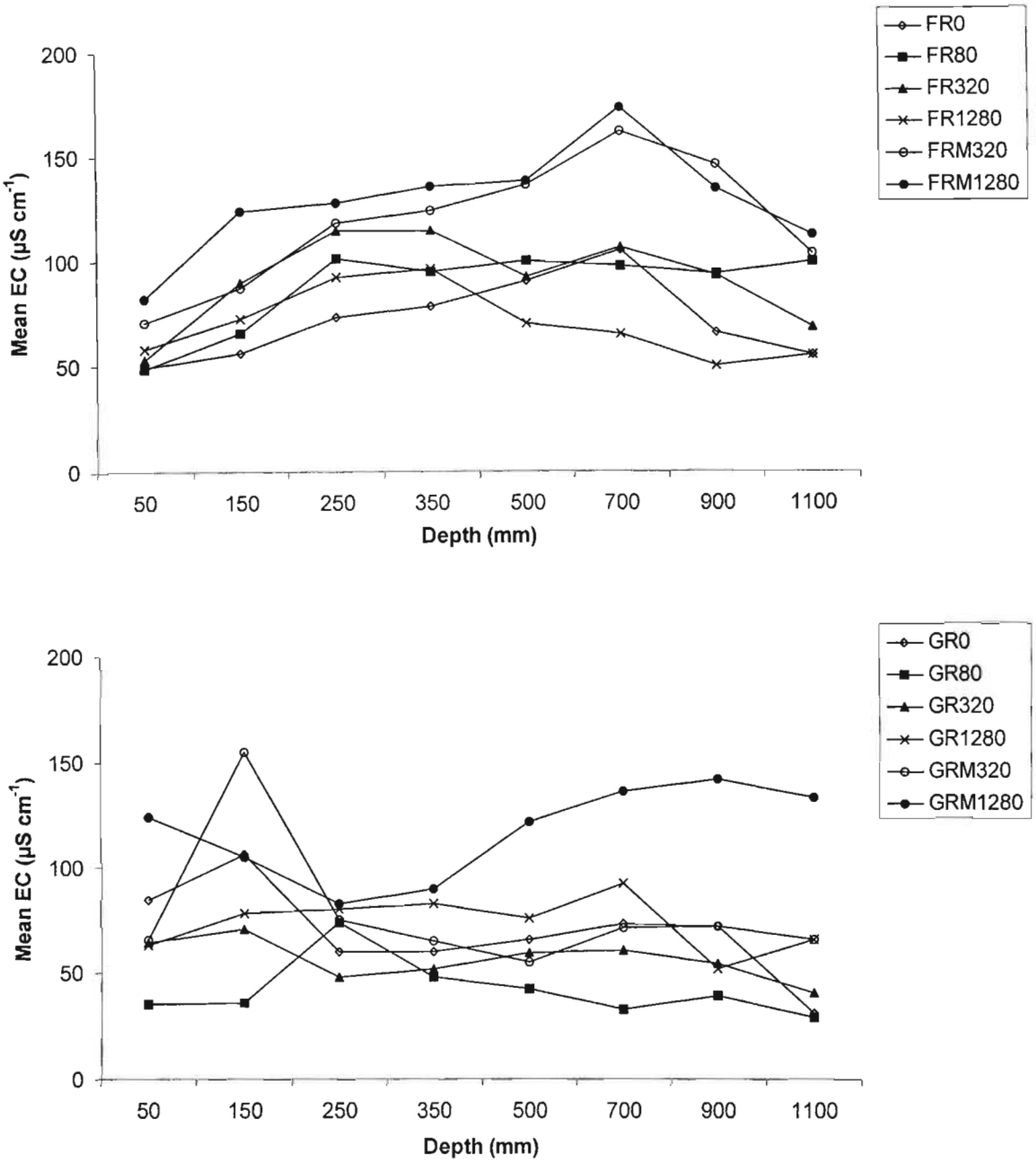


Figure 4.18. The trend in mean electrical conductivity (EC) under fallow (F) and grassed (G) conditions at Brookdale Farm at the different water treatment residue (R) application rates sampled in September 2001 (M = mulch).

4.1.4 Microbial activity

4.1.4.1 Main effects

At Ukulinga the change in microbial activity with depth was significant in May 2002, only. However, at Brookdale the change in microbial activity with depth was significant in both September 2001 and May 2002 (Appendix 4.10). In September 2001 and May 2002 microbial activity at Ukulinga ranged between 0.2 and 0.4μMol g^{-1} hr^{-1} (Figure 4.19a), while at Brookdale microbial activity ranged from 0 to 1.5μMol g^{-1} hr^{-1} (Figure 4.19b). In September 2001 and May 2002 microbial activity increased with increasing soil depth at both Ukulinga and Brookdale, reaching peak activity at the 300-400mm and 300-600mm depth, respectively. The range of microbial activity values recorded at Ukulinga and Brookdale is given in Appendix 4.11 and 4.12, respectively.

The change in microbial activity with the different application rates of WTR was non-significant at Ukulinga at both sampling times (Figure 4.20a) but was significant at Brookdale in September 2001 (Figure 4.20b). Additionally, at Ukulinga the mean microbial activity recorded in September 2001and May 2002 was very similar. Despite the statistical significance of the WTR effect on microbial activity of the Brookdale September 2001 samples, Figure 4.20b shows only marginal differences in microbial activity between treatments. At Brookdale microbial activity was higher in September 2001 than in May 2002.

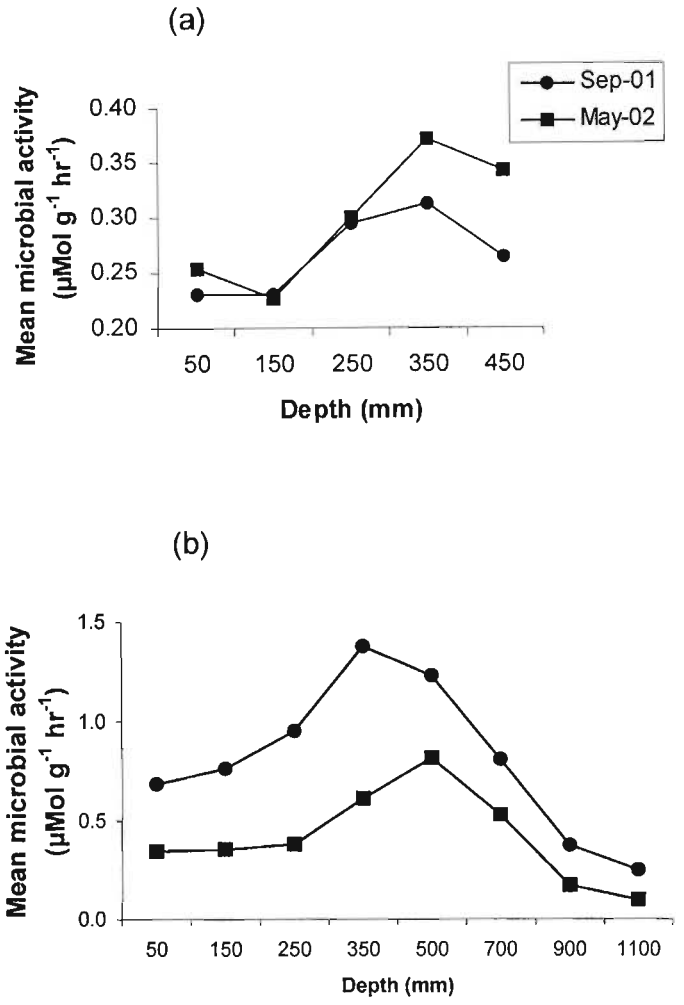


Figure 4.19. The trend in mean microbial activity in the soil profile at (a) Ukulinga Research Farm and (b) Brookdale Farm sampled in September 2001 and May 2002.

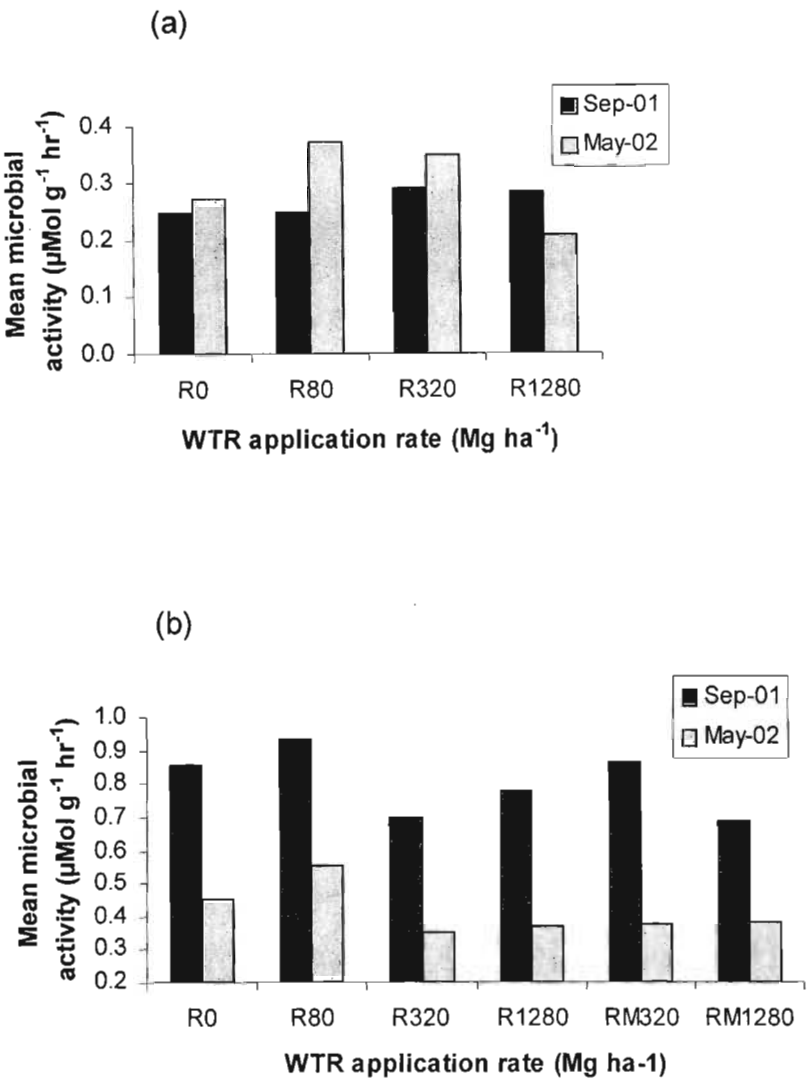


Figure 4.20. The trend in mean microbial activity at (a) Ukulinga Research Farm and (b) Brookdale Farm at the different application rates of water treatment residue (R) sampled in September 2001 and May 2002 (M = mulch).

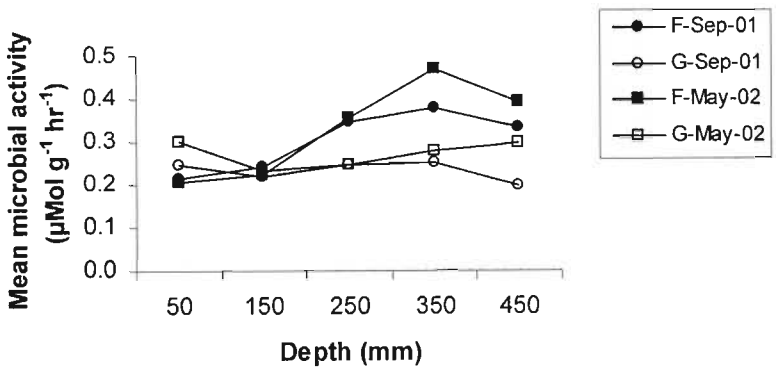
4.1.4.2 Interaction effects

At Ukulinga the (status x depth) interaction (Figure 4.21a) was significant in May 2002, while at Brookdale this interaction (Figure 4.21b) was significant at both sampling times (Appendix 4.10). At Ukulinga the fallow plots displayed marginally higher activity than the grassed treatments, with a peak in activity at a depth of 300-400mm. At Ukulinga the grassed plots displayed fairly constant microbial activity throughout the soil profile. The grassed treatments at Brookdale had marginally higher mean microbial activity in the 0-600mm soil depth.

At Ukulinga the (WTR x depth) interaction (Figure 4.22) was non-significant in September 2001 and May 2002 while at Brookdale this interaction (Figure 4.23) was significant at both sampling times (Appendix 4.10). At Brookdale all treatments showed an increase in microbial activity with increasing soil depth, reaching peak activity in the 300-600mm soil samples. This peak activity declined from between 1 and 2µMol g^{-1} hr^{-1} in September 2001 to between 0.5 and 1.4µMol g^{-1} hr^{1} in May 2002.

At Ukulinga the (status x WTR) interaction was non-significant while at Brookdale Farm it was significant at both sampling times (Appendix 4.10). However, plots of these relationships indicated only small differences in microbial activity across all application rates of WTR under grassed and fallow conditions at Ukulinga (Figure 4.24a) and Brookdale (Figure 4.24b).

(a)



(b)

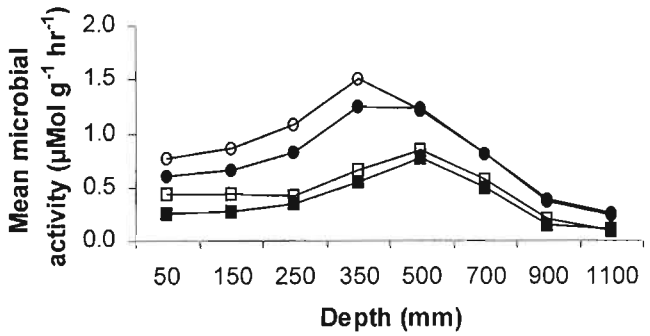


Figure 4.21. The trend in mean microbial activity at (a) Ukulinga Research Farm and (b) Brookdale Farm under fallow (F) and grassed (G) conditions sampled in September 2001 and May 2002.

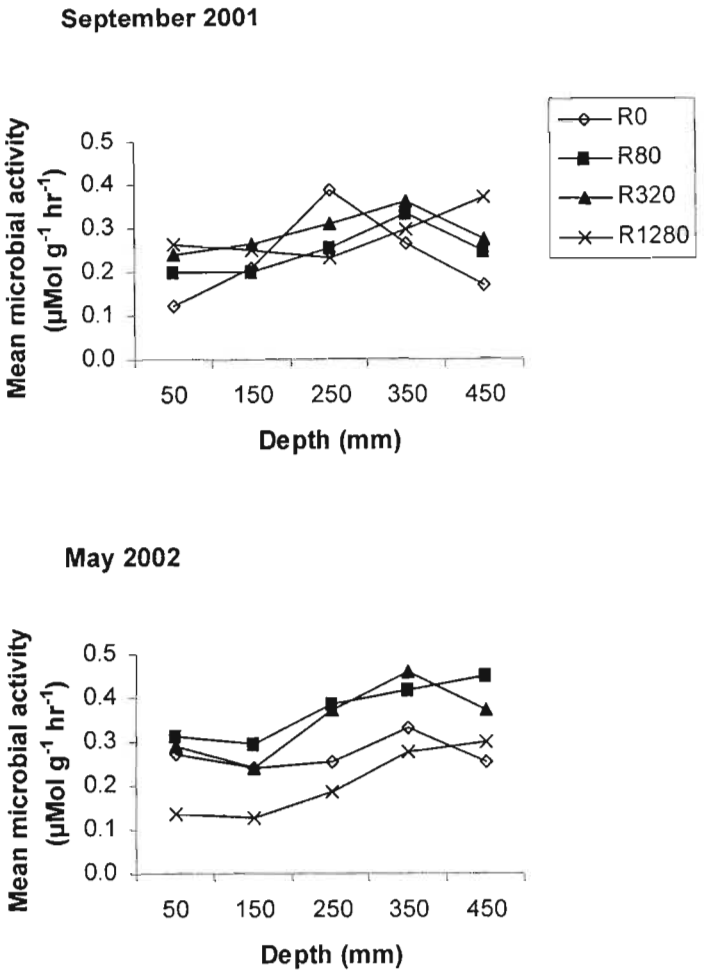


Figure 4.22. The trend in mean microbial activity in the soil profile at the different application rates of water treatment residue (R) at Ukulinga Research Farm sampled in September 2001 and May 2002.

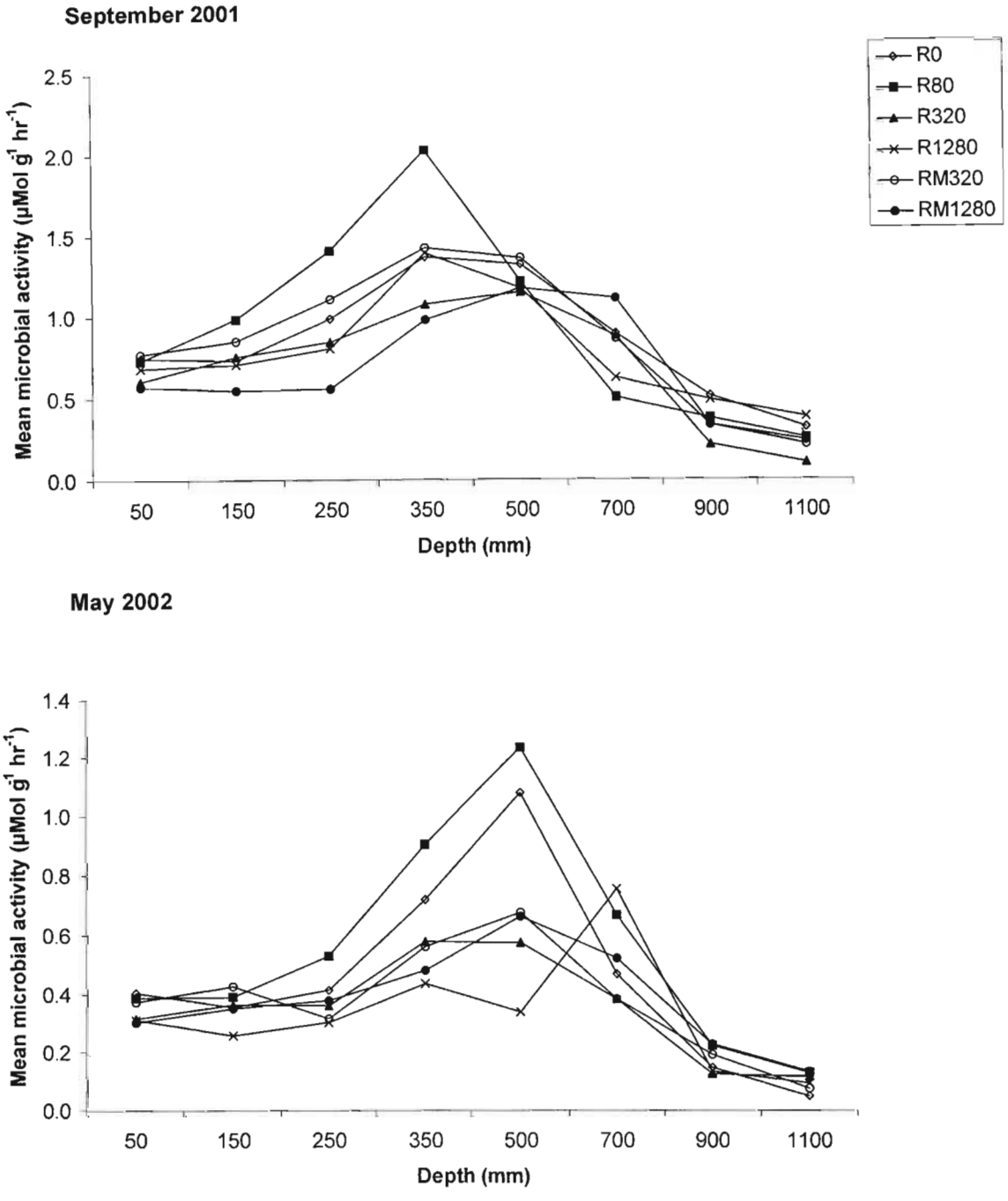
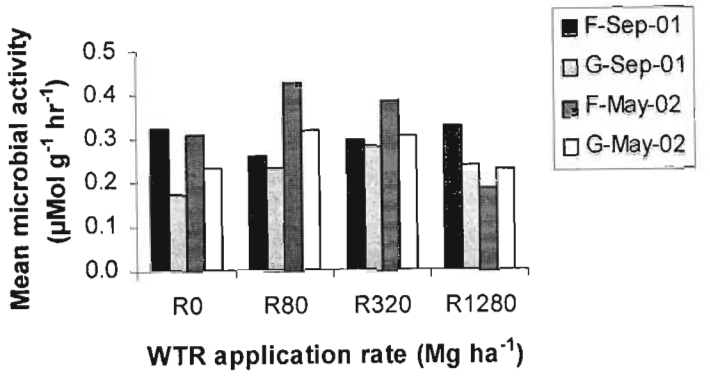


Figure 4.23. The trend in mean microbial activity in the soil profile at the different application rates of water treatment residue (R) at Brookdale Farm sampled in September 2001 and May 2002 (M = mulch).

(a)



(b)

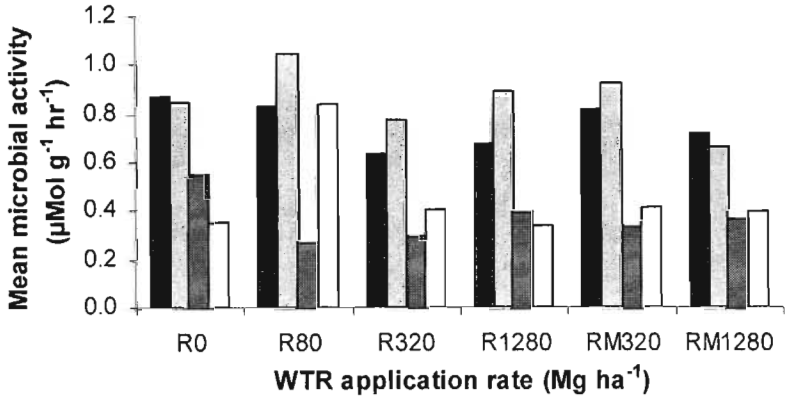


Figure 4.24. The trend in mean microbial activity under fallow and grassed treatments at the different application rates of water treatment residue (R) at (a) Ukulinga Research Farm and (b) Brookdale Farm sampled in September 2001 and May 2002 (M = mulch).

4.2 Discussion

4.2.1 Organic carbon

Gregorich *et al.* (1994) considered that the amount and rate of change in total OC is dependent more on the initial level of carbon than on the treatment or management practice imposed on the soil. Hence when looking at soil OC as an indicator of soil quality it is important to take into consideration the inherent soil properties. For example, soil texture plays an important role in determining the amount of organic matter that may be stabilized in soil. As a result soils with relatively high clay contents tend to stabilize and retain more organic matter than those with low clay contents (Jenkinson, 1977; Sparling, 1992). Thus, the unamended Hutton soil at Brookdale Farm reflected a higher OC content in comparison to the unamended Westleigh soil at Ukulinga Research Farm (Table 3.1).

When looking at any statistical evaluation, one needs to be mindful that statistical significance does not necessarily result in changes to the studied ecosystem and its functioning. With the exception of the September 2001 sampling at Brookdale Farm, the addition of the WTR resulted in significant changes in OC. However, these changes are more likely a result of the heterogeneity of the soil as opposed to WTR addition. The minimal effect of WTR on soil OC content can be attributed to the very similar OC contents of the Umgeni WTR (2.8%) and the Westleigh soil at Ukulinga Research Farm, which ranged between 2 and 2.5% in the top 200 mm. The same reasoning can be applied to Brookdale Farm, where the mean OC content of the control soils averaged 3.2% in the top 200mm.

Campbell *et al.* (1991) suggested that soils with an initially high level of organic matter may require prolonged and intense perturbation to show significant degradation compared to soils with initially lower organic matter contents. The implication is a change in soil quality, more specifically soil OC, would be

detected earlier on the poorly structured Westleigh soil form at Ukulinga Research Farm than on the more strongly structured Hutton soil form at Brookdale Farm. Although the field trials at Ukulinga and Brookdale have been running for almost 3 and 5 years, respectively, neither indicated marked changes in soil OC (Appendix 4.2 and 4.3). Additionally, one needs to consider the availability of the carbon source contributed by the WTR. Since some years have elapsed between WTR addition to the field experiments and the OC analyses, it is possible that the available OC has been depleted.

At both sites there was a decline in OC with increasing soil depth at both sampling times. This trend is supported by findings of Lavahun *et al.* (1996) that stated that the decreasing supply of fresh and easily decomposable plant material down the soil profile resulted in a corresponding decline in soil OC. Further, Anderson and Domsch (1980) stated that crop and plant residues are a source of C input to soils. However, for the benefits of such input to be fully utilized, tillage is essential since it increases contact between soil and plant residues, and promotes decomposition (Haynes, 1999). Since both field trials have been maintained undisturbed, it is not surprising that little difference exists between fallow and grassed treatments, more especially at Brookdale Farm.

However, at Ukulinga a significant (status x depth) interaction was noted at both sampling times, with marginally higher amounts of OC under the fallow treatment as opposed to the grassed treatment (Figure 4.3). Although this is perhaps an unexpected trend, the fact that it was noted on the poorly structured Westleigh soil is in keeping with the statement by Campbell *et al.* (1991) that soils of poor quality would be more likely to reflect management changes. Further, Gregorich *et al.* (1994) found that the OC content of soil is a result of the interaction between substrate additions of C in fertilizers and in plant and animal residues, and losses of C through microbial decomposition and mineralization and erosion. Since the grassed treatments contribute to soil OC by input of above and below ground plant residues, the C losses from grassed treatments are higher due to

stimulation of microbial activity resulting in a subsequent decline in soil OC. This is also supported by findings of Witter *et al.* (1993) that showed that total C losses in fertilized soils were six times higher than in fallow soils.

An important point to note is that since the OC content of a WTR is generally from source water sediment, activated carbon, polymeric coagulants and flocculants, it is variable in nature (Elliot and Dempsey, 1991). Typically WTRs consist of 3% or less OC by mass. This value is representative of agricultural soils, and is much lower than found in sewage sludge (United States Environmental Protection Agency, 1996). The Umgeni WTR, used in the both field experiments, conformed to this data containing 2.8% m/m OC.

While many concerns are voiced regarding the possibility of groundwater pollution by heavy metals in WTS, heavy metal toxicity may also affect C mineralization processes. Chander *et al.* (1995) indicated that soils amended with low metal WTS contained less OC than soils amended with a high metal WTS. The indication is that mineralization of organic matter was decreased in metal-enriched soils. These results concur with findings by Chander and Brookes (1993). However, the trace metal concentration of WTRs is generally higher than soils (Appendix 3.1), but lower than WTS and sewage sludge. In addition, low availability of metals is characteristic of WTRs due to their neutral or basic properties (Elliott *et al.,* 1990).

The heavy metal content of WTRs depends on factors such as the catchment characteristics and the treatment process. Titshall (2003) showed that although significant amounts of heavy metals were extracted under laboratory conditions, the mobility of the heavy metals in the Umgeni WTR was severely restricted under field conditions. Therefore, despite the acidic nature of the Hutton and Westleigh soils, phytotoxicity resulting from WTR addition is not likely.

4.2.2 pH

The application of WTR to acidic soil has been shown to increase soil pH (Ahmed *et al.,* 1998). Ahmed *et al.* (1997) suggested that the acid-neutralizing power of WTR was due to the presence of lime, which is used in the water treatment process to precipitate soluble species of aluminium. Similarly, lime was used in the generation of the Umgeni WTR collected and used in this study (Table 3.2).

The Hutton and Westleigh soils used in the field experiments were acidic in nature (Table 3.1). In South Africa, soil acidity poses a serious problem, with 5 million ha displaying a pH_{KCl} < 4.5 and a further 11 million ha ranging between 4.5 and 5.5. Three of the main reasons suggested for the acidity of South African soils are (i) nitrification following the application of nitrogen fertilization; (ii) cultivation, which enhances aeration and promotes bacterial oxidation of soil organic matter, resulting in the production of organic acids; and (iii) forest plantations which acidify the soil by the accumulation of basic cations in the forest biomass (Beukes, 1995).

In view of the acidity problems of South African soils, the potential of WTR to act as a soil ameliorant (Rengasamy *et al.,* 1980; Bugbee and Frink, 1985) is very useful. For successful farming, the application of nitrogen fertilizers is often essential. However, the reduction in pH resulting from associated nitrification processes, can decrease crop yield if optimal pH conditions are not maintained (Vaughan and Ord, 1985). Despite the use of fertilization on the grassed treatments at both field trials, no reduction in pH was noted. Conversely, at both field trials a pH increase was noted in the top 200mm, into which the WTR was incorporated.

However, the magnitude of any increase in pH depends on several factors such as the original soil pH, the pH of the WTR, and its application rate (Ahmed *et al.,*

1997). While the mean pH of the Umgeni WTR was 7.7, a slightly acidic pH was noted at both field trials, with pH ranging between 5 and 6. At both field trials, an increase in pH was noted with increasing application rates of WTR, with a maximum increase of 2 pH units noted at the highest application rate of WTR (1280Mg ha^{-1}).

Heil and Barbarick (1989) conducted a greenhouse study on the effect of an iron WTR (pH 7.3) on the growth of sorghum-sudangrass (*Sorghum sudanese*) in an acidic soil (pH 5.2). Following a 113 day incubation period, the highest WTR application rate (20g kg^{-1}) raised soil pH from 4.7 in the control treatments to a maximum of 7.0. This is equivalent to 40Mg ha^{-1}, and so in comparison to the pH increases noted at the Brookdale and Ukulinga Farms, is higher. However, both field experiments have been running for much longer than the 113 day period used by Heil and Barbarick (1989). The Umgeni WTR could have resulted in greater pH increases at the start of the field experiments, which have since decreased.

Alum WTRs are generated from a water treatment process that uses alum (aluminium sulphate) and are also capable of producing increases in soil pH. For example, a year after application of an alum WTR (pH 7) at an application rate of 1170m^3 ha^{-1} to forest soil (pH 5), Bugbee and Frink (1985) showed that soil pH was raised by 0.5 to 1.0 units in the top 100mm soil. However, there are concerns regarding aluminium (Al) mobility and phytotoxicity arising from the land disposal of alum WTR (Ahmed *et al.,* 1997). Alum has not been used in the generation of the Umgeni WTR and so should not pose any problems in this regard.

Although all soils contain substantial amounts of Al, phytotoxicity is related to the availability of Al and not the total amount present (Elliot and Dempsey, 1991). The hydrolysis of Al in solution is pH-related and certain species of Al are more harmful to biota than others. Regardless of pH, the organically bound and

polymeric forms are less toxic and considered to be essentially harmless (Ahmed *et al.,* 1997).

Poor plant growth in acidic soils is generally associated with the inhibition of root growth due to high concentrations of Al (Bohn *et al.,* 1985). However, if soil pH is maintained in the 6–6.5 range, Al toxicity does not pose a problem (Elliot and Dempsey, 1991). The Umgeni WTR has a pH-neutralizing potential although the effect is less pronounced over time (Figures 4.8 and 4.9). At the highest WTR application rate pH of the topsoil is raised to above pH 6.0. However, if the pH of the WTR is not high enough to produce a significant liming effect, the soil acidity problem would then translate to an issue of management, and lime application may have to be carried out to combat problems caused by acidity.

One of the most pH sensitive reactions is biological nitrification, which is related to soil pH and is often used as an index of soil fertility. Since the optimum pH for nitrification may vary from 6.6 to 8.0, the increase in pH resulting from WTR additions on the Hutton and Westleigh soils is desirable for nitrification. In agricultural soils nitrification rates decrease below pH 6, and become negligible below pH 4.5, while high pH values inhibit the transformation of nitrite to nitrate. Further, most known bacterial populations grow within the pH range from 4 to 9, or within smaller segments of that range while fungi are more tolerant to lower pH and grow within the pH range from 4 to 6 (Paul and Clark, 1996). The pH increase resulting from WTR addition to both field experiments is unlikely to have a detrimental effect on microbial growth and activity, since most bacteria grow best at neutral pH (Brock and Madigan, 1991).

4.2.3 Electrical conductivity

Due to the presence of elements such as calcium (Ca), magnesium (Mg), sodium (Na) and potassium (K) in the Umgeni WTR, one would expect the EC of WTR-amended treatments to be higher than the controls. However, at both field trials

this trend is not apparent. This is possibly due to the long period of time that has elapsed since the addition of the WTR to the trials and subsequent sampling and measurement of EC. The salts may have been leached, and a fraction may have been bound to exchange sites on the soil colloids, and are therefore no longer water-soluble.

The Ukulinga and Brookdale data indicated a fairly constant EC throughout the soil profile across all treatments. Although there is no field-based study to which the current EC data of WTR-amended soils can be compared, the most important finding is that, across all treatments, EC is substantially lower than 4dS m^{-1}. This is significant since an EC higher than 4dS m^{-1} is associated with saline soil and is therefore detrimental to crop growth (Dayton and Basta, 2001). This is supported by the findings of Ahmed *et al.* (1997) that indicated that an Australian WTR contained modest amounts of salts which were not sufficient to pose a salinity risk.

4.2.4 Microbial activity

There is only sparse literature regarding the microbial status of WTR. However, Elliot *et al.* (1990) conducted a comprehensive study into the land application of 20 United States WTRs. Although the bulk of the research focussed on the physical and chemical properties of WTR, they stated that since WTR was in contact with disinfectants, the possibility of contamination with pathogens was unlikely. Additionally, none of the WTRs tested positive for the presence of coliform organisms. Similarly, the Umgeni WTR used in the present study tested negative for the presence of *Escherichia coli* and *Salmonella* species (section 3.1.1). To date, research effort on the microbial aspect of land disposal of WTR has only focussed on tests to determine the presence of pathogens in the WTR, with no research conducted into the effect of WTR on microbial activity and diversity in field and laboratory studies.

Although microbial activity is a sensitive indicator of changes or disturbances resulting from management practices (Schnurer and Rosswall, 1982), there is little information regarding the microbial status of South African soils (Nsabimana, 2002). Microbial activity in the Hutton soil at Brookdale Farm was higher than in the Westleigh soil at Ukulinga Research Farm probably due to higher OC content of the former. Also, it is likely that the apedal Hutton soil provided better soil aeration and moisture conditions for microbial growth.

The effect of WTR on microbial activity was only significant at Brookdale Farm at the September 2001 sampling. Although statistical differences in activity were noted, these differences were very small (Appendix 4.12). The highly significant (WTR x depth) relationship was probably due to the significance of the depth effect, as indicated by the similar trends across all treatments.

However, the most apparent trend at both field trials was the relationship between microbial activity and depth. Soil enzyme activities have been found to decrease with soil depth (Ladd, 1985). Also, the numbers of all microbial populations usually decreases with increasing soil depth (Turco *et al.,* 1994). However, the bulk of the data pertaining to microbial activity is based on surface soil measurements (Kaiser *et al.,* 1992), and therefore cannot be directly compared to current data. Although the microbial activity of the topsoil is directly correlated with organic matter (C and N) contents (Ladd, 1985), there are few data on the relationship between microbial activity and soil organic carbon at depths of between 30 and 90cm (Lavahun *et al.,* 1996).

When explaining microbial activity data, assessment of soil OC is necessary since activity is dependent on the quantity, quality and distribution of carbon sources. These factors vary with time and depth (Kaiser and Heinmeyer, 1993). While the OC trend at both trials showed a decline in OC with increasing soil depth, microbial activity increased with increasing soil depth to 300-400 mm, and 300-600 mm at Ukulinga and Brookdale, respectively, before declining with

greater depth. This increase in activity with increasing soil depth may be attributed to a number of factors although it is considered that soil moisture was particularly important, since visual observation of the topsoil revealed very dry aggregates at both sampling times.

The relationship between microbial activity and soil moisture is not a new concept since Harris (1981) indicated that soil microorganisms require a water environment for nutrient uptake and cell surface integrity. This was supported by Burns (1989) who showed that water films were the principal sites of microbial activity. Water content also closely relates to soil aeration, since drying of soils allows for more air-filled porosity (Stotzsky and Burns, 1982). Surface soils tend to be more aerated (Ladd, 1985), with the implication that, since soil moisture increases with increasing depth, aeration at lower depths is greatly reduced.

Although Schnurer and Rosswall (1982) indicated that microbial activity increases with increasing soil organic matter (or organic carbon) content, an interplay of a wide range of factors affects activity. While soil enzyme activities vary seasonally and with type of vegetation cover (Ladd, 1985), other factors affecting microbial activity may include soil pH, the ionic strength of the soil solution, and redox potential. Additionally, even in aerated soils, the composition of the air may vary, subsequently stimulating different microorganisms (Smith *et al.*, 2000).

Looking at the chemical parameters measured at both trials, the EC from 0mm to the sampling depth corresponding with peak activity was constant, indicating that the effect of EC on activity was negligible. Although application of WTR at both trials resulted in an increase in pH in the top 200mm, it is unlikely that the pH change is responsible for the lower microbial activity in the topsoil. This statement is based on the fact that soils microorganisms can survive within the pH range from 4 to 9 (Paul and Clark, 1996). With the exception of the highest

WTR treatment, the increase in pH was not in excess of 1 pH unit, and therefore not likely to result in drastic changes to the microbial populations present.

Microbial activity helps to aggregate the soil which reduces erosion, allows for good water infiltration and maintains adequate aeration of the soil. However, an increase in microbial activity does not imply an increase in soil quality (Kennedy and Papendick, 1995), with more emphasis being placed on the stability of microbial populations. Since at both trials the range of microbial activity recorded at both sampling times was similar, the implication is that stable microbial populations are present thus indicating good soil quality.

Lastly, due to the number of samples processed, soils were stored at 4°C for up to four months prior to microbial activity testing. Thus the effect of storage on microbial activity may be considered a cause for concern. However, it has been shown that holding field moist soils at or near 5°C, causes little change in activities over many months and appears to be a satisfactory procedure for many enzymes (Ladd, 1985).

CHAPTER 5

Short term respiratory response of soils amended with water treatment residue

5.1 Results

5.1.1 Microbial respiration

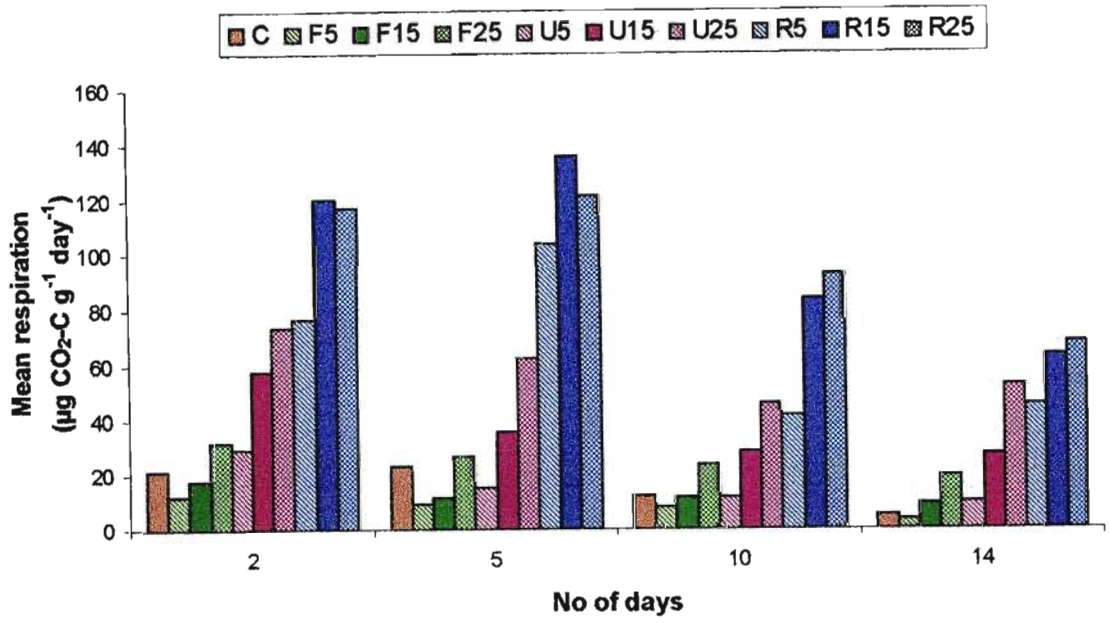
5.1.1.1 Experiment 1

The ANOVA indicated significant differences in respiration between the controls, the different WTRs, WTR application rate, soil type and sampling interval (Appendix 5.1). Respiration of the unamended Hutton (Figure 5.1a) and Westleigh soils (Figure 5.1b) was similar throughout the two week sampling period. Basal respiration appeared to decrease with time, with the Hutton control declining from 21.2µg CO_2-C g^{-1} day^{-1} at Day 2 to 5.5µg CO_2-C g^{-1} day^{-1} by Day 14. In comparison, the Westleigh control basal respiration peaked at Day 5 (30.6µg CO_2-C g^{-1} day^{-1}) and declined to 8.2µg CO_2-C g^{-1} day^{-1} by Day 14.

Respiration was influenced by the type of WTR and followed the sequence: Faure[1] WTR < Umgeni WTR < Rand WTR. The effect of Faure[1] WTR on the respiration activity was unclear and only small differences were noted in both the Hutton and Westleigh soils. The Umgeni and Rand WTR resulted in pronounced increases in respiration compared to the controls throughout the two week sampling period. However, this increase became less pronounced with time.

Respiration of soil amended with Rand and Umgeni WTR increased with increasing WTR application rate and these amendments resulted in higher respiration in the Hutton soil compared to the Westleigh soil.

(a)



(b)

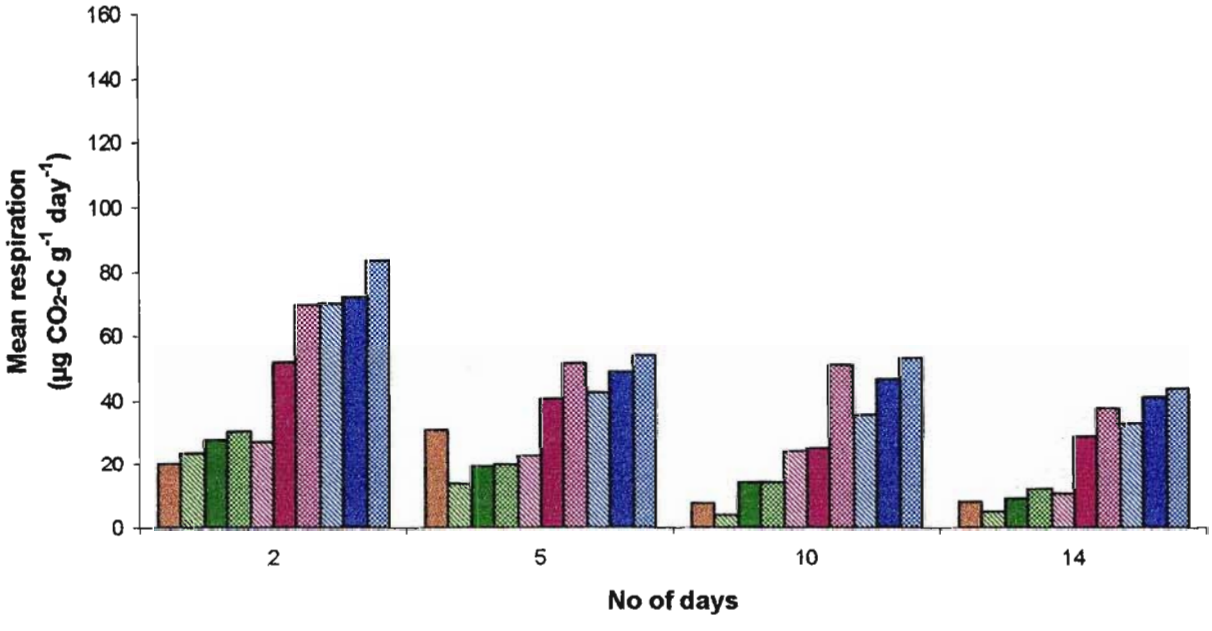


Figure 5.1. The effect of Faure[1] (F), Umgeni (U) and Rand (R) WTR at rates of 5, 15 and 25% (m/m) on the mean respiration (n=2) of (a) Hutton, and (b) Westleigh soil.

5.1.1.2 Experiment 2

The ANOVA indicated significant differences in respiration between the controls, the different WTRs, WTR application rate, and sampling interval (Appendix 5.2).

Respiration of the unamended Hutton (Figure 5.2a) and Westleigh soil (Figure 5.2b) was similar throughout the two week sampling period, and was consistently less than 10µg CO_2-C g^{-1} day^{-1}.

Respiration was influenced by the type of WTR and followed the sequence: Amatola WTR < Faure[2] WTR < Midvaal WTR. The Amatola WTR had no effect on the respiration activity of the Hutton soil, while it produced small increases in respiration in the Westleigh soil. Throughout the two week sampling period the Faure[2] and Midvaal WTRs resulted in pronounced increases in respiration compared to the controls with the highest respiration achieved at the 15% WTR treatment. However, this increase was less pronounced with time.

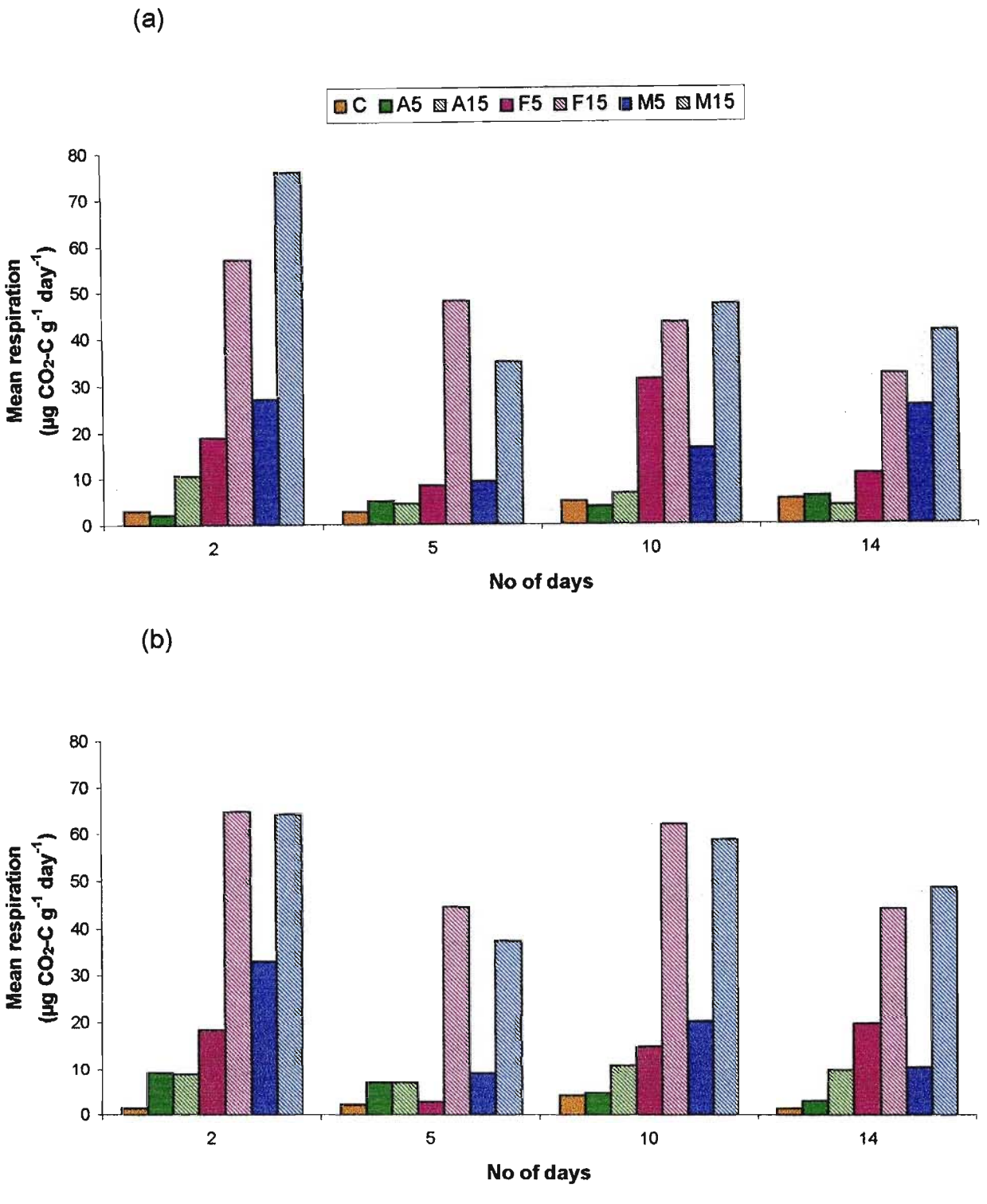


Figure 5.2. The effect of Amatola (A), Faure[2] (F) and Midvaal (M) WTR at rates of 5 and 15% (m/m) the mean respiration (n=2) of (a) Hutton, and (b) Westleigh soil.

5.1.2 pH (composite samples)

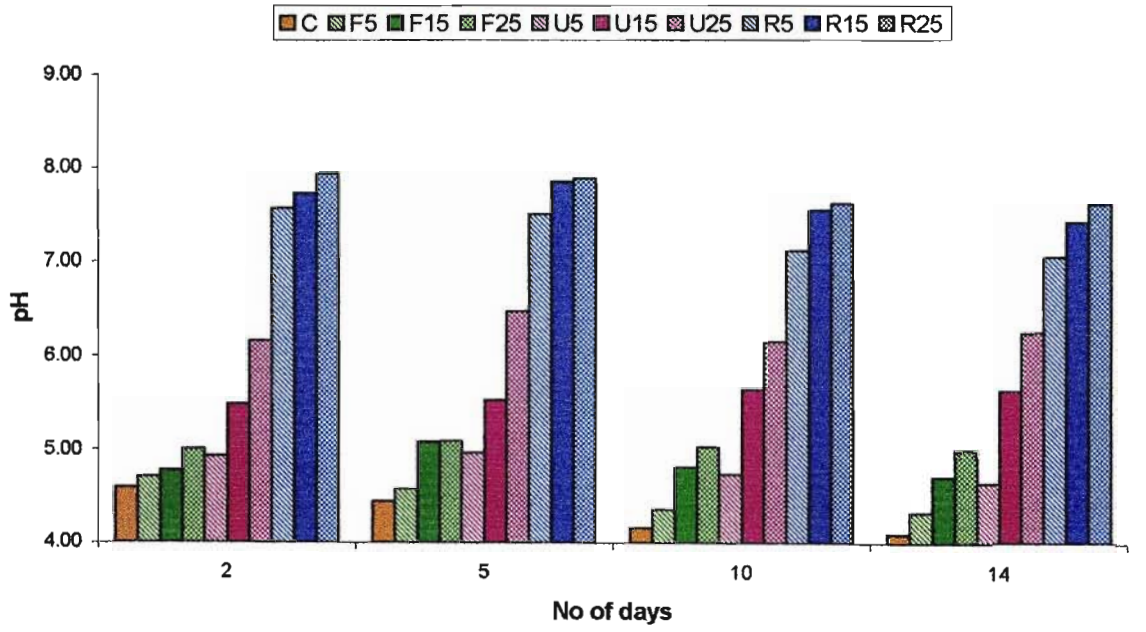
5.1.2.1 Experiment 1

The pH of the Hutton control ranged between 4.0 and 4.5 (Figure 5.3a), whereas the mean pH of the Westleigh control ranged between 5.0 and 5.5 (Figure 5.3b). Addition of WTR produced increases in pH, which were influenced by the type of WTR and application rate. The Rand WTR produced the greatest change in pH whereas the Faure[1] WTR had the least influence on pH. Similar pH changes were noted for both soil types throughout the sampling period.

5.1.2.2 Experiment 2

The pH of the Hutton control ranged between 4.3 and 4.8 (Figure 5.4a), whereas the mean pH of the Westleigh control ranged between 5.3 and 5.8 (Figure 5.4b). On both soil types, the Amatola WTR resulted in small increases in soil pH. The Faure[2] and Midvaal WTR treatments produced pH increases of between 1 and 2 pH units. Additionally, pH was influenced by WTR application rate with the 15% (m/m) WTR treatments producing the greatest pH increases.

(a)



(b)

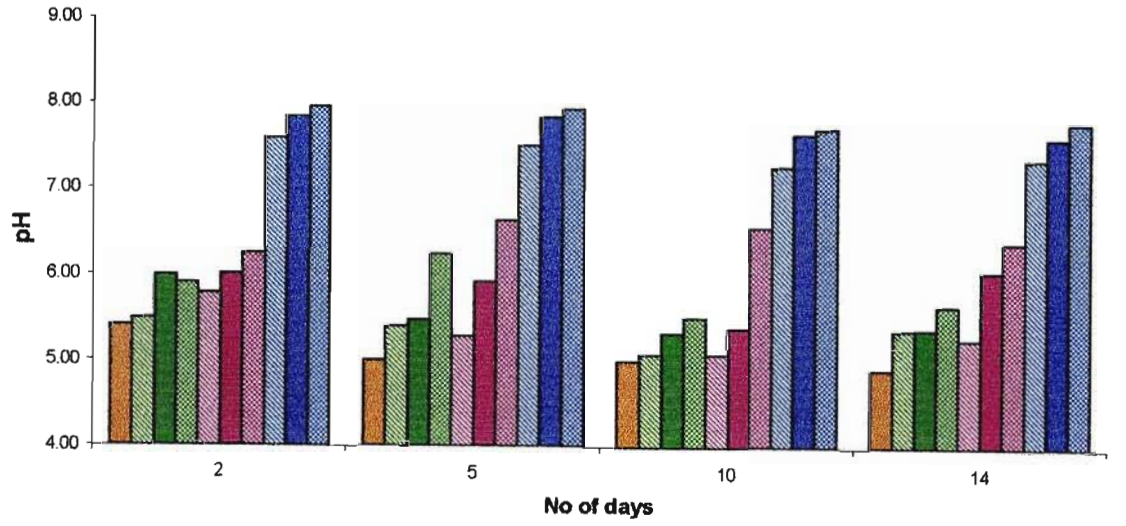
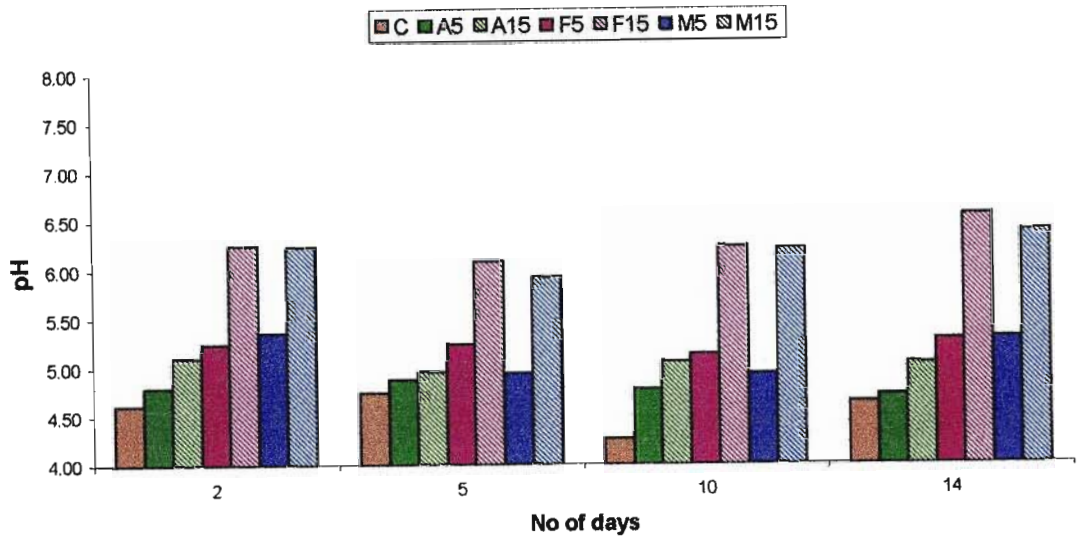


Figure 5.3. The effect of Faure[1] (F), Umgeni (U) and Rand (R) WTR at rates of 5, 15 and 25% (m/m) on pH of (a) Hutton, and (b) Westleigh soil.

(a)



(b)

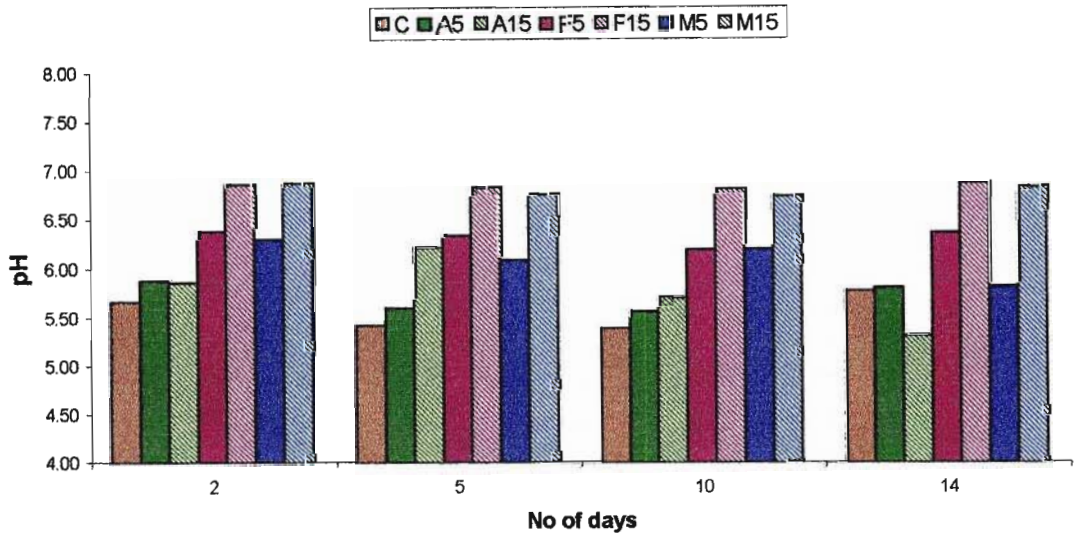


Figure 5.4. The effect of Amatola (A), Faure[2] (F) and Midvaal (M) WTR at rates of 5 and 15% (m/m) on pH of (a) Hutton, and (b) Westleigh soil.

5.1.3 Electrical conductivity (composite samples)

5.1.3.1 Experiment 1

The EC of the Hutton (Figure 5.5a) and Westleigh (Figure 5.5b) controls ranged between 180 and 230µS cm^{-1}, and from 100 to 140µS cm^{-1}, respectively. The addition of Faure[1] WTR did not appear to influence the EC of either soil. However, the Rand and Umgeni WTR-amended treatments resulted in marked increases in EC. For both soil types the 25% (m/m) Umgeni WTR amendment produced the most pronounced increase in EC.

5.1.3.2 Experiment 2

The EC of the Hutton (Figure 5.6a) and Westleigh (Figure 5.6b) controls ranged from 150 to 300µS cm^{-1}, and from 150 to 250µS cm^{-1}, respectively. All WTRs had only small effects on the EC of the Westleigh soil, whereas WTR addition to the Hutton soil resulted in an increase in EC on days 2 and 5. Higher EC values were generally measured on the 15% (m/m) treatments as opposed to the 5%(m/m) treatments, although this increase in EC was not consistently observed throughout the sampling period.

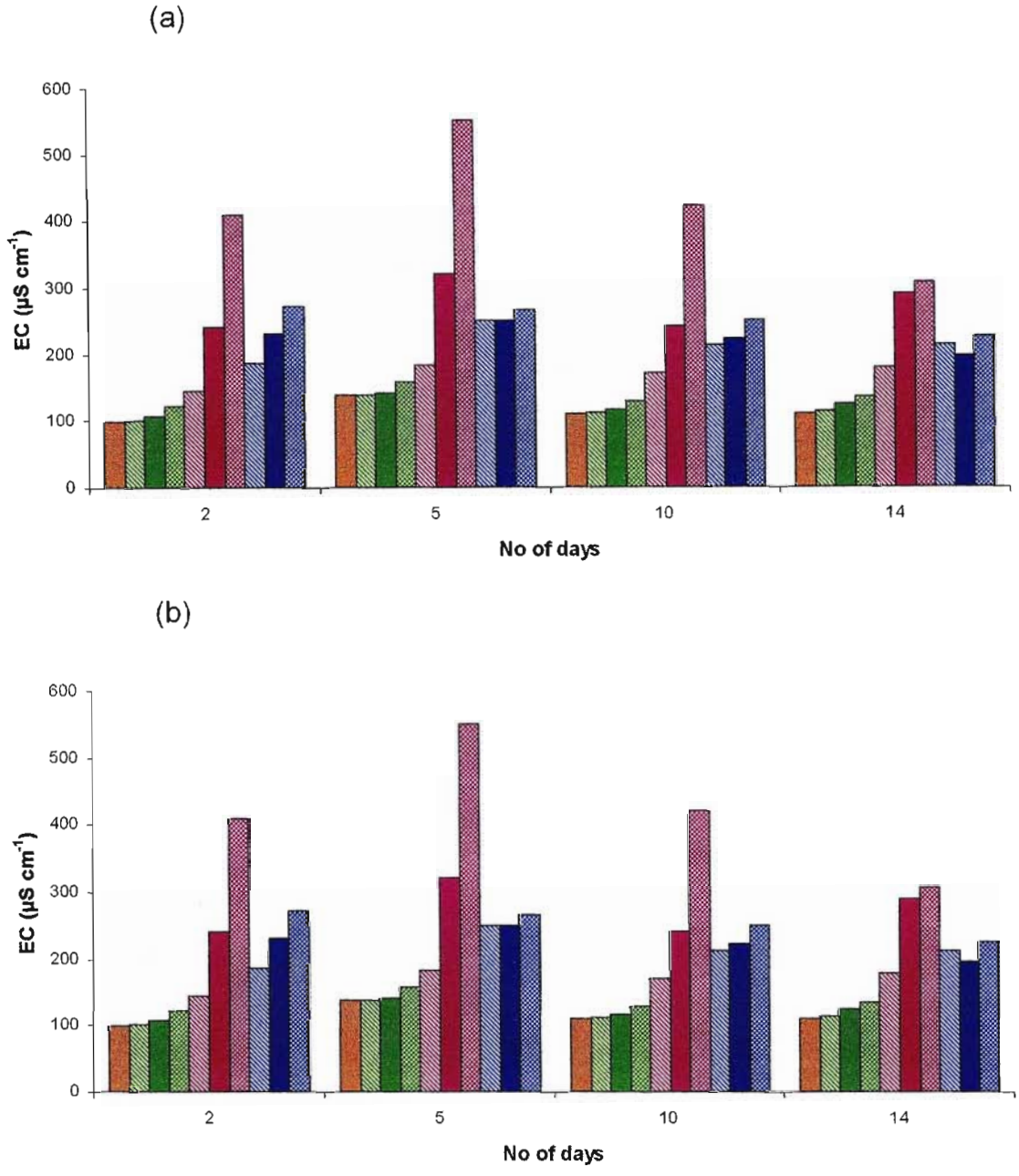


Figure 5.5. The effect of Faure[1] (F), Umgeni (U) and Rand (R) WTR at rates of 5, 15 and 25% (m/m) on electrical conductivity (EC) of (a) Hutton, and (b) Westleigh soil.

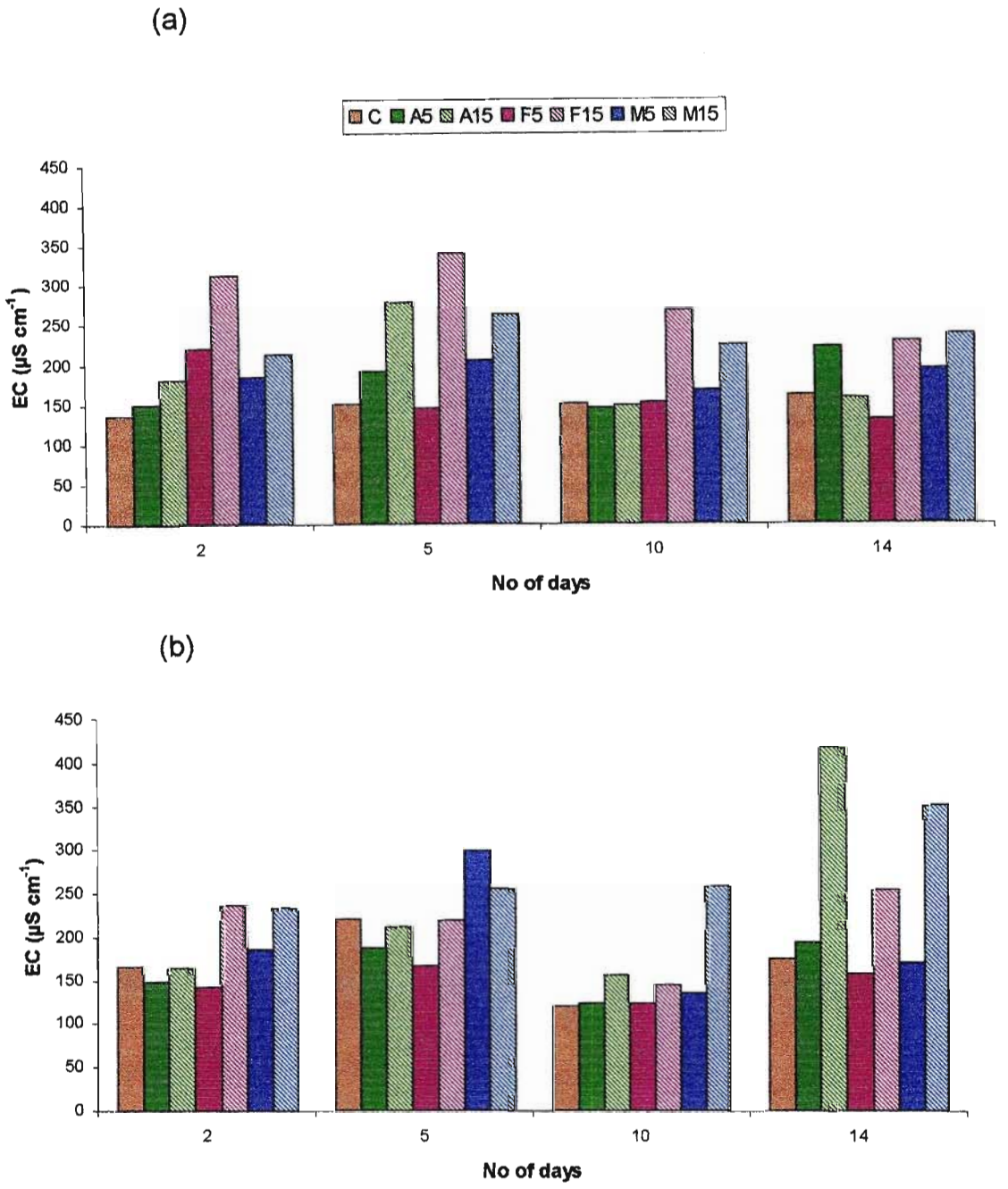


Figure 5.6. The effect of Amatola (A), Faure[2] (F) and Midvaal (M) WTR at rates of 5 and 15% (m/m) on electrical conductivity (EC of (a) Hutton, and (b) Westleigh soil.

5.2 Discussion

5.2.1 Microbial respiration

The rate of hydrolysis of fluorescein diacetate (used for the field experiment samples) and the measurement in the laboratory of respiration activity have been extensively used to quantify microbial activity in soil, and are closely related (Schnurer and Rosswall, 1982). The measurement of respiration has been used as an index of microbial activity due to its sensitivity and ease of use (Beare *et al.,* 1990; Gray, 1990; Nay *et al.,* 1994). However, apart from testing WTRs for the presence or absence of pathogenic microorganisms (Elliot *et al.,* 1990), relatively little attention has been paid to the effects of WTR on soil microbial properties.

There has been a number of investigations into the effect of wastewater and sewage sludge on microbial respiration (Chander and Brookes, 1993; Chander *et al.,* 1995; Pascual *et al.,* 1997; Roy and Couillard, 1998). For example, Pascual *et al.* (1997) showed that the addition of sewage sludge (SS) and municipal solid waste (MSW) to land, resulted in a general increase in microbial respiration due to the presence of high quantities of labile organic materials. These increases in respiration were followed by a subsequent decrease, dependant on the treatment carried out. The increase in respiration is stimulated by the presence of easily metabolized organic carbon, which enables an increase in the fraction of microbial biomass that is active (Keift, 1994). Similarly, Ritz *et al.* (1997) showed initial increases in respiration in response to animal manure application.

With the exception of the Faure[1] and Amatola WTRs, the tested WTRs produced initial increases in respiration, which declined with time. Since the WTRs are predominantly inorganic in nature (Elliot *et al.,* 1990), the stimulated respiration effect was short-lived, as shown by marked decreases in respiration by Day 14. Further, findings from field experiments (section 4.2.1) indicated non-significant

differences in microbial activity across all treatments 3-5 years after the addition of WTR.

All the WTRs contained substantially lower amounts of organic material than SS and MSW. The two samples of WTR collected from Faure Water Treatment Plant displayed higher OC (7.4% and 10.3% m/m), while other collected WTRs used in the respiration experiments displayed <3% m/m OC (Table 3.3). This reflects the variable nature of WTRs, and highlights the need for South African studies on the chemical nature of locally produced WTRs, and their disposal to land.

All un-amended WTRs displayed higher respiration activity than both test soils (Table 3.3), which indicates the ability of the WTRs to support microbial activity, if for only a short period of time. The respiration activity is likely to be supported by the presence of easily available carbon sources including non-living microorganisms.

Although statistical comparison between both respiration experiments was not possible, the trends in respiration can be compared. In Experiment 1, the Faure[1] WTR (which had the highest OC of 7.4% m/m) exhibited the lowest respiration rates on both the Hutton and Westleigh soils (Figure 5.1). A possible explanation for the absence of increased respiration on soils amended with Faure[1] and Amatola WTR was that the OC present was unavailable. This is supported by findings of Elliot *et al.* (1990) which suggested that the OC associated with WTRs is often stable and resistant to breakdown. Conversely, the Rand WTR (0.8% OC m/m) showed the highest increases in respiration on both soils. Thus, the OC content of a WTR does not necessarily correlate to the amount or extent of respiration recorded. Since the Rand WTR has a high lime content, it is likely that the high respiration rates recorded on the Rand treatments have resulted from the breakdown of the lime, which releases carbon dioxide.

In Experiment 2 the Faure^2 WTR significantly increased the respiration of both the Hutton and Westleigh soils. Visual observations of the two Faure WTR samples revealed differences between these WTRs. The Faure^1 WTR aggregates were resistant to milling, whereas the Faure^2 WTR broke down easily into a fine powder. The powdery texture of the Faure^2 WTR and its higher OC content (10.3% m/m) in comparison to the Faure^1 WTR, is possibly due to the presence of more activated carbon. Higher dosing with activated carbon was necessary to combat taste and odour problems resulting from the presence of blue-green algae, which posed a problem at the time of sampling. Further, blue-green algae are present in the WTR and would present an available source of OC, thus stimulating respiration activity of soils amended with Faure^2 WTR.

In cases where WTR addition resulted in significant increases in respiration (Umgeni, Rand, Midvaal and Faure^1), it was also found that respiration rates increased with increasing application rate of WTR. While the magnitude of the respiration increase varied with the type of WTR a general trend shown was that the respiration rate of WTR-amended treatments decreased with time. This trend is likely to persist until such time that the available carbon is completely utilized, following which respiration of WTR-amended treatments would return to conditions of the controls (unless the physical and chemical conditions are dramatically altered).

The data from the field experiments revealed higher activity on the Hutton soil, as opposed to the Westleigh soil, and the current data revealed the same trend. However, both Experiments 1 and 2 revealed only small differences in basal respiration between the two soil types (Figures 5.1 and 5.2). It is possible that the apedal structured Hutton soil allows it to support a greater active microbial biomass, due to its better drainage and aeration properties. While sieving of soils for laboratory experiments has been shown to disrupt soil structure (Anderson, 1982), it is likely that sieving of the Westleigh soil improved aeration. The improved aeration status, in combination with favourable incubation

conditions of moisture and temperature, thus improved the ability of the Westleigh soil to support microbial activity. Therefore, the difference in basal respiration activity between the Hutton and Westleigh soils was small, in comparison to the field activity measurements.

5.2.2 pH

In Experiment 1 mean pHs of approximately 4 and 5 were recorded for the Hutton and Westleigh soils, respectively, whereas in Experiment 2 an increase of 0.5 pH units was recorded for both soils. The pH of all the WTRs was higher than the test soils, ranging from 6.6 to 8.5. Addition of WTR resulted in increases in pH that were stable for the duration of both experiments (Figures 5.3 and 5.4). Ahmed *et al.* (1997) suggested that the magnitude of the pH increase depends on several factors such as the original soil pH, the pH of the WTR itself, and the application rate of the residue.

The present research supports these findings with greater pH increases noted at the higher WTR application rates. Further, the Faure[1] WTR which had the lowest pH (6.6) produced the smallest increase in pH while the Rand WTR (pH 8.5, a result of the large quantities of lime used in the water treatment process) produced the largest increases in pH of up to 3 pH units at the 25%(m/m) treatment.

Ahmed *et al.* (1997) suggested that WTRs containing lime have potential for use as agricultural liming agents. All of the WTRs tested included lime as part of the water treatment process (Table 3.2). The high increase in pH displayed by the Rand WTR-treatments was due to the difference in the water treatment process. At Rand Water a higher dosage of lime was used in the water treatment process to boost the pH to between 10.5 and 11.0, which is optimal for precipitation when using activated silica as a coagulant. Also, the higher pH of the Faure[2] WTR in comparison to the Faure[1] WTR (Table 3.3) is possibly due to the addition of more

lime to the Faure[2] WTR due to blue green algae problems experienced at the time of sampling.

While the field experiments were indicative of the acid-neutralizing effect of the Umgeni WTR, both respiration experiments confirmed that the other tested WTRs produced similar pH increases. Since both experimental soils were acidic in nature, the WTR-induced increases in pH were not detrimental to soil health. Additionally, the effect of increasing pH on respiration activity is likely to support microbial growth, since most soil bacteria grow best at neutral pH (Brock and Madigan, 1991). Bacteria are also tolerant to a wide range of pH and growth occurs within the pH range from 4 to 9 (Paul and Clark, 1996).

5.2.3 Electrical conductivity

The Rand and Umgeni WTR resulted in increases in EC of the Hutton and Westleigh soil, while the effect of the Amatola, Faure[1], Faure[2] and Midvaal WTRs on soil EC was unclear. Calcium, magnesium, sodium and potassium salts are often added as coagulants or precipitants during the water treatment process. These salts may be lost through leaching or adsorbed onto clay colloids and the effect of EC on microbial activity is therefore difficult to assess. However, as with pH and respiration which generally increased with increasing WTR application rate, the same trend was noted for EC. Therefore, a likely assumption is that changes to soil EC do not have any detrimental effects on microbial activity.

CHAPTER 6

Microbial community structure analysis of the Hutton soil amended with water treatment residue

6.1 Results

Successful amplification of PCR product for all samples, except from the Rand WTR, was confirmed by 1.5 % (w/v) agarose gel electrophoresis. The amplicon size of all products was found to be marginally larger than the 200 bp marker.

The DGGE profiles of the various treatments are shown in Figure 6.1. Distinctive banding patterns were revealed for the various treatments. This indicates that a denaturing gradient of 30-70% formamide was sufficient for resolving (or separating) bands. Although the positive control (Lane 6) yielded multiple bands, the results were consistent with previous electrophoresis runs (data not shown). The negative control (Lane 7) displayed a band at location 'a'.

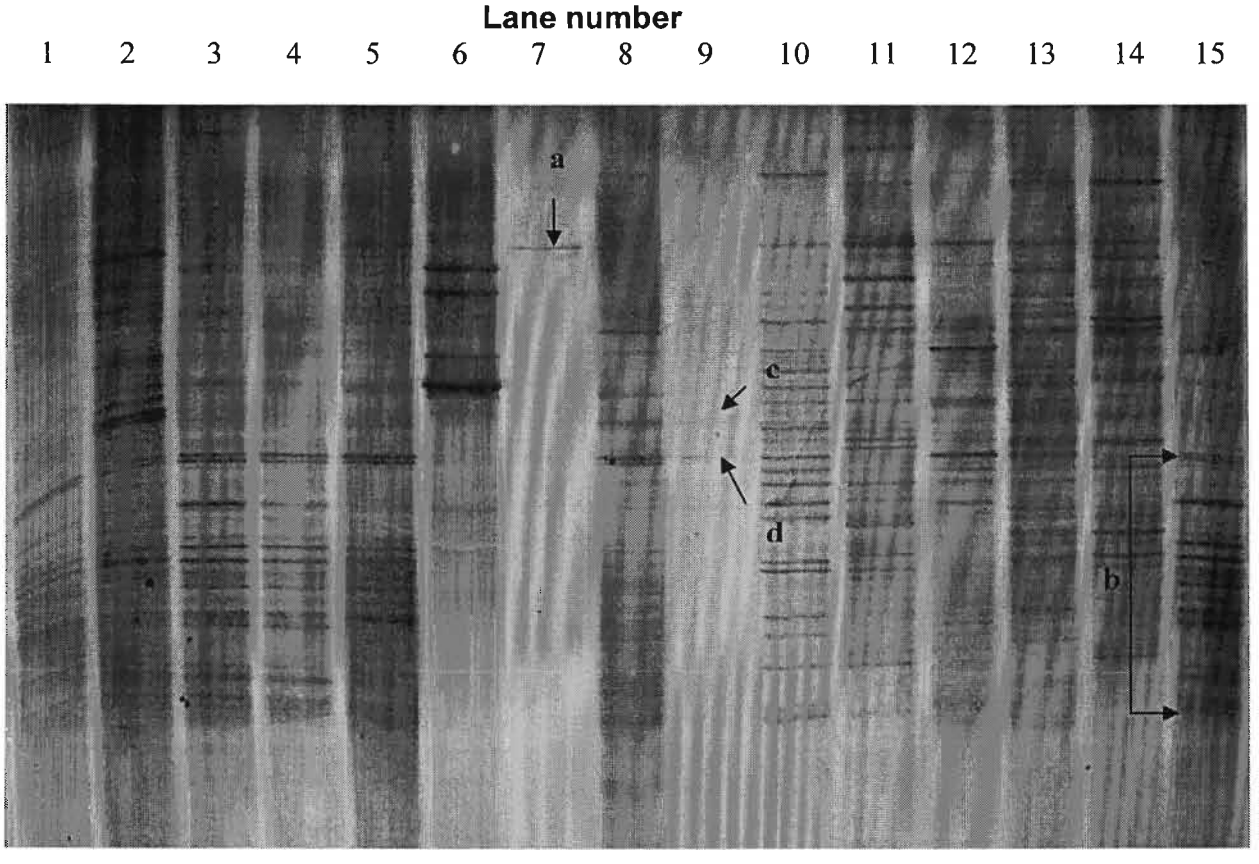
Bands occurred at 74 distinct locations on the denaturing gradient gel, and the number of bands observed in each treatment lane is shown in Table 6.1. With the exception of the Rand WTR (Lane 9), the WTR samples (Lanes 10-14) displayed complex banding patterns. The Midvaal, Umgeni, Amatola, Faure[1] and Faure[2] WTRs contained 28, 31, 21, 24, and 29 bands, respectively. However, the DGGE profiles of the WTRs were distinctly different (Figure 6.2). The Rand WTR displayed two clear but faint bands at locations 'c' and 'd' (Figures 6.1 and 6.2). These bands corresponded to prominent bands in Lane 8 (Hutton/Rand WTR) and were therefore attributed to sample carry over during loading of the DGGE gel..

The DNA banding pattern of the Hutton soil at location 'b' (Figures 6.1 and 6.2) appeared to be conserved following the addition of the WTRs (Figures 6.1 and

6.2). Specific points of comparison (or DNA bands) are indicated by arrows on Figure 6.3, which provides an enlarged view of the DGGE gel and indicates similarities between the soil/WTR treatments.

The similarity matrix (Table 6.2) compares the similarity of all treatment lanes to one another. If there are N treatment lanes then the similarity matrix is an N by N matrix. The diagonal elements of a similarity matrix always have values of 100, because a treatment lane is always 100 percent similar to itself. With the exception of the Midvaal WTR, Soil/WTR treatments had greater similarity with the Hutton soil, than with the relevant WTRs. Soil amended with the Umgeni, Faure[1], Faure[2] and Amatola WTRs displayed 25.5, 48.4, 25.7, and 56.1% similarity to the Hutton control treatment, respectively. The Midvaal WTR displayed 34.7% and 30.7% similarity to the Hutton control treatment and the soil/WTR treatment, respectively.

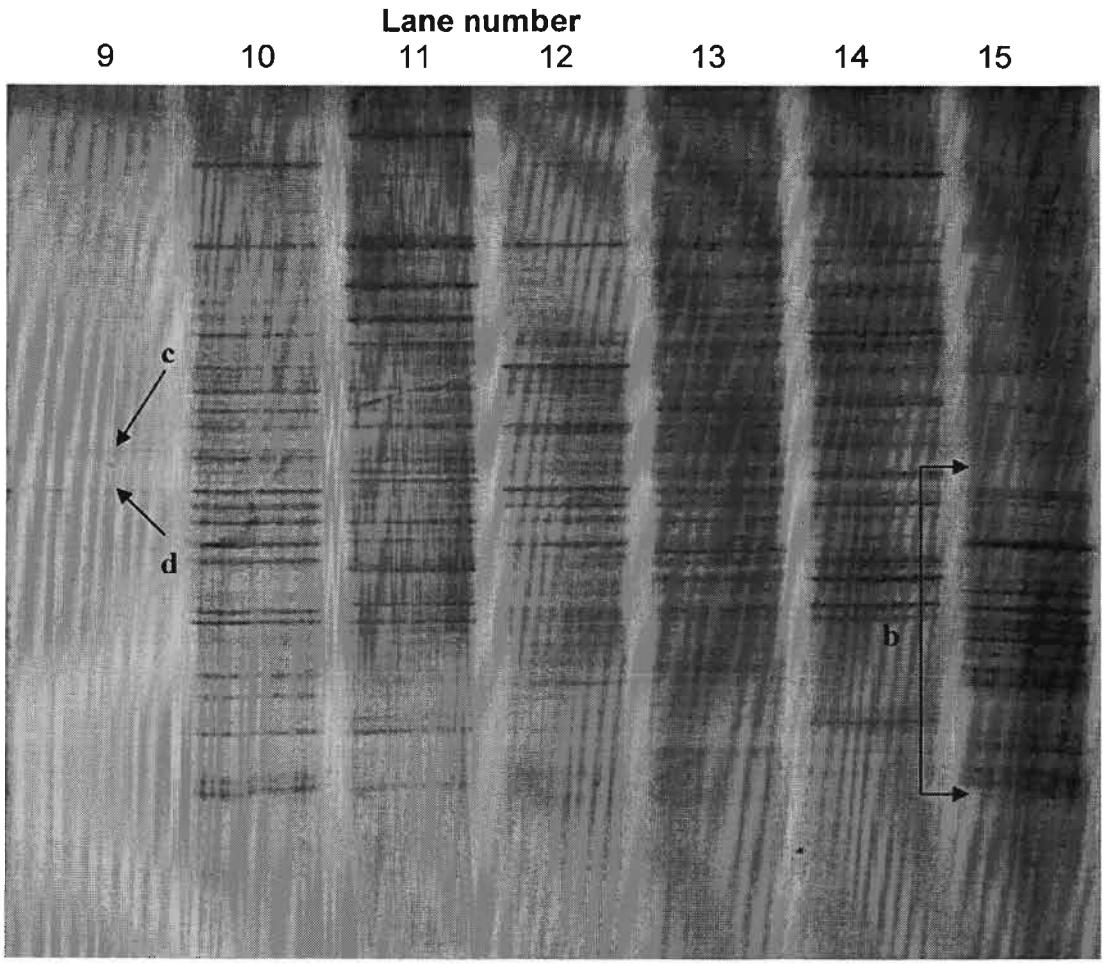
With the exception of the Midvaal WTR, similarity comparisons between the remaining WTRs and the Hutton control treatment were <23%. The two Faure WTRs displayed only 26% similarity to each other, despite being produced at the same water treatment plant. However, the Amatola and Midvaal WTRs displayed 47% similarity to each other.



Key:

1. Hutton/Umgeni mixture
2. Hutton/Faure[2] mixture
3. Hutton/Faure[1] mixture
4. Hutton/Amatola mixture
5. Hutton/Midvaal mixture
6. Positive control (*Bacillus* B81)
7. Negative control
8. Hutton/Rand mixture
9. Rand WTR
10. Midvaal WTR
11. Umgeni WTR
12. Amatola WTR
13. Faure[1] WTR
14. Faure[2] WTR
15. Hutton soil control

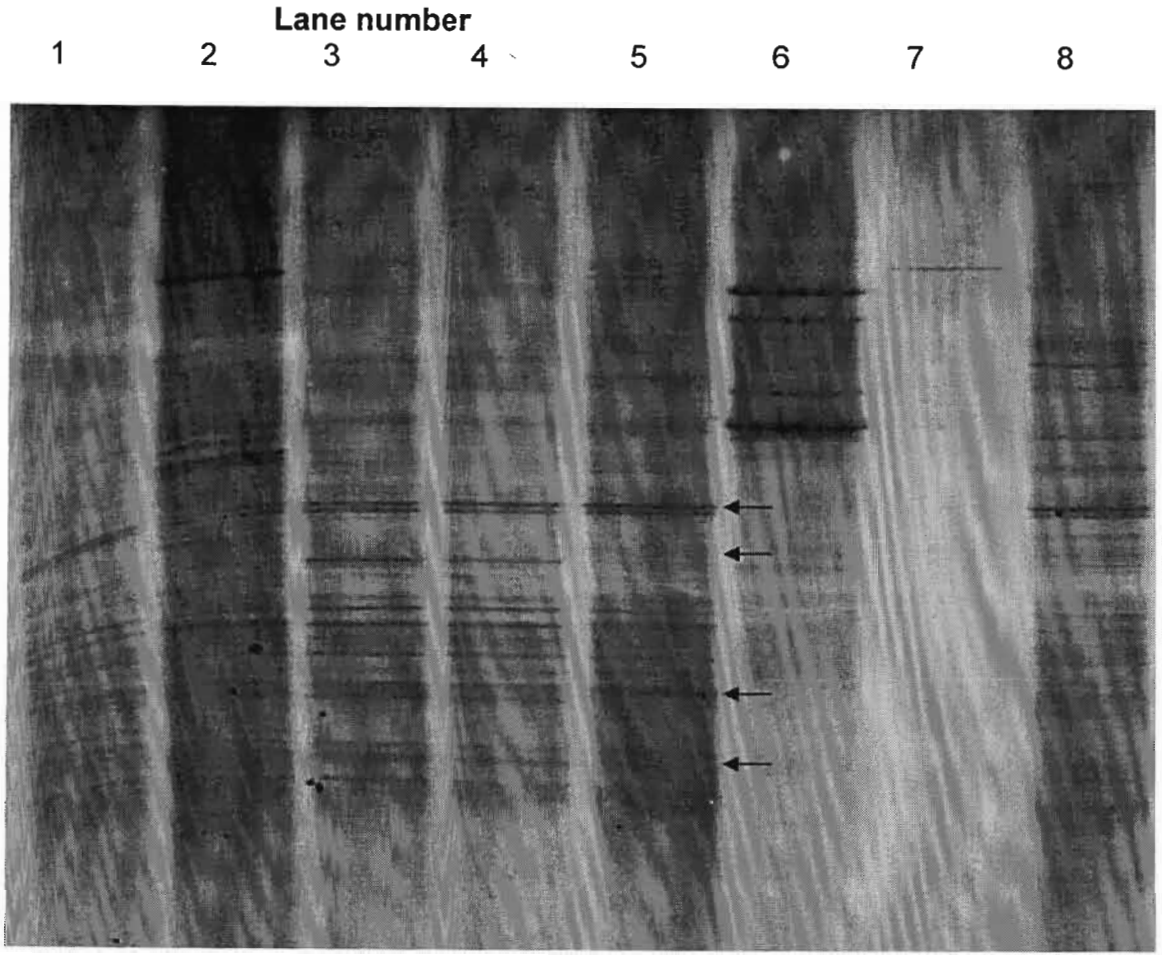
Figure 6.1. Silver-stained denaturing gradient gel showing the DNA banding patterns of the tested water treatment residues (WTRs), soil/WTR treatments, and relevant controls. Lines indicated by a, b, c, and d are referred to in the text.



Key:

9: Rand WTR
10: Midvaal WTR
11: Umgeni WTR
12: Amatola WTR
13: Faure[1] WTR
14: Faure[2] WTR
15: Hutton soil control

Figure 6.2. Silver-stained denaturing gradient gel showing the DNA banding patterns of the tested water treatment residues (WTRs) in comparison to the Hutton soil control. Lines indicated by b, c, and d are referred to in the text.



Key:

1: Hutton/Umgeni mixture
2: Hutton/Faure[2] mixture
3: Hutton/Faure[1] mixture
4: Hutton/Amatola mixture
5: Hutton/Midvaal mixture
6: *Bacillus* B81
7: Negative control
8: Hutton/Rand mixture

Figure 6.3. Silver-stained denaturing gradient gel showing the DNA banding patterns of the soil/water treatment residue treatments. Arrows indicate specific points of comparison between the treatments.

Table 6.1. The number of bands detected in each treatment lane using the BioRad Quantity 1D software.

LANE	*HU*	*HF²*	*HF¹*	*HA*	*HM*	*(+) control*	*(-) control*	*HR*	*R*	*M*	*U*	*A*	*F1*	*F2*	*H*
	26	25	25	20	23	9	1	23	0	28	31	21	24	29	21

Table 6.2. Similarity matrix report showing the similarity (%) of all lanes to one another.

LANE	*HU*	*HF²*	*HF¹*	*HA*	*HM*	*(+) control*	*(-) control*	*HR*	*R*	*M*	*U*	*A*	*F1*	*F2*	*H*
HU	100	33.6	20.7	20	25	3.5	0	26.6	0	12.1	14.1	8.3	22.4	16.7	25.5
HF2	33.6	100	30.1	22.9	31.4	5.1	0	35.1	0	21.9	15.7	12.4	20.4	17.6	25.7
HF1	20.7	30.1	100	51.7	28.9	13.9	0.1	46.5	0	26	11.6	23.8	11	9.6	48.4
HA	20	22.9	51.7	100	42.6	8.3	0	55.4	0	36.1	6.4	32	8.4	9.4	56.1
HM	25	31.4	28.9	42.6	100	7.1	4.4	33.4	0	20.5	11.4	13.6	16	15.5	30.7
(+) control	3.5	5.1	13.9	8.3	7.1	100	11.9	9.6	0	14.4	15.3	20.4	12.9	8.5	9.8
(-) control	0	0	0.1	0	4.4	11.9	100	0	0	2.3	0.9	19	9.4	2.6	0
HR	26.6	35.1	46.5	55.4	33.4	9.6	0	100	0	40.1	21.3	35	17.1	21.3	39.7
R	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0
M	12.1	21.9	26	36.1	20.5	14.4	2.3	40.1	0	100	34.3	47.1	22	20.5	34.7
U	14.1	15.7	11.6	6.4	11.4	15.3	0.9	21.3	0	34.3	100	15.3	14.3	24.9	19.2
A	8.3	12.4	23.8	32	13.6	20.4	19	35	0	47.1	15.3	100	24.1	24.7	22.5
F¹	22.4	20.4	11	8.4	16	12.9	9.4	17.1	0	22	14.3	24.1	100	25.6	12
F²	16.7	17.6	9.6	9.4	15.5	8.5	2.6	21.3	0	20.5	24.9	24.7	25.6	100	7.4
H	**25.5**	**25.7**	**48.4**	**56.1**	**30.7**	9.8	0	39.7	**0**	**34.7**	**19.2**	**22.5**	**12**	**7.4**	100

* where H = Hutton control soil; U = Umgeni WTR; F^2 = Faure ^{2} WTR; F^1 = Faure ^{1} WTR; A = Amatola WTR; M = Midvaal WTR.

6.2 Discussion

The poor recovery of DNA may be attributed to selective lysis of DNA and adsorption of DNA onto clay colloids (Holben, 1997). Low bacterial numbers can also affect the concentration of DNA extracted. Further, it is often difficult to scale up a method to improve DNA recovery (Holben, 1997). In addition, contaminants such as polyphenolic compounds (e.g. humic or fulvic materials) complicate recovery because they have significant absorbance at 260nm.

The PCR-DGGE technique is a useful tool that allows one to distinguish between microorganisms present in a mixed population. It has the advantage of simultaneously analyzing up to 18 samples, and presenting the data in a qualitative and semi-quantitative manner (Muyzer *et al.,* 1993). Theoretically, during electrophoresis the PCR-amplified DNA fragments, having the same size, but different sequences, migrate towards an increased denaturant concentration. The denaturant concentration at which a fragment ceases to migrate is used to distinguish between different species of bacteria present. Therefore, the number of bands present in a DGGE profile is indicative of the number of bacterial species present, and darker bands correlate to a higher concentration of bacterial DNA than lighter bands (Zhang and Fang, 2000). However, in the research presented herein density comparisons are only applicable to bands occurring within a lane, since DGGE was used to provide a qualitative assessment of data.

With the exception of the Rand WTR, isolation and detection of bacterial DNA was successful, and the PCR-DGGE technique yielded distinct banding patterns. The positive and negative controls were included to validate the results obtained. Gerstein (2001) suggested that the negative control should not yield a band because it does not contain bacterial DNA. The presence of a single band in the negative control (Lane 7; Figure 6.1) indicates the presence of a contaminant and corresponding bands occurring at this location in the other lanes were therefore disregarded. Although the banding pattern of the positive control was consistent

with previous runs, it should have yielded one band. A likely explanation for the presence of numerous bands in the positive control (Table 6.1) is the presence of contaminant. In addition there may have been heteroduplex formation due to the presence of a high concentration of DNA, and/or non-specific binding of the primers. A heteroduplex DNA molecule has strands from two different PCR products, and is formed by re-annealing of the denatured PCR products (Murray *et al.,* 1996).

The absence of DNA bands in the Rand WTR suggests that the Rand WTR does not constitute a favourable environment for bacterial growth, possibly due its low OC content (Table 3.3). The water treatment process at Rand Water employs a high dosage of lime (50 to 60mg L^{-1}) resulting in a high pH (Table 3.3), which could further inhibit microbial proliferation.

The presence of numerous DNA bands in the Midvaal, Umgeni, Amatola, Faure[1] and Faure[2] WTRs indicates a high diversity of bacterial species (Figure 6.2, Table 6.1). This was an expected trend since at the start of the water purification and treatment process, the initial waste material has a organic carbon (OC) content, which supports a high diversity and concentration of bacterial species. Despite the effectiveness of the water treatment process at removing and disinfecting these microorganisms, it is possible that some bacteria may persist in the final waste product (i.e., WTR) derived from the water treatment process.

The diversity indicated by the DGGE profiles of these WTRs is due in part to the ability of PCR to amplify low density bacterial populations (Fournier *et al.,* 1998), and to the ability of DGGE to allow the resolution of both viable and non-viable bacterial species (Kandeler *et al.,* 2000). Further, there are concerns regarding the over-estimation of microbial diversity using the PCR-DGGE technique, due to the presence of heteroduplex molecules (Liesack *et al.,* 1991). However, Murray *et al.* (1996) concluded that the formation of heteroduplex molecules is not a

significant problem in mixed populations, and so should not pose a problem in the present research.

All WTRs displayed low degrees of similarity (< 50%) to each other (Table 6.2). This can be attributed to the variable nature of the tested WTRs resulting from differences in waste quality, water quality, and the treatment process. However, the DGGE method did not distinguish between viable and non-viable DNA and so it is possible that the active microbial populations within each treatment may have a higher degree of similarity than reflected in the similarity matrix.

The nature of the WTRs is such that its composition may vary between sampling times within a specified water treatment plant. For example, the Faure[1] (Lane 13; Figure 6.2) and Faure[2] (Lane 14; Figure 6.2) WTRs that were obtained from the Faure Water Treatment Plant showed only 26% similarity to each other (Table 6.2). This disparity was attributed to a blue-green algal bloom when the Faure[2] WTR was collected. The abundance of blue green algae indicates possible eutrophication of the water source which may have supported a different microbial community. Further, a higher dosing of Faure[2] WTR with activated charcoal was required to remedy resultant taste and odour problems. This may have also contributed to the changes in the microbial community structure of the Faure WTRs as seen on the denaturing gel.

The banding patterns of all the soil/WTR treatments displayed greater similarity to the Hutton soil control, than the respective WTRs. Irrespective of the type of WTR added to the Hutton soil, the prominent banding patterns of the Hutton control (Figures 6.1 and 6.2; location 'b') also occurred in the soil/WTR treatments. This provides an indication of the competitive nature of soil microbial community resident in the Hutton soil subsequent to the application of WTR.

The soil/WTR treatments displayed additional bands to those found in the Hutton control soil, indicating that the addition of the tested WTRs had altered the

community structure of WTR-amended treatments. This change in structure may be directly due to the contribution of bacterial species from the WTRs, and/or indirectly due to physico-chemical changes in the soil following WTR addition, such as increased pH (Section 5.1.2) and OC.

The PCR-DGGE results reflect changes in the microbial community structure of WTR-amended soils after a period of only five days. However, the extrapolation of such data to field conditions is difficult and warrants further research to examine spatial and temporal components of the microbial community structure.

CHAPTER 7

Conclusions and future directions

7.1 Conclusions

This research attempted to evaluate the effect of WTR on soil microbial activity and community structure following land application or land disposal. At the onset there was a lack of information on the chemical composition of South African WTRs. Research efforts therefore sought to incorporate the measurement of selected soil chemical and microbial properties as indicators of soil quality of WTR-amended soils.

For good statistical representation of field data triplicate soil cores were sampled from each of two duplicate treatment plots and analyzed without compositing. In addition, sampling throughout the soil profile was carried out to determine the possibility of WTR-induced depth effects. As a result of the sampling protocol, the analysis of samples from Ukulinga and Brookdale was tedious and time consuming.

At both field experiments measurement of pH, EC, OC, and microbial activity indicated that soil quality was not adversely affected by the addition of even very high rates (1280Mg ha^{-1}) of WTR. However, the Umgeni WTR produced increases in pH in the topsoil (0-200mm). This is in keeping with the findings of numerous studies that suggest that WTRs possess the potential to act as a soil conditioner or acid-neutralizing agent (Bugbee and Frink, 1985; Heil and Barbarick, 1989; Ahmed *et al.,* 1997).

Additionally, the heavy metal concentrations of WTR are lower than in WTS, but are higher than the levels generally found in soil (Elliot *et al.,* 1990b). Since soil pH is the main controlling factor concerned with the chemical reactions of metals in soils (Roy and Couillard, 1998), the pH of WTR-amended soils is a significant

issue that must be addressed by guidelines regarding the disposal of WTRs. However, since heavy metals are less available and less mobile in soils of neutral or basic pH, the increase in pH caused by WTR addition can be considered to be a positive factor. With regards to conditions suitable for plant growth, a desirable pH of between 5 and 8 and an EC < 4dS m^{-1} are commonly suggested (Dayton and Basta, 2001). Although the effect of WTR on soil EC was variable, at both field experiments pH and EC were well within the limits defined by Dayton and Basta (2001).

At Ukulinga and Brookdale small differences were observed (in all measured variables) between the fallow and grassed treatments. The fallow treatments were originally included in the experimental design to investigate the effect of WTR on soil physical properties only (Moodley, 2001), and it is apparent that fallow management does not constitute a sustainable land use. While research findings show that grass can be maintained on WTR-amended soil under no-till conditions, investigation into the use of WTR in other cropping or management systems is suggested.

Microbial activity of soil samples from both field experiments was determined by the fluorescein diacetate (FDA) hydrolysis method because it provides an indication of general microbial activity (Schnurer and Rosswall, 1982). It is especially useful when processing a large number of samples, and therefore has been recommended for this purpose. At both field experiments similar trends in microbial activity were observed in the soil profile across all treatments.

Research findings further indicate that time and expense can be reduced by restricting sampling to the rooting depth, since WTR-influenced changes to microbial activity, pH, EC, and OC were not seen at lower depths. While the variable nature of microbial activity justifies the sampling criteria adopted, soil OC did not display the same degree of variability. Thus, the analysis of OC on

composite samples could have been employed without compromising the statistical quality of the data.

The respiration studies were designed to examine the short-term changes in microbial activity of the field trial soils in response to the addition of various WTRs. Ideally, it was hoped that the laboratory respiration experiment would be conducted as a single experiment inclusive of all collected WTRs. However, the staggered arrival of WTRs and the short supply of the Amatola and Midvaal WTRs, which resulted in the set-up of two experiments, were unavoidable. Consequently, trends in respiration between the WTRs were efficiently compared whereas statistical comparisons were neglected.

The field experiments demonstrated the ability of the Umgeni WTR to raise soil pH, and the respiration experiments showed that all the tested WTRs produced immediate increases in pH, of varying amounts. The increases in pH were most pronounced on the Rand, Midvaal, Umgeni, and Faure[2] treatments. Although laboratory experiments showed that the tested WTRs displayed liming potential, the laboratory assessment of changes in EC of the various WTR treatments was unclear but the EC never reached unacceptable values.

Field assessments indicated that the long-term effects of WTR on microbial activity were negligible, while the laboratory respiration experiments displayed immediate changes in the activity of the microbial populations. With the exception of the Faure[1] and Amatola WTRs, the other tested WTRs produced initial increases in microbial activity. However, the increased respiration activity of WTR-amended soil was short-lived, possibly due to the depletion of available carbon sources in the WTR. From the perspective of microbial activity, the decline in respiration activity by Day 14 is evidence that WTR treatments would return to the conditions of the controls. While this is promising the measurements of activity did not address concerns regarding the possibility of altered microbial community structure resulting from WTR addition.

The use of PCR-DGGE showed that the microbial community structure of all soil/WTR treatments in comparison to the Hutton control soil had been altered. However, there was evidence to show that the dominant soil microorganisms were still present in the soil/WTR treatments, but in lower quantities.

In the South African context, the idea of land application of WTR is still in its infancy and will need to be followed up by much research. The field trials that have been running for approximately three and five years at Ukulinga and Brookdale, respectively, have indicated that land disposal of the Umgeni WTR is a feasible option. Further, laboratory studies suggest that the tested WTRs have value as liming agents. From a perspective of microbial ecology the tested soil/WTR treatments showed an ability to adapt to changes resulting from WTR addition.

7.2 Future directions

Although, chemical characterization of the WTRs used in this study has been carried out (Water Research Commission, 2003), data on the chemical nature of South African WTRs is sparse. Therefore, an essential step leading to future research in this field should be the characterization of WTRs produced by South African water utilities. This would allow for an unambiguous distinction to be drawn between WTR and WTS, ultimately allowing for more effective regulation of WTR disposal.

Due to the high costs involved in setting up and running field experiments, it is suggested that further studies proceed via glasshouse experiments. This would provide a more cost-effective way of evaluating the possibility of growing cash crops on various soil/WTR treatments. While current research has examined general microbial activity, specific enzyme assays can be carried out to determine the influence of WTR on specific groups of microorganisms, or the research focus can incorporate rhizosphere/microbial interactions.

It should also be stressed that successful land disposal of WTRs translates to an issue of efficient management and this is an issue that warrants further investigation. It would be interesting to observe the effects of tillage, crop rotation, and ploughing of crop residues, on WTR breakdown and related microbial response.

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Appendix 3.1. XRF analysis of the Umgeni, Midvaal, Amatola, Rand and Faure[1] water treatment residues.

Sample	SiO_2	Al_2O_3	Fe_2O_3	MnO	MgO	CaO	Na_2O	K_2O	TiO_2	P_2O_5	Total	LOI
	%											
Umgeni	54.57	22.60	11.95	1.53	1.92	4.20	0.15	1.47	0.86	0.24	99.49	23.91
Midvaal	53.07	22.36	14.24	0.41	1.83	4.45	0.33	1.89	0.74	0.47	99.79	27.70
Amatola	52.59	29.06	10.31	0.07	1.82	1.55	0.43	2.98	0.86	0.12	99.79	25.63
Rand	24.36	9.89	4.85	0.66	5.25	53.19	0.61	0.82	0.31	0.09	100.03	36.93
Faure[1]	29.93	8.70	53.80	0.96	0.72	3.15	bd	0.93	0.78	0.39	9.37	43.07
	Cd	Co	Cr	Cu	Ni	Pb	Zn	S	Cl	As	V	Sr
	mg kg^{-1}											
Umgeni	4.00	39.00	161.00	44.00	53.00	37.00	84.00	720.00	1575.00	17.00	154.00	71.00
Midvaal	nd	32.00	246.60	53.20	117.70	26.00	142.10	2402.00	nd	5.00	187.80	66.20
Amatola	nd	19.00	134.30	23.00	39.70	36.00	84.50	911.00	nd	11.00	175.80	91.40
Rand	nd	3.50	77.30	5.40	22.90	5.80	33.10	700.00	nd	1.70	61.10	273.40
Faure[1]	nd	nd	43.00	bd	21.00	nd	182.00	2500.00	nd	nd	93.00	nd
	Nb	Ce	Nd	Zr	Ba	Sc	Th	Y	La	Rb	Ga	U
	mg kg^{-1}											
Umgeni	11.00	96.00	25.00	126.00	1007.00	33.00	10.00	27.00	19.00	94.00	20.00	bd
Midvaal	7.80	51.00	24.00	101.60	438.70	30.70	7.90	23.10	5.40	109.20	19.00	0.20
Amatola	10.60	71.00	38.00	105.10	705.70	30.10	16.10	29.40	30.60	171.50	23.00	3.80
Rand	2.70	5.10	bd	32.20	398.40	13.50	5.00	38.10	bd	38.10	8.90	2.10
Faure[1]	nd	nd	nd	nd	nd	nd	nd	nd	38.00	nd	nd	nd

Where: nd = not determined, bd = below detection, and LOI = Loss on ignition

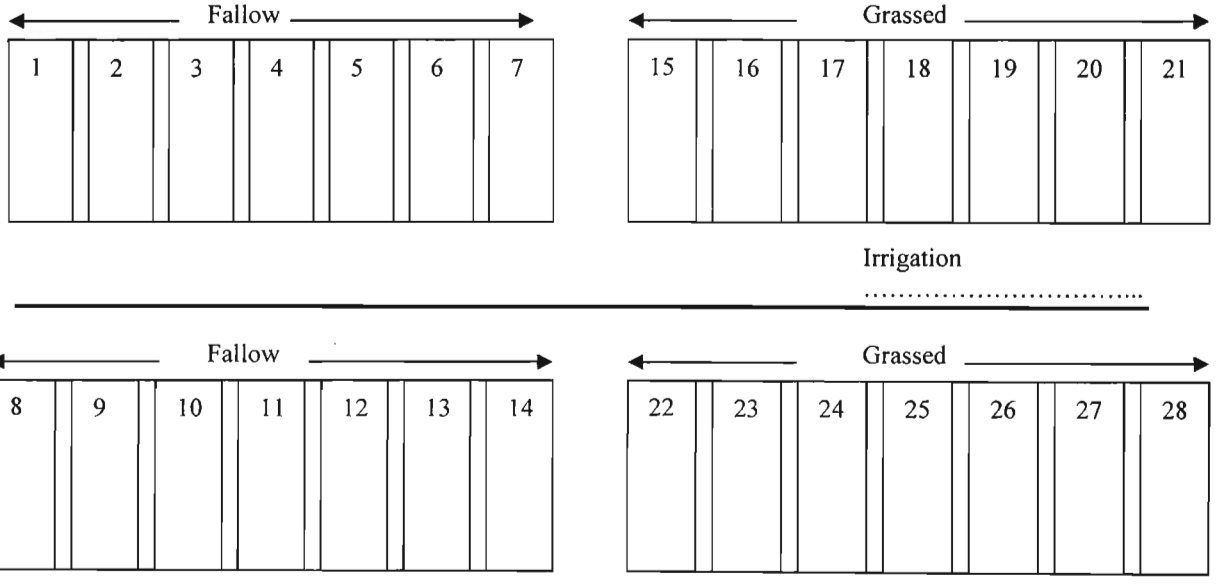
Appendix 3.2. Field layout at Ukulinga Research Farm and treatments investigated.

Fallow		Grassed	
Plot	**Treatment**	**Plot**	**Treatment**
1	Control	15	80
2	320	16	P30
3	G10	17	80
4	1280	18	320
5	L10	19	1280
6	320	20	G10
7	P30	21	Control
8	80	22	1280
9	G10	23	L10
10	1280	24	320
11	P30	25	L10
12	80	26	Control
13	L10	27	G10
14	Control	28	P30

For a description of the treatments see the key given below

KEY

Codes used in Table above	Description of Treatment
L10	Lime applied at 10Mg ha^{-1}. Incorporated with the top 0.2m of the soil by discing.
G10	Gypsum applied at 10Mg ha^{-1}. . Incorporated with the top 0.2m of the soil by discing.
P30	Anionic polyacrylamide applied at 30kg ha^{-1}. Sprayed onto the plot surface as a 0.1g L^{-1} solution.
80, 320,1280	WTR applied (Mg ha^{-1}). Incorporated with the top 0.2m of the soil by discing



Appendix 3.3. Field layout at Brookdale Farm and treatments investigated.

Fallow		Grassed	
Plot	**Treatment**	**Plot**	**Treatment**
1	P15	33	Control
2	L10	34	P15
3	1280	35	40
4	G10	36	L2
5	M_{320}	37	160
6	40	38	320
7	G5	39	M_{1280}
8	1280	40	L10
9	M_{640}	41	P30
10	M_{1280}	42	160
11	L2	43	640
12	320	44	M_{320}
13	80	45	Control
14	P30	46	P15
15	Control	47	G10
16	160	48	40
17	640	49	P30
18	80	50	320
19	160	51	G5
20	Control	52	G5
21	640	53	80
22	L10	54	L10
23	M_{640}	55	M_{320}
24	G5	56	G10
25	320	57	640
26	40	58	80
27	M_{320}	59	L2
28	P15	60	1280
29	G10	61	M_{640}
30	P30	62	M_{1280}
31	M_{1280}	63	M_{640}
32	L10	64	1280

NB The plot size is 6 m X 4 m. For a description of the treatments see the key below.

KEY

Codes used in Table above	Description of Treatment
L2	Lime applied at 2Mg ha^{-1}. Incorporated with the top 0.2m of the soil by discing.
L10	Lime applied at 10Mg ha^{-1}. Incorporated with the top 0.2m of the soil by discing.
G5	Gypsum applied at 5Mg ha^{-1}. Incorporated with the top 0.2m of the soil by discing.
G10	Gypsum applied at 10Mg ha^{-1}. Incorporated with the top 0.2m of the soil by discing.
P15	Anionic polyacrylamide applied at 15kg ha^{-1}. Sprayed onto the plot surface as a 0.1 g L^{-1} solution.
P30	Anionic polyacrylamide applied at 30kg ha^{-1}. Sprayed onto the plot surface as a 0.1g L^{-1} solution.
40, 80, 160, 320, 640, 1280	WTR content (Mg ha^{-1}). Incorporated with the top 0.2m of the soil by discing.
M_{320}, M_{640}, M_{1280}	Subscript refers to WTR content (Mg ha^{-1}). The WTR was applied to the plot as a mulch (M) i.e. no incorporation of the WTR with the soil was undertaken.

Appendix 3.4. Preparation of solutions used in microbial community analysis.

10% APS: Make a 10% (w/v) solution in Milli-Q (1g in 10mL) and store 0.5mL alliquots at -20°C.

10M NaOH: Dissolve 400g NaOH in 450mL water and add water to make up to 1L.

0.5M EDTA: Dissolve 186.1g $Na_2EDTA.2H_2O$ in 700mL water and adjust pH to 8 with 10M NaOH. Make volume up to 1L, autoclave for 20 minutes at 120°C, and store at room temperature.

50x TAE buffer: Mix 242g Tris-base (final conc. 2M), 57.1mL acetic acid, and 200mL 0.5M EDTA or 37.2g $Na_2EDTA.2H_2O$ (final 0.1M). Adjust volume to 1L (solution pH 8). Autoclave for 20 minutes at 120°C and store at room temperature.

1x TAE running buffer: Mix 20mL 50X TAE with 980mL H_2O.

Gel-dye: Mix 0.05g bromophenol blue in 10mL 1x TAE.

Loading buffer: Mix 0.05% (w/v) bromophenol blue (0.05g), 40% sucrose (40g), 0.1M EDTA (20mL 0.5M EDTA), and 0.5% SDS (0.5g) and adjust to 100mL.

Ethidium bromide: Mix 10mg ethidium bromide in 1mL H_2O.

Appendix 3.5. Silver staining of DNA polyacrylgels.

Solutions required:

Fixation solution: 10% ethanol and 0.5% acetic acid in water.

Part of developing solution: 1.5% NaOH

Stop mix: 0.75% Na_2CO_3

Freshly prepared: 250mL of 0.1% $AGNO_3$

Developing solution: 25mg $NaBH_4$, 1mL formaldehyde in 250mL 1.5% NaOH

Procedure:

Use two clean trays.

In Tray 1:

- Destain a Ethidium bromide stained gel on a glass plate by agitation in bidest for 30minutes.
- Fixate the gel for 2 times 3 minutes (at least) with fixation solution (100-200mL), with gentile agitation.
- Remove fixation solution. Rinse gel with bidest. Add 250mL of freshly prepared 0.1% $AGNO_3$ solution and agitate in the dark for 15 minutes.
- Remive solution and rinse with bidest. Fill tray with amount of bidest and position the gel well on the glass plate. Transfer the glass plate with gel to second tray.

In Tray 2:

- Develop the gel by agitation with 250mL of developing solution for about 30 minutes in the dark, in a daylight protected tray. Directly after addition shake the solution well for uniform development. Monitor the development in time, if necessary stop the development earlier.
- Remove developing solution and rinse with bidest. Add 200mL stop mix and agitate for at least 10 minutes. Make a photo. Store sealed in plastic.

Appendix 4.1. ANOVA results of organic carbon as affected by water treatment residue (WTR), status and soil depth at Ukulinga Research Farm and Brookdale Farm (sampled in September 2001 and May 2002).

Ukulinga	**September 2001**			**May 2002**		
Stratum	degrees of freedom	mean square	Probability	degrees of freedom	mean square	Probability
Main effects						
WTR	3	3.34	<0.001***	3	0.96	0.042*
Depth	4	7.73	<0.001***	4	3.62	<0.001***
Interactions						
Status.WTR	3	1.84	0.004**	3	0.38	0.209^{NS}
Status.Depth	4	0.14	0.021*	4	0.31	0.002**
WTR.Depth	12	0.62	<0.001***	12	0.09	0.188^{NS}
Status.WTR.Depth	11(1)	0.31	<0.001***	12	0.04	0.702^{NS}
Brookdale	**September 2001**			**May 2002**		
Stratum	degrees of freedom	mean square	Probability	degrees of freedom	mean square	Probability
Main effects						
WTR	5	1.41	0.107^{NS}	5	1.11	0.020*
Depth	7	72.71	<0.001***	7	60.80	<0.001***
Interactions						
Status.WTR	5	0.67	0.389^{NS}	5	0.36	0.278^{NS}
Status.Depth	7	0.24	0.010**	7	0.30	0.363^{NS}
WTR.Depth	35	0.23	<0.001***	35	0.52	0.008**
Status.WTR.Depth	35	0.06	0.903^{NS}	34(1)	0.19	0.852^{NS}

Status = condition of plots i.e. grassed or fallow; WTR = water treatment residue;
NS = non-significant (P>0.05); * = significant (P≤0.05); ** = highly significant (P≤0.01); *** = very highly significant (P≤0.001)

Appendix 4.2. The effect of water treatment residue (WTR) and depth on mean soil organic carbon (%; n=6) at Ukulinga Research Farm (sampled in September 2001 and May 2002).

September 2001						
WTR (Mg ha^{-1})	**Mean depth (mm)**					**Means**
	50	**150**	**250**	**350**	**450**	
0	2.30	1.84	1.39	0.91	0.64	1.42a
80	2.19	2.02	1.96	1.71	1.79	1.94b
320	2.20	2.02	1.95	1.72	1.32	1.84b
1280	2.41	2.07	1.91	1.74	1.21	1.87b
Means	2.27a	1.99b	1.80c	1.52d	1.24e	
Least significant differences of means (5% level)						
		WTR		DEPTH		WTR * DEPTH
rep.		60		48		12
l.s.d.		0.16		0.08		0.20
d.f		6		31		22.91
May 2002						
WTR (Mg ha^{-1})	**Mean depth (mm)**					**Means**
	50	**150**	**250**	**350**	**450**	
0	2.21	2.00	1.90	1.64	1.27	1.81a
80	2.14	1.95	1.96	1.92	1.58	1.91a
320	2.20	2.01	1.99	1.73	1.53	1.89a
1280	2.49	2.23	2.08	1.98	1.73	2.11b
Means	2.26a	2.05b	1.99b	1.81c	1.53d	
Least significant differences of means (5% level)						
		WTR		DEPTH		WTR * DEPTH
rep.		60		48		12
l.s.d.		0.19		0.10		0.24
d.f		6		32		23.65

• Means with the same letter are not significantly different at the 5% level of probability

Appendix 4.3. The effect of water treatment residue (WTR) and depth on mean organic carbon (%; n=6) at Brookdale Farm (sampled in September 2001 and May 2002).

September 2001									
WTR	**Depth (mm)**								
(Mg ha^{-1})	**50**	**150**	**250**	**350**	**500**	**700**	**900**	**1100**	**Means**
0	3.33	3.34	2.89	2.40	2.04	1.47	1.03	0.64	2.14
80	3.23	3.01	2.75	2.35	1.91	1.03	0.76	0.58	1.95
320	3.11	2.97	2.55	2.13	1.85	1.05	0.70	0.52	1.86
M320	3.22	2.98	2.95	2.69	2.04	1.59	0.93	0.67	2.13
1280	3.38	3.23	3.06	2.54	2.05	1.25	0.91	0.74	2.14
M1280	3.12	3.09	2.88	2.64	1.99	1.38	0.88	0.55	1.98
Means	3.23a	3.25a	2.85b	2.46c	1.98d	1.29e	0.87f	0.62g	
Least significant differences of means (5% level)									
				WTR		DEPTH		WTR*DEPTH	
rep.				96		72		12	
l.s.d.				0.24		0.09		0.32	
d.f				10		82		36.45	
May 2002									
WTR	**Depth (mm)**								
(Mg ha^{-1})	**50**	**150**	**250**	**350**	**500**	**700**	**900**	**1100**	Mean
0	3.16	3.16	2.95	2.46	1.88	1.45	1.07	0.94	2.32a
80	3.23	3.16	3.01	2.72	2.13	1.43	0.95	0.77	2.18ab
320	3.26	3.08	2.89	2.27	1.86	1.29	0.90	0.83	2.05b
M320	3.22	3.05	2.81	2.45	1.95	1.31	0.89	0.75	2.05b
1280	3.21	3.08	2.99	2.86	2.25	1.76	1.15	0.75	2.26a
M1280	3.26	3.11	3.06	2.64	2.16	1.51	1.03	0.79	2.20ab
Means	3.23a	3.11ab	2.95b	2.57c	2.04d	1.46e	1.07f	0.97f	
Least significant differences of means (5% level)									
				WTR		DEPTH		WTR*DEPTH	
rep.				96		72		12	
l.s.d.				0.16		0.17		0.42	
d.f				10		83		92.95	

*Means with the same letter are not significantly different at the 5% level of probability

Appendix 4.4. ANOVA results of pH as affected by water treatment residue (WTR), status and soil depth at Ukulinga Research Farm and Brookdale Farm (sampled in September 2001 and May 2002).

Ukulinga	**September 2001**			**May 2002**		
Stratum	degrees of freedom	mean square	Probability	degrees of freedom	mean square	Probability
Main effects						
WTR	3	1.51	0.171 ^{NS}	3	5.12	<0.001***
Depth	4	1.07	<0.001***	4	2.18	<0.001***
Interactions						
Status.WTR	3	0.61	0.472 ^{NS}	3	0.09	0.398 ^{NS}
Status.Depth	4	1.22	<0.001***	4	0.49	<0.001***
WTR.Depth	12	0.47	<0.001***	12	1.60	<0.001***
Status.WTR.Depth	11(1)	0.35	<0.001***	12	0.11	0.076 ^{NS}
Brookdale	**September 2001**			**May 2002**		
Stratum	degrees of freedom	mean square	Probability	degrees of freedom	mean square	Probability
Main effects						
WTR	5	3.54	0.092 ^{NS}	5	3.35	0.066 ^{NS}
Depth	7	2.46	<0.001***	7	2.46	<0.001***
Interactions						
Status.WTR	5	3.36	0.104 ^{NS}	5	1.18	0.439 ^{NS}
Status.Depth	7	0.36	0.024*	7	0.36	0.552 ^{NS}
WTR.Depth	35	0.52	<0.001***	35	0.52	<0.001***
Status.WTR.Depth	35	0.20	0.123 ^{NS}	34(1)	0.20	0.558 ^{NS}

Status = condition of plots i.e. grassed or fallow; WTR = water treatment residue;
NS = non-significant (P>0.05); * = significant (P≤0.05); ** = highly significant (P≤0.01); *** = very highly significant (P≤0.001)

Appendix 4.5. The effect of water treatment residue (WTR) and depth on mean soil pH (n=6) at Ukulinga Research Farm (sampled in September 2001 and May 2002).

September 2001						
WTR	**Mean depth (mm)**					
(Mg ha^{-1})	**50**	**150**	**250**	**350**	**450**	**Means**
0	6.12	6.15	6.21	6.34	6.48	6.27
80	6.04	5.83	5.94	6.17	6.42	6.41
320	6.68	6.39	6.09	6.42	6.52	6.42
1280	6.83	6.52	6.13	6.15	6.42	6.08
Means	6.42a	6.22b	6.09c	6.29b	6.46a	
Least significant differences of means (5% level)						
		WTR		DEPTH		WTR * DEPTH
rep.		60		48		12
l.s.d.		0.36		0.07		0.36
d.f		6		31		8.40
May 2002						
WTR	**Mean depth (mm)**					
(Mg ha^{-1})	**50**	**150**	**250**	**350**	**450**	**Means**
0	5.74	5.90	6.01	6.27	6.38	6.06a
80	6.39	6.08	6.01	6.18	6.29	6.19b
320	6.99	6.67	6.14	6.29	6.45	6.51c
1280	7.41	7.31	6.24	6.10	6.44	6.70c
Means	6.63a	6.49b	6.10c	6.21d	6.39e	
Least significant differences of means (5% level)						
		WTR		DEPTH		WTR * DEPTH
rep.		60		48		12
l.s.d.		0.13		0.10		0.21
d.f		6		32		35.33

• Means with the same letter are not significantly different at the 5% level of probability

Appendix 4.6. The effect of water treatment residue (WTR) and depth on mean soil pH (n=6) at Brookdale Farm (sampled in September 2001 and May 2002).

September 2001									
WTR	**Depth (mm)**								
(Mg ha^{-1})	**50**	**150**	**250**	**350**	**500**	**700**	**900**	**1100**	**Means**
0	5.23	5.36	5.62	5.77	5.84	5.82	5.89	5.86	5.68
80	5.98	5.66	5.26	5.55	5.68	5.79	5.53	5.26	5.56
320	5.42	5.28	5.41	5.58	5.59	5.56	5.24	5.25	5.42
M320	5.72	5.45	5.50	5.88	5.96	6.04	6.07	5.96	5.82
1280	6.86	6.68	5.51	5.56	5.70	5.77	5.55	5.45	5.89
M1280	6.61	6.28	5.89	5.85	6.04	6.07	5.98	5.93	6.08
Means	5.97a	5.79bc	5.53e	5.70cd	5.80b	5.84ab	5.71bcd	5.62de	
Least significant differences of means (5% level)									
					DEPTH				WTR*DEPTH
rep.					72				12
l.s.d.					0.13				0.45
d.f					82				28.89
May 2002									
WTR	**Depth (mm)**								
(Mg ha^{-1})	**50**	**150**	**250**	**350**	**500**	**700**	**900**	**1100**	Means
0	4.87	5.04	5.34	5.49	5.67	5.64	5.66	5.59	5.41
80	5.02	5.37	5.31	5.64	5.60	5.71	5.78	5.74	5.52
320	5.33	5.36	5.63	5.80	5.90	5.92	5.87	5.84	5.71
M320	5.12	5.31	5.37	5.64	5.69	5.55	5.27	5.27	5.40
1280	5.24	5.14	5.42	5.77	5.92	5.87	5.90	5.96	5.65
M1280	5.53	5.21	5.38	5.56	5.71	5.85	5.83	5.62	5.58
Means	5.19a	5.24a	5.41b	5.65c	5.75c	5.76c	5.72c	5.67c	
Least significant differences of means (5% level)									
					DEPTH				WTR*DEPTH
rep.					72				12
l.s.d.					0.13				0.43
d.f					83				32.80

*Means with the same letter are not significantly different at the 5% level

Appendix 4.7. ANOVA results of electrical conductivity as affected by water treatment residue (WTR), status and soil depth at Ukulinga Research Farm and Brookdale Farm (sampled in September 2001 and May 2002).

Ukulinga	**September 2001**			**May 2002**		
Stratum	degrees of freedom	mean square	Probability	degrees of freedom	mean square	Probability
Main effects						
WTR	3	112320.1	<0.001***	3	7629.4	0.112^{NS}
Depth	4	2963.0	0.001**	4	13937.9	<0.001***
Interactions						
Status.WTR	3	6766.7	0.244^{NS}	3	416.8	0.914^{NS}
Status.Depth	4	199.7	0.806^{NS}	4	5321.9	<0.001***
WTR.Depth	12	1671.5	0.003**	12	812.3	0.480^{NS}
Status.WTR.Depth	11(1)	1244.5	0.022*	12	465.5	0.852^{NS}
Brookdale	**September 2001**			**May 2002**		
Stratum	degrees of freedom	mean square	Probability	degrees of freedom	mean square	Probability
Main effects						
WTR	5	109913.8	<0.001***	5	24841.8	0.003**
Depth	7	9076.2	<0.001***	7	12802.0	<0.001***
Interactions						
Status.WTR	5	13501.1	0.074^{NS}	5	6453.3	0.169^{NS}
Status.Depth	7	8049.0	<0.001***	7	1127.5	0.172^{NS}
WTR.Depth	35	9528.2	<0.001***	35	3462.5	<0.001***
Status.WTR.Depth	35	2313.6	0.040*	34(1)	650.4	0.659^{NS}

Status = condition of plots i.e. grassed or fallow; WTR = water treatment residue;
NS = non-significant (P>0.05); * = significant (P≤0.05); ** = highly significant (P≤0.01); *** = very highly significant (P≤0.001)

Appendix 4.8. The effect of water treatment residue (WTR) and depth on mean soil electrical conductivity (μS cm^{-1}; n=6) at Ukulinga Research Farm (sampled in September 2001 and May 2002).

September 2001						
WTR (Mg ha^{-1})	**Mean depth (mm)**					Means
	50	**150**	**250**	**350**	**450**	
0	98.74	104.82	106.50	108.12	107.16	105.07a
80	64.14	58.08	55.40	44.14	54.46	55.25b
320	69.98	65.99	66.91	54.37	49.22	61.29b
1280	184.33	149.91	133.07	125.14	148.27	148.14c
Means	104.30a	94.70b	90.47bc	82.94c	89.78bc	
Least significant differences of means (5% level)						
		WTR		DEPTH		WTR * DEPTH
rep.		60		48		12
l.s.d.		27.25		9.28		29.71
d.f		6		31		13.37
May 2002						
WTR (Mg ha^{-1})	**Mean depth (mm)**					**Means**
	50	**150**	**250**	**350**	**450**	
0	62.99	30.86	28.62	22.08	30.63	35.04
80	68.61	48.40	42.28	39.29	38.32	46.88
320	71.38	50.71	43.38	34.39	38.32	47.64
1280	99.83	81.85	47.98	46.50	36.66	62.56
Means	75.70a	52.95b	40.56c	35.57c	35.36c	
Least significant differences of means (5% level)						
		WTR		DEPTH		WTR * DEPTH
rep.		60		48		12
l.s.d.		22.23		11.92		28.58
d.f		6		32		24.44

• Means with the same letter are not significantly different at the 5% level of probability

Appendix 4.9. The effect of water treatment residue (WTR) and depth on mean soil electrical conductivity (μS cm^{-1}; n=6) at Brookdale Farm (sampled in September 2001 and May 2002).

September 2001

WTR ($Mgha^{-1}$)	Depth (mm)								Means
	50	150	250	350	500	700	900	1100	
0	66.9	81.5	67	69.3	78.1	89.1	68.9	57.8	72.3a
80	42.1	50.8	87.4	71.3	71.3	65.4	66.4	64.3	64.9a
320	58.8	80.5	81.4	83.1	76.2	83.3	73.7	54.3	73.9a
M320	68.3	121.5	96.7	94.5	95.4	116.7	108.4	84.5	98.3b
1280	61.0	75.7	86.4	89.7	73.1	78.8	51.5	60.5	72.0a
M1280	103.3	114.7	105.5	112.7	130.0	145	148	135	154.3c
Means	66.7a	87.4b	87.4b	86.8b	87.3b	100.9c	103.2c	94.6bc	

Least significant differences of means (5% level)

	WTR	DEPTH	WTR*DEPTH
rep.	96	72	12
l.s.d.	22.10	12.56	35.05
d.f	10	82	63.04

May 2002

WTR ($Mgha^{-1}$)	Depth (mm)								Means
	50	150	250	350	500	700	900	1100	
0	66.92	45.33	38.70	40.73	44.33	57.43	56.75	51.74	50.24ab
80	60.80	52.63	54.67	56.09	64.83	77.92	78.57	85.17	66.33b
320	37.58	42.44	42.18	63.78	55.71	78.10	69.83	70.23	57.48ab
M320	56.76	37.45	39.47	42.53	49.34	55.44	54.58	46.66	47.78a
1280	81.18	82.85	64.95	53.62	48.48	76.68	89.5	112	90.36c
M1280	54.33	46.00	38.90	42.08	43.62	59.61	58.54	64.08	50.90ab
Means	59.59 bc	51.12ac	46.48a	49.80a	51.05ac	67.53b	78.47d	80.08d	

Least significant differences of means (5% level)

	WTR	DEPTH	WTR*DEPTH
rep.	96	72	12
l.s.d.	18.40	9.03	26.66
d.f	10	83	51.33

*Means with the same letter are not significantly different at the 5% level

Appendix 4.10. ANOVA results of microbial activity as affected by water treatment residue (WTR), status and soil depth at Ukulinga Research Farm and Brookdale Farm (sampled in September 2001 and May 2002).

Ukulinga	**September 2001**			**May 2002**		
Stratum	degrees of freedom	mean square	Probability	degrees of freedom	mean square	Probability
Main effects						
WTR	3	0.03	0.868 ^{NS}	3	0.34	0.134 ^{NS}
Depth	4	0.07	0.083 ^{NS}	4	0.18	<0.001***
Interactions						
Status.WTR	3	0.06	0.711 ^{NS}	3	0.07	0.679 ^{NS}
Status.Depth	4	0.06	0.105 ^{NS}	4	0.15	<0.001***
WTR.Depth	12	0.04	0.282 ^{NS}	12	0.02	0.269 ^{NS}
Status.WTR.Depth	11(1)	0.06	0.071 ^{NS}	12	0.01	0.521 ^{NS}
Brookdale	**September 2001**			**May 2002**		
Stratum	degrees of freedom	mean square	Probability	degrees of freedom	mean square	Probability
Main effects						
WTR	5	0.932	<0.001***	5	0.590	0.092 ^{NS}
Depth	7	10.60	<0.001***	7	3.87	<0.001***
Interactions						
Status.WTR	5	0.319	0.005**	5	1.600	0.004**
Status.Depth	7	0.32	<0.001***	7	0.08	0.110 ^{NS}
WTR.Depth	35	0.42	<0.001***	35	0.16	<0.001***
Status.WTR.Depth	35	0.13	<0.001***	34(1)	0.19	<0.001***

Status = condition of plots i.e. grassed or fallow; WTR = water treatment residue;
NS = non-significant (P>0.05); * = significant (P≤0.05); ** = highly significant (P≤0.01); *** = very highly significant (P≤0.001)

Appendix 4.11. The effect of water treatment residue (WTR) and depth on mean microbial activity (μMol g^{-1} hr^{-1}; n=6) at Ukulinga Research Farm (sampled in September 2001 and May 2002).

September 2001						
WTR (Mg ha^{-1})	**Mean depth (mm)**					**Means**
	50	**150**	**250**	**350**	**450**	
0	0.22	0.21	0.38	0.26	0.17	0.25
80	0.20	0.20	0.25	0.33	0.25	0.25
320	0.24	0.26	0.31	0.36	0.27	0.29
1280	0.26	0.25	0.23	0.29	0.37	0.28
Means	0.23	0.23	0.29	0.31	0.26	
Least significant differences of means (5% level)						
	WTR		DEPTH		WTR * DEPTH	
rep.	60		48		12	
l.s.d.	0.16		0.07		0.19	
d.f	6		31		18.94	
May 2002						
WTR (Mg ha^{-1})	**Mean depth (mm)**					**Means**
	50	**150**	**250**	**350**	**450**	
0	0.27	0.24	0.25	0.33	0.25	0.27
80	0.31	0.29	0.39	0.42	0.45	0.37
320	0.29	0.24	0.37	0.46	0.37	0.35
1280	0.14	0.13	0.19	0.28	0.30	0.21
Means	0.25ac	0.23a	0.30bc	0.37d	0.34bd	
Least significant differences of means (5% level)						
	WTR		DEPTH		WTR * DEPTH	
rep.	60		48		12	
l.s.d.	0.16		0.05		0.17	
d.f	6		32		12	

• Means with the same letter are not significantly different at the 5% level of probability

Appendix 4.12. The effect of water treatment residue (WTR) and depth on mean microbial activity (μMol g^{-1} hr^{-1}; n=6) at Brookdale Farm (sampled in September 2001 and May 2002).

September 2001									
WTR	**Depth (mm)**								
(Mg ha^{-1})	**50**	**150**	**250**	**350**	**500**	**700**	**900**	**1100**	**Means**
0	0.75	0.73	0.99	1.36	1.32	0.89	0.51	0.31	0.86a
80	0.73	0.99	1.40	2.03	1.21	0.50	0.37	0.25	0.94b
320	0.61	0.75	0.85	1.07	1.15	0.87	0.21	0.10	0.70c
M320	0.78	0.85	1.10	1.42	1.35	0.86	0.34	0.22	0.86a
1280	0.69	0.71	0.80	1.39	1.17	0.62	0.48	0.38	0.78d
M1280	0.57	0.55	0.56	0.98	1.17	1.10	0.34	0.24	0.69c
Means	0.69a	0.76b	0.95c	1.37d	1.23d	0.81e	0.38f	0.25g	
Least significant differences of means (5% level)									
				WTR		DEPTH		WTR*DEPTH	
rep.				96		72		12	
l.s.d.				0.07		0.04		0.11	
d.f				10		82		68.23	
May 2002									
WTR	**Depth (mm)**								
(Mg ha^{-1})	**50**	**150**	**250**	**350**	**500**	**700**	**900**	**1100**	**Means**
0	0.40	0.35	0.41	0.72	1.08	0.47	0.14	0.05	0.45
80	0.39	0.39	0.53	0.90	1.23	0.67	0.22	0.13	0.56
320	0.31	0.36	0.36	0.58	0.57	0.38	0.12	0.11	0.35
M320	0.37	0.43	0.31	0.57	0.67	0.38	0.19	0.08	0.37
1280	0.31	0.26	0.30	0.43	0.67	0.75	0.13	0.10	0.37
M1280	0.30	0.35	0.38	0.48	0.66	0.52	0.23	0.13	0.38
Means	0.35a	0.3571a	0.38a	0.61b	0.81c	0.53d	0.17e	0.10f	
Least significant differences of means (5% level)									
				WTR		DEPTH		WTR*DEPTH	
rep.				96		72		12	
l.s.d.				0.15		0.07		0.21	
d.f				10		83		46.21	

*Means with the same letter are not significantly different at the 5% level

Appendix 5.1. ANOVA results of respiration as affected by Faure[1], Umgeni and Rand WTR.

Source of variation	Degrees of freedom	Mean square	F probability
CONTROL	2	11155.2	<0.001***
CONTROL.RATE	2	7028.1	<0.001***
CONTROL.WTR	2	36502	<0.001***
CONTROL.SOIL	1	5544.4	<0.001***
CONTROL.DATE	9	2074.9	<0.001***
CONTROL.RATE.WTR	4	719.8	0.007**
CONTROL.RATE.SOIL	2	537.0	0.066^{NS}
CONTROL.WTR.SOIL	2	5386.6	<0.001***
CONTROL.RATE.DATE	6	41.4	0.971^{NS}
CONTROL.WTR.DATE	6	677.1	0.003**
CONTROL.SOIL.DATE	3	454.6	0.075^{NS}
CONTROL.RATE.WTR.SOIL	4	236.0	0.303^{NS}
CONTROL.RATE.WTR.DATE	12	59.9	0.986^{NS}
CONTROL.RATE.SOIL.DATE	6	25.8	0.992^{NS}
CONTROL.WTR.SOIL.DATE	6	657.0	0.004**
CONTROL.RATE.WTR.SOIL.DATE	12	55.2	0.990^{NS}

WTR = water treatment residue;
NS = non-significant (P>0.05); * = significant (P≤0.05); ** = highly significant (P≤0.01); *** = very highly significant (P≤0.001)

Appendix 5.2. ANOVA results of respiration as affected by Faure[2], Amatola and Midvaal WTR.

Source of variation	Degrees of freedom	Mean square	F probability
CONTROL	2	3143.64	<0.001***
CONTROL.RATE	1	12513.58	<0.001***
CONTROL.WTR	2	7952.39	<0.001***
CONTROL.SOIL	1	86.75	0.184^{NS}
CONTROL.DATE	9	322.82	<0.001***
CONTROL.RATE.WTR	2	2459.64	0.001***
CONTROL.RATE.SOIL	1	189.62	0.051^{NS}
CONTROL.WTR.SOIL	2	10.66	0.801^{NS}
CONTROL.RATE.DATE	3	133.91	0.048^{NS}
CONTROL.WTR.DATE	6	257.86	0.001**
CONTROL.SOIL.DATE	3	21.73	0.715^{NS}
CONTROL.RATE.WTR.SOIL	2	68.39	0.248^{NS}
CONTROL.RATE.WTR.DATE	6	64.76	0.249^{NS}
CONTROL.RATE.SOIL.DATE	3	140.86	0.041^{NS}
CONTROL.WTR.SOIL.DATE	6	55.68	0.339**
CONTROL.RATE.WTR.SOIL.DATE	6	70.94	0.201^{NS}

WTR = water treatment residue;
NS = non-significant (P>0.05); * = significant (P≤0.05); ** = highly significant (P≤0.01); *** = very highly significant (P≤0.001)