DENITRIFICATION IN THE UPLAND SOILS OF A FORESTED LAND TREATMENT SYSTEM

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Louise Barton

The University of Waikato
Te Whare Wānanga o Waikato

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Frontispiece -Measuring *in situ* denitrification rates in the upland soils of the Rotorua Land Treatment System. (Photo J. Barran).

ABSTRACT

The contribution of upland denitrification to nitrate removal in soils, and the factors controlling denitrification, were investigated in the Rotorua Land Treatment System (RLTS), New Zealand. The RLTS is forested with radiata pine and located on free-draining soils formed from pumiceous parent materials. In land treatment systems, a large proportion of the nitrogen added in the wastewater is thought to either be utilised by the cover crop, or by soil microbial processes. An important soil microbial process that is often assumed to occur is denitrification. However, the contribution of upland denitrification to nitrogen renovation, and the effects of wastewater application on the soil denitrifying population, is poorly understood in forested land treatment systems.

An initial study was undertaken to establish a suitable method for measuring *in situ* denitrification rates in the RLTS. Denitrification enzyme activity (DEA) was measured at different soil depths (litter, 0-5, 5-10, 10-20 and 20-40 cm) in three topographic positions of the RLTS (ridge, midslope and toeslope). In addition, *in situ* denitrification rates were measured, using an acetylene-inhibition technique, at various time intervals before and after irrigation to determine how frequently soil cores needed to be taken to quantify denitrification losses after wastewater irrigation. It was concluded that it was necessary to collect cores from the uppermost 10 cm of the soil profile (including the litter layer), on a daily basis between irrigation events, and repeatedly throughout the year, to accurately estimate annual denitrification rates in the RLTS.

Denitrification rates were measured in the RLTS over a period of 12 months. The spatial variability of *in situ* denitrification rates was investigated by using a nested field design that divided the RLTS into four stages (irrigation block, topographic position, field site and sample point). *In situ* denitrification rates were measured between irrigation events, to establish how daily denitrification rates varied after wastewater irrigation, and on 21 different occasions during the year, to establish how daily denitrification rates varied seasonally. Annual denitrification rates of 2.4 and 1.7 kg N ha^{-1} yr^{-1} were recorded for the wastewater-irrigated and unirrigated soils, respectively. Daily denitrification rates were spatially and temporally variable, with coefficients of variation greater than 100%. Differences in denitrification rates between irrigation blocks contributed significantly more to spatial variability than differences between or

within topographic positions. Denitrification rates varied seasonally, with greatest losses occurring in the late summer and autumn. Daily denitrification rates also varied from day-to-day after irrigation. However, the day-to-day pattern of denitrification after irrigation changed throughout the year. Over 12 months, temporal effects contributed more than spatial effects to the overall variation in denitrification rates.

Soil moisture content, nitrate concentration, respiration, DEA and temperature were measured during the 12 month field trial to determine their effects on *in situ* denitrification rates. Using multiple regression analysis, soil and environmental properties could only explain up to 29% of the variation in *in situ* denitrification rates.

Laboratory studies showed that denitrification rates were very small when soil moisture contents were less than 80%. During the field trial, water-filled porosity was low, and in 84% of the samples collected (n = 4527), soil moisture contents were less than the critical threshold value required for denitrification. Therefore, it was proposed that *in situ* denitrification rates were small in the RLTS because soil moisture contents were low and generally less than the critical moisture content required for denitrification.

The size of the denitrifying population was also found to be small in the RLTS. Under optimum laboratory conditions, potential denitrification rates at 25 °C were 13.4 kg N ha^{-1} yr^{-1} in the wastewater irrigated soils. However, potential denitrification rates would be expected to be less at average field temperatures (11 °C). Laboratory studies, using disturbed soil samples, suggested that the size of the denitrifying population in the wastewater irrigated soils was limited by soil aeration. When oxygen availability in irrigated soils was limited, the size of the denitrifying population increased to a greater extent than measured in the field. However, adding carbon and nitrate to anaerobic soils did not further increase the denitrifying population in comparison to controls in the irrigated soils. Wastewater-irrigation changed the factors limiting denitrifiers in the RLTS. In the irrigated soils, denitrification is limited by soil aeration, while in the unirrigated soils denitrifiers were limited by both soil aeration and nitrate. Furthermore, wastewater irrigation altered the short-term response of denitrifiers to anaerobiosis. Under low oxygen conditions, in the laboratory, denitrifiers in the wastewater-irrigated soils produced enzymes earlier, and at a greater rate, than soil that had no history of wastewater irrigation.

It was concluded that wastewater needs to contain sufficient nitrogen to increase soil nitrate concentrations and should be applied to soils which are less free-draining soils than the soils used in this study, if upland denitrification is to contribute significantly to nitrogen removal in a forested land treatment system. A conceptual model is proposed to assist in establishing the likelihood of upland denitrification significantly contributing to nitrogen removal in a forested land treatment system.

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TABLE OF ABBREVIATIONS

CI	confidence interval
COD	chemical oxygen demand
CV	coefficient of variation
DEA	denitrifying enzyme activity
h	hour
ln	log normal
MC	moisture content
min	minute
MPN	most probable number
N	nitrogen
n	number of observations
PVC	polyvinyl chloride
R	rainfall
Resp	soil respiration
RLTS	Rotorua Land Treatment System
SE	standard error
Temp	soil temperature
WFP	water-filled porosity

CHAPTER 1

GENERAL INTRODUCTION

CHAPTER 1

GENERAL INTRODUCTION

1.1 INTRODUCTION

The disposal of wastewater is a concern in many countries, including New Zealand. Often municipal wastewater is treated, and then directly discharged into local surface waters. In some cases this practice may be unacceptable as nitrate contained in the waste may lead to surface water pollution, algal blooms and water unfit for human consumption. In New Zealand, Section 70 of the Resource Management Act (1991) states:

" any discharge of a contaminant or water; or discharge of a contaminant onto or into land under circumstances which may result in the contamination entering water must not: i) produce conspicuous oil or grease, scums or foams, or floatable or suspended materials; ii) produce conspicuous change in colour or visual clarity; iii) produce any emission of objectionable odour; iv) render fresh water unsuitable for the consumption by farm animals; or v) produce significant adverse effects on aquatic life."

Consequently, land application of wastewater has become an increasingly popular alternative to directly discharging municipal wastewater into local surface waters which may be sensitive to nutrient additions. Furthermore, in New Zealand, land treatment of wastes conforms with the environmental ethics of the indigenous Maori people.

Located in the Rotorua Lakes region, New Zealand, is one of the world's largest land-based systems for treatment of municipal wastewater (Tomer *et al.* 1997). The Rotorua Land Treatment System (RLTS) has been in operation since 1991, and serves a population of 60, 000. Wastewater is sprinkler-applied through an irrigation scheme that covers 242 ha of nearby Whakarewarewa Forest, which is currently managed as a radiata pine plantation. The upland soils are well drained and were formed from ash and tephra deposited from intermittent volcanic eruptions during the last 20, 000 years. The upland soils are, in turn, dissected by small streams that are often associated with

wetlands. The combination of upland and wetland soils in the RLTS matches the conceptual land treatment system model depicted in Figure 1.1.

Land treatment has been defined as *"the controlled application of wastewater onto the land surface to achieve a designed degree of treatment through natural, physical, chemical, and biological processes in the plant-soil-water matrix"* (USEPA 1981). A large proportion of the nitrogen added by the wastewater is thought to either be utilised by the cover crop, or removed by soil microbial processes (Figure 1.1). In land treatment systems, an important soil microbial process that is often assumed to occur is upland soil denitrification. Denitrification provides a sink for nitrate, converting aqueous nitrate to gaseous dinitrogen, and thereby removing it as a potential water pollutant. However, the annual contribution of upland denitrification to nitrogen removal in land treatment systems is generally unknown.

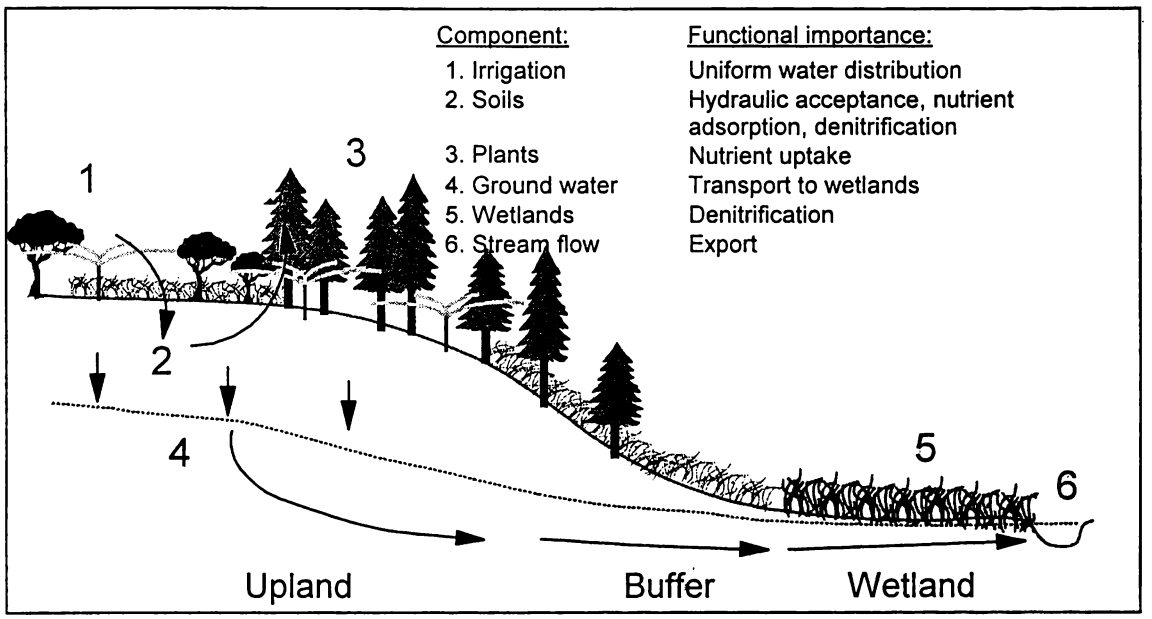

Figure 1.1 Schematic cross-section of a hillslope and a wetland in a land treatment system, showing major components and their functions within a land treatment system. (Reproduced from Tomer *et al.* 1997, with permission.)

1.2 DENITRIFICATION IN SOILS

1.2.1 The denitrification process

Denitrification is the reduction of nitrogen oxides to dinitrogen gases by soil microorganisms (Firestone 1982):

NO_3^- (aq)	→	NO_2^- (aq)	→	NO (gas)	→	N_2O (gas)	→	N_2 (gas)
Nitrate		*Nitrite*		*Nitric oxide*		*Nitrous Oxide*		*Dinitrogen*

The occurrence of NO as a free obligate intermediate and a precursor of N_2O has been widely debated, however recently studies have concluded that NO is produced in soils by denitrification (Skiba *et al.* 1993; Ye *et al.* 1994).

The previous definition of denitrification includes both biological and chemical denitrification. Chemodenitrification is the production of nitrogen oxides and dinitrogen gases via reactions catalysed by abiological agents (Chalk and Smith 1983). Biological denitrification includes respiratory and non-respiratory denitrification. For the purposes of this thesis, 'denitrification' refers to the biological denitrification only. Non-respiratory denitrification is the production of N_2O by various organisms including algae, bacteria, fungi and yeasts (Tiedje 1988).

In soils, respiratory denitrification is considered the most significant process by which nitrate is reduced, and is often simply referred to as denitrification (Tiedje 1988). Respiratory denitrification results from heterotrophic bacteria using NO_3^-, NO_2^- or N_2O as a terminal electron acceptor, while simultaneously oxidising organic compounds to gain energy (Tiedje 1988). Denitrifying bacteria are generally facultative and can use either nitrate or oxygen as the electron acceptor during respiration. Under aerobic conditions, oxygen is the preferred terminal electron acceptor as it yields more energy for the bacteria than nitrogen oxides (Brock *et al.* 1984). Consequently, as oxygen supply becomes limiting, denitrifying bacteria use nitrogen oxides as the electron acceptor if it is available.

Denitrification is considered to be the last step in the nitrogen cycle because it returns fixed N_2 to the atmosphere. However, not all denitrifiers can complete the entire reduction of nitrate to dinitrogen gas; the enzymes mostly commonly missing are the

nitrate reductase or nitrous oxide reductase (Robertson and Kuenen 1991). In addition, environmental conditions (e.g. soil pH) may also restrict the reduction of nitrate by denitrifiers, resulting in the accumulation of nitrate, nitrite or nitrous oxide end products (Firestone 1982). If nitrous oxide is the end product of denitrification, the emissions can contribute to ozone depletion and global warming (Crutzen and Ehhalt 1977; Wang *et al.* 1976).

1.2.2 Factors regulating denitrification

Conditions required for respiratory denitrification include the presence of denitrifying microbes, absence of oxygen, sufficient nitrate (or other nitrogen oxides), and organic carbon. Denitrifiers are widely distributed throughout a variety of soil environments and, consequently, denitrification is generally not limited by the absence of denitrifiers (Tiedje 1988). Instead, soil oxygen, nitrate and carbon are the main regulators of denitrification and directly affect the activity of denitrifiers (i.e. 'proximal factors', Groffman *et al.* 1987). In the field, proximal factors are, in turn, affected by various physical and biological factors (i.e 'distal factors', Groffman *et al.* 1987; Figure 1.2).

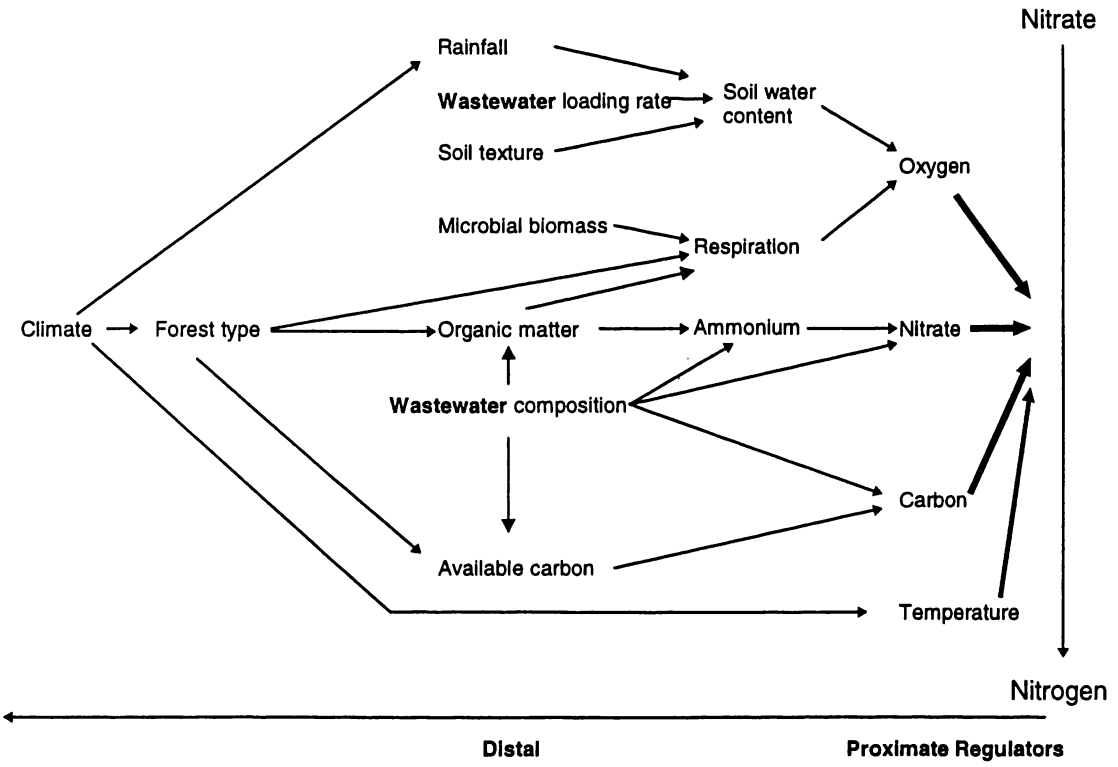

Figure 1.2 A simplified model of factors affecting denitrification in a land treatment system (Adapted from Tiedje 1988). Note how wastewater application influences the three proximate factors.

In forested land treatment systems, oxygen supply will depend upon soil water contents, and the rate of oxygen consumption by plant roots and soils microbes (respiration). Soil moisture contents will depend upon the frequency and loading-rate of wastewater, annual rainfall, and soil texture. Soil nitrate content and soil carbon will be depend upon the composition and application rate of the wastewater, and will also vary with tree species (Davidson *et al.* 1990).

1.2.3 Denitrification in upland soils

In upland soils, *in situ* denitrification rates have been mainly measured in forest and agricultural soils (Chapter 2). The contribution of upland denitrification to nitrogen removal varies with land use. Annual denitrification losses have ranged from less than 0.1 to 40 kg N ha^{-1} yr^{-1} in forest soils (Davidson *et al.* 1990), and from 0 to 204 kg N ha^{-1} yr^{-1} in agricultural soils (Colbourn and Dowell 1984). Greater denitrification losses have generally occurred on finer textured than coarser textured soil types. Altering soil conditions which cause the availability of oxygen to decrease (e.g. application of water or increased respiration rates)(e.g. Rolsten *et al.* 1982; Sexstone *et al.* 1985), or the availability of nitrate (e.g. Ryden 1983) or carbon (Rolsten *et al.* 1982) to increase, has generally increased denitrification rates when other factors have not be limiting. In northern temperate forests, soil moisture content, soil nitrate and soil temperature are the most important factors limiting soil denitrification (Davidson *et al.* 1990), whereas in northern temperate grassland soils, moisture contents and temperature generally limit denitrification (Ryden 1985).

In situ denitrification rates are spatially and temporally variable with coefficients of variation often greater than 100% factors (e.g. Burton and Beauchamp 1985; Parkin 1987). Denitrification rates are spatially variable due to the non-homogenous distribution of carbon and nitrate and other regulating factors (Parkin 1987; Groffman and Tiedje 1989). Temporally, denitrification rates also vary seasonally and in response to rainfall, irrigation and the application of N fertilisers (e.g. Jarvis *et al.* 1991; de Klein and van Logtestijn 1994). Due to the high spatial and temporal variability associated with denitrification, intensive sampling schemes are required to measure annual denitrification rates. The development of soil models that can predict denitrification is considered to be one way to avoid measuring *in situ* denitrification rates. For example,

in upland soil, *in situ* denitrification rates have been related to soil moisture contents, nitrate and available carbon with varying success (Robertson and Klemedtsson 1996).

In forested land treatment systems, *in situ* denitrification in upland soils have generally not been measured. Consequently the annual contribution of denitrification to nitrogen removal, the spatial and temporal variability of *in situ* denitrification rates, and the relationship between *in situ* denitrification rates and factors that regulate denitrification has not been extensively investigated in forested land treatment systems.

1.3 OBJECTIVES OF THIS STUDY

The contribution of upland denitrification to nitrogen removal and the effects of wastewater application on the soil denitrifying population is poorly understood in forested land treatment systems, including the RLTS. The overall aim of this study, therefore, was to characterise rates and patterns of denitrification, and to identify factors regulating denitrification in the upland soils of the RLTS.

Specifically, the objectives of this study were to:

1. Develop a sampling design for determining *in situ* denitrification rates in a forested land treatment system;

2. Determine the annual rate of denitrification, and investigate the spatial and temporal variability of denitrification occurring in the upland soils, of the RLTS;

3. Relate soil and environmental factors in the RLTS to *in situ* denitrification rates; and

4. Investigate the effects of wastewater irrigation on the denitrifying population in the upland soils of the RLTS.

1.4 THESIS OUTLINE

This thesis contains six chapters. Following the Introduction, Chapter Two reviews literature relevant to upland denitrification. The review discusses approaches to measuring *in situ* denitrification rates, reports annual denitrification rates, and identifies factors shown to limit denitrification rates in various upland soils. In addition, areas in which our understanding of upland denitrification is lacking are identified in Chapter Two. Chapters Three to Five contain the main research results of this thesis. Chapter Six syntheses findings presented in the previous chapters in order to make general conclusions regarding upland denitrification in wastewater-irrigated forest soils, and discusses the implications of this study to land treatment design.

The thesis is structured so that each chapter is 'stand alone'. Consequently, in some chapters minor repetition exists between sections (e.g. parts of the introduction, and soil and site details). In addition, each chapter contains a separate reference list and, therefore, there is not an inclusive reference list at the end of the thesis.

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CHAPTER 2

A REVIEW OF ANNUAL DENITRIFICATION RATES IN UPLAND SOILS

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2.1 ABSTRACT

This review considers the role of denitrification in the removal of nitrate from upland soils. Current knowledge of upland denitrification comes mainly from studies in temperate forest and grassland soils. For studies which measured *in situ* denitrification, annual denitrification rates were found to range from less than 0.1 to 28 kg N ha^{-1} yr^{-1} in undisturbed forest sites, and from 0 to 204 kg N ha^{-1} yr^{-1} in agricultural soils. The largest denitrification rate reported was for an irrigated, nitrogen fertilised loam soil, cropped with vegetables. In forest soils, soil aeration and nitrate availability often restricted denitrification because of the accumulation of organic matter and low availability of nitrate. In comparison, in fertilised grassland soil, soil aeration mainly limited denitrification rates. In forest soils, site factors (e.g. leaf-drop, clear-felling, fertiliser applications) which increased the availability of nitrate increased annual denitrification rates. In addition, applying nitrogen and irrigation increased *in situ* denitrification rates in both agricultural soils, and in a single study of a wastewater-irrigated forest soil. Annual denitrification rates and the factors controlling denitrification need to be examined across a greater variety of land uses and climatic regions, to further increase our understanding of upland denitrification.

2.2 INTRODUCTION

Denitrification is the reduction of nitrogen oxides to dinitrogen gas by microorganisms. It is a respiration process by which denitrifying microbes couple nitrogen oxide reduction with oxidation of organic matter to produce energy by phosphorylation (Firestone 1982). Denitrifying microbes can use either nitrate or oxygen as the electron acceptor, but only reduce nitrate when oxygen is unavailable (Roberston and Kluenen 1991). Conditions required for respiratory denitrification therefore include, the presence of denitrifying microbes, absence of oxygen, sufficient nitrate (or other nitrogen oxides),

organic carbon (or another electron donor), and other environmental conditions suited to soil microbes, such as soil temperatures greater than 4 °C (Focht 1974).

Soil denitrification can be viewed as either a beneficial or detrimental process. In land-based wastewater treatment systems, removal of excess nitrate from the soil is considered a beneficial process that can aid in the protection of ground and surface waters (Kim and Burger 1997). In agricultural and forest soils, however, loss of soil nitrate may be costly and detrimental to crop production. Furthermore, if nitrous oxide is the end-product of denitrification, the emissions can contribute to ozone depletion and global warming (Crutzen and Ehhalt 1977; Wang *et al.* 1976). Quantifying the annual contribution of denitrification to nitrate removal is, therefore, of interest in a variety of ecosystems.

Knowledge of *in situ* denitrification rates was hindered by the lack of suitable methodology (Tiedje *et al.* 1989). However, with the advent of acetylene methods and recommended protocols for measuring denitrification rates (Tiedje *et al.* 1989), the number of annual rates reported for upland soils have increased since rates were last reviewed for agricultural (Colbourn and Dowell 1984) and forest soils (Davidson *et al.* 1990). The purpose of this chapter is to review and evaluate our understanding of annual upland denitrification rates. In particular, the review focuses on a comparison between denitrification in forest and agricultural soils, as this is where most upland denitrification rates have been measured. Only annual denitrification rates that have been calculated using *in situ* measurements are included in this chapter, and no attempt has been made to extrapolate rates from papers in which authors have not presented an annual rate. The first section of the review briefly discusses the methodology for measuring annual denitrification rates in the field. Following a discussion of annual rates in forest and agricultural ecosystems, field regulators of denitrification are examined. The chapter concludes by indicating future research directions that can improve our understanding of upland denitrification and its contribution to nitrate removal in upland soil.

2.3 DETERMINING ANNUAL DENITRIFICATION RATES

In situ denitrification rates generally exhibit high spatial and temporal variability. Consequently, the sampling approach and method for measuring denitrification must be carefully considered when quantifying annual denitrification rates. The following section discusses spatial and temporal variability associated with *in situ* denitrification rates, and the implications it has for choosing sampling approaches and methods for measuring *in situ* denitrification rates.

2.3.1 Spatial and temporal variation of *in situ* denitrification rates

In situ denitrification rates are subject to considerable spatial and temporal variability. In agricultural and forest soils, coefficients of variation are often greater than 100% (Burton and Beauchamp 1985; Parkin 1987; Groffman and Tiedje 1989a). Spatial variability results from 'hot spots' of denitrifier activity (Parkin 1987). 'Hot spots' are caused by non-homogenous distribution of available carbon (Parkin 1987) and other factors that regulate denitrification, such as nitrate and soil aeration (Groffman and Tiedje 1989b), in the soil. Overcoming spatial variability requires taking a large number of soil cores or using soil covers which integrate over a large surface area. From a spatial variability study, Parkin *et al.* (1987) concluded that 10 to 15 kg of soil (using > 4.2 cm diameter cores) was required to obtain a reasonable estimate of *in situ* denitrification rates. Alternatively, Tiedje *et al.* (1989) suggested that at least 20 soil cores per sample date be taken when measuring *in situ* denitrification rates using soil cores. However, the recommendations of Tiedje *et al.* (1989) appears to rarely have been applied in upland denitrification studies (Tables 2.1 and 2.2).

Denitrification rates vary temporally with both seasonal and daily changes in soil temperature and the distribution of regulating factors (Myrold 1988; Groffman and Tiedje 1989b). The largest daily denitrification rates generally occur at those times of the year when soils are warm, moist, and soil nitrate and carbon are available. The season in which denitrification rates are largest, however, can vary with climate and land use. For example, denitrification rates have peaked in spring and autumn in northern temperate forests (e.g. Davidson and Swank 1987; Groffman and Tiedje 1989ab; Vermes and Myrold 1992) and northern temperate agricultural soils (Ryden

1983; Groffman *et al.* 1987; Myrold 1988; Parsons *et al.* 1991; Estavillo *et al.* 1994; de Klein and van Logtestijn 1994; Schnabel and Stout 1994), summer in fertilised soils in a semi-arid region in Canada (Corre *et al.* 1996), and winter in fertilised and unfertilised pasture soils in New Zealand (Luo *et al.* 1994; Ruz-Jerez 1994; Ledgard *et al.* 1996). As a consequence of the seasonal variability in daily denitrification rates, a large proportion of the annual denitrification loss may occur over a small period of the year. For example, in a northern temperate forest, over 80% of the annual denitrification rate occurred during a three to six week period during spring and late autumn (Groffman and Tiedje 1989a).

In addition to seasonal fluctuations, daily denitrification rates may also vary due to irrigation (Rolsten *et al.* 1982), rainfall (Jarvis *et al.* 1991) and nitrogen fertiliser application (Hulm and Killham 1988). For example, denitrification rates often increase with irrigation or rainfall, with denitrification rates remaining elevated while soil moisture contents are high, and decreasing thereafter until another irrigation or precipitation event (Rolsten *et al.* 1982; Jarvis *et al.* 1991; de Klein and van Logtestijn 1994). The denitrification response to irrigation, or precipitation, varies with soil type and soil conditions. For example, poor responses to irrigation or precipitation events have been recorded when nitrate availability has been low (Rolsten *et al.* 1982; Ryden and Lund 1980), soil temperatures have been limiting (Nommik and Larsson 1989) or when studies have been conducted on free-draining sands where high moisture contents have been difficult to maintain for an extended period after irrigation (Sexstone *et al.* 1985; Bijay-Singh *et al.* 1989).

As a result of seasonal and short-term changes in daily denitrification rates, the pattern of denitrification over a year is often irregular. Therefore, when quantifying annual denitrification rates, measurements need to be taken throughout the year. Tiedje *et al.* (1989) recommended 12 to 20 sampling dates per year when determining annual denitrification rates using soil cores. In field studies determining annual denitrification rates, soil cores have been collected from between two and 200 days per year (Table 2.1 and 2.2).

2.3.2 Methods for measuring denitrification in upland soils

Denitrification rates are generally determined by measuring the amount of gaseous end-products. Direct measurement of N_2 is very difficult as the background atmosphere consists of approximately 80% nitrogen. Consequently, techniques have been developed to avoid the need to measure N_2 directly. Techniques for field measuring denitrification are reviewed elsewhere (e.g Hauck 1986; Smith 1987; Tiedje *et al.* 1989) and the following comments will focus on those methods that are useful when quantifying *in situ*, annual denitrification rates.

Soil chambers and soil core methods, both with acetylene inhibition to block the reduction of nitrous oxide to nitrogen gas (Yoshinari *et al.* 1977), are commonly used to measure *in situ* denitrification rates (Tables 2.1 and 2.2). Using soil chambers, measurements of nitrous oxide flux from the soil to the atmosphere are taken after placing a sealed cover on the soil surface. Acetylene is either injected into the head-space above the soil or added directly to the soil using probes. The rate at which nitrous oxide accumulates in the cover head-space is then calculated (Tiedje *et al.* 1989). Soil core measurements involve extracting an intact soil core from the field and placing it in a chamber to which acetylene is added (e.g. Ryden *et al.* 1987). The rate of nitrous oxide evolution is then measured for a fixed period of time. Cores can either be incubated in the field (e.g. de Klein and van Logtestijn 1994) or in the laboratory (e.g. Robertson *et al.* 1987) before a gas sample of the head-space is taken. Soils incubated in the laboratory generally have rates adjusted to account for differences in field and laboratory temperatures (e.g. Knowles 1981; Rolsten *et al.* 1984).

The static core method, with acetylene inhibition, appears to have been the most commonly used method for measuring *in situ* denitrification rates (Table 2.1 and 2.2). The technique enables more samples to be collected and over a shorter period of time than other methods; an important consideration given the large spatial and temporal variability associated with denitrification. Denitrification rates determined using soil cores have compared favourably with results obtained using chamber techniques (Ryden *et al.* 1987), and ^{15}N methods (Rolsten *et al.* 1982; Mosier *et al.* 1986; Malone *et al.* 1988) and over a range of denitrification rates, despite some concerns that the oxygen regime will change after the soil core is removed from the soil profile (de Klein and van Logtestijn 1994). Potentially, acetylene inhibition techniques can underestimate

denitrification rates if the acetylene does not thoroughly diffuse through the soil, or if the denitrifiers become tolerant to the acetylene (Tiedje *et al.* 1989). In addition, acetylene inhibition may cause denitrification rates to become overestimated if denitrifiers use the acetylene as a carbon substrate (Tiedje *et al.* 1989). In a limited number of soils, however, studies have shown that incubating soil cores for 24 h allows time for acetylene to diffuse through the soil core (Ineson *et al.* 1991), but not so much time that it becomes a carbon substrate source (Smith *et al.* 1978).

In nearly all of the studies reported, annual denitrification rates have been quantified using acetylene inhibition methods (Tables 2.1 and 2.2). However, there have been recent suggestions that nitric oxide, a precursor to nitrous oxide during denitrification, reacts with acetylene and oxygen (McKenny *et al.* 1996; Bollman *et al.* 1997; McKenney *et al.* 1997). If such a reaction was to occur while measuring denitrification, annual denitrification rates could be underestimated. However, the significance of the reaction between nitric oxide, acetylene and oxygen, needs investigating to assess its significance at field temperatures. To date, studies have only examined this interference at laboratory temperatures (e.g. 25 °C, Bollman *et al.* 1997; 20 °C, McKenney *et al.* 1997), which are greater than most field soil temperatures in which annual denitrification rates are measured. In some soils, acetylene has not affected nitric oxide production during the first 24 h of the incubation (the time commonly used to incubate soil cores when measuring *in situ* denitrification rates) (McKenney *et al.* 1996). At lower temperatures, therefore, acetylene may take longer to react with nitric oxide. While there is strong evidence for a reaction between nitric oxide and acetylene in disturbed aerated soil samples, denitrification rates determined using acetylene inhibition have compared favourably with ^{15}N techniques (Rolsten *et al.* 1982; Mosier *et al.* 1986; Malone *et al.* 1998).

2.4 ANNUAL DENITRIFICATION RATES IN UPLAND FOREST AND AGRICULTURAL SOILS

Annual denitrification rates that have been reported for upland forest and agricultural soils are shown in Tables 2.1 and 2.2, respectively. Where authors reported a range of annual rates from the same site, only the average is presented in Tables 2.1 and 2.2. The annual denitrification rates found were from 29 locations, in 13 countries and predominantly in temperate climates.

Annual denitrification rates have been reported for forest soils under coniferous, deciduous and tropical forests (Table 2.1). In addition, researchers have reported the effects of stand type, stand age, site preparation, fertilisation and wastewater-irrigation on annual denitrification rates in forest soils. In the following discussion, 'disturbed' forests refers to sites that were subject to clear felling, site preparation or fertilisation within 10 years of annual denitrification rates being measured. Davidson *et al.* (1990) suggested acetylene inhibition of soil cores is the most practical method for measuring *in situ* denitrification rates in forest soils, as it does not require transportation of large amounts of equipment into the forest. Consequently most measurements of *in situ* denitrification rates in forest soils have been made using acetylene inhibition of soil cores (Table 2.1).

Overall, annual denitrification rates in forest soils ranged from less than 0.1 to 40 kg N ha^{-1} yr^{-1} (Figure 2.1a), with the highest rate occurring in a clear-felled Sitka spruce stand (Dutch and Ineson. 1990). One-third of the denitrification rates reported for forest soils are close to, or below, the detection limit of the acetylene inhibition technique (i.e. < 0.1 kg ha^{-1} yr^{-1}). More than half the rates were less than 1 kg N ha^{-1} yr^{-1} and 80% of the rates were less than 10 kg N ha^{-1} yr^{-1} (Figure 2.1a). In undisturbed forests, denitrification rates are generally greater in deciduous than coniferous forest soils (Table 2.3), and have been attributed to greater nitrogen and carbon availability through more frequent litter inputs (Davidson *et al.* 1990). In addition, Davidson *et al.* (1990) also suggested denitrification rates are larger in deciduous forest soils than coniferous forest soils because deciduous forest soils are finer-textured, with higher soil pH.

Table 2.1. Estimates of annual denitrification rates in forest soils

Forest system and location	Rate (kg N ha^{-1} yr^{-1})	Study duration (months)	No. of observation days (replicates per day)	Method	Comments	Reference
Undisturbed conifer						
Spruce *Norway*	<0.1	13	26 (12)	C_2H_2 block, intact soil cores at field temp		Henrich and Haselwandter (1997)
White spruce *Alberta, Canada.*	0.11	3 June-August	4 (12)	C_2H_2 block, intact soil cores at 15°C		Blew and Parkinson (1993)
Mixed conifers *Oregon, Canada.*	<0.1	6 May-October	3 (15)	C_2H_2 block, intact soil cores at field temp		Vermes and Myrold (1992)
Sitka spruce *Scotland.*	2.4	24	year 1: 26 (6) year 2: 17 (6)	C_2H_2 block, intact soil cores at 15°C (corrected to field temp)		Dutch and Ineson (1990)
Douglas-fir *British Columbia.*	<0.1	12	24 (5-12)	C_2H_2 block, soils mixed, field temp		Cushon and Feller (1989)
Western hemlock, douglas-fir, mixed conifers, *Juniperus* spp. *Oregon, U.S.A.*	<0.1	12	12 (18)	C_2H_2 block, intact soil cores, field temp		Myrold *et al.* (1989)
Sitka spruce *Scotland.*	<0.1	14	7 (5)	C_2H_2 block, intact soil cores at field temp		Hulm and Killman (1988)
Loblolly pine *North Carolina, U.S.A.*	0.6	6 May, September	2 (20)	C_2H_2 block, intact soil cores at 20-22°C		Robertson *et al.* (1987)

Table 2.1. Estimates of annual denitrification rates in forest soils (continued)

Forest system and location	Rate (kg N ha^{-1} yr^{-1})	Study duration (months)	No. of observation days (replicates per day)	Method	Comments	Reference
Mixed Conifer *California. U.S.A.*	<0.1	10	not stated	C_2H_2 block, soil cores		Strauss and Firestone (1982)
Undisturbed deciduous						
Northern red oak, white oak, red maple *Appalachians, U.S.A.*	11	24	12 (3)	C_2H_2 block, chamber		Kim and Burger (1997)
Sugar maple red oak Sugar maple brasswood Silver and red maples *Michigan, U.S.A*	0.4 0.7 0.15	9 March-November	9 (1)	C_2H_2 block, soil mixed, Q_{10} correction		Merrill and Zak (1992)
Alder Oregon, U.S.A.	1.0	6 May-October	3 (15)	C_2H_2 block, intact soil cores at field temp	stand ages 31-78 years	Vermes and Myrold (1992)
Mixed hardwood *Michigan, U.S.A.*	sand: 0.6 loam: 17 clay loam: 28	7 April-October	12 (15)	C_2H_2 block, intact soil cores at 22°C (Q_{10} correction) or field temp	means averaged across drainage classes	Groffman and Tiedje (1989a)
Northern hardwood *New Hampshire, U.S.A.*	0.4	1	1 (12)	C_2H_2 block, soil mixed, field temp		Melillo *et al.* (1983)
Disturbed conifer						
Douglas-fir *Oregon, U.S.A.*	2.1	6 May-October	3 (15)	C_2H_2 block, intact soil cores at field temp	clear-felled, 2 year old stand;average of three sites	Vermes and Myrold (1992)

Table 2.1. Estimates of annual denitrification rates in forest soils (continued)

Forest system and location	Rate (kg N ha^{-1} yr^{-1})	Study duration (months)	No. of observation days (replicates per day)	Method	Comments	Reference
Sitka spruce *Scotland.*	site 1: 9.9 site 2: 40	12	26 (6)	C_2H_2 block, intact soil cores at 15°C (corrected to field temp)	clear-felled, 1 (site 1) and 2 (site 2) year old stands	Dutch and Ineson (1990)
Sitka spruce *Scotland.*	0.1	14	7 (5)	C_2H_2 block, intact soil cores at field temp	160 kg N ha^{-1} applied	Hulm and Killman (1988)
Mixed conifers *Sweden*	site 1: <0.1 site 2: <0.1	5	5 (2)	C_2H_2 block, intact soil cores at 15°C	site1 : 2800 kg dolomite ha^{-1} applied site 2: dolomite and 150 kg N ha^{-1} applied	Nohrstedt (1988)
Loblolly pine *North Carolina, U.S.A.*	4.5	6 May, September	2 (20)	C_2H_2 block, intact soil cores at 20-22°C	2 year old stands, previously felled using various techniques	Robertson *et al.* (1987)
Mixed conifer *California, U.S.A.*	<0.1	10	not stated	C_2H_2 block, soil cores	clearfelled	Strauss and Firestone (1982)
Disturbed deciduous						
Mixed conifer *Oregon, U.S.A.*	5.4	6 May-October	3 (15)	C_2H_2 block, intact soil cores at field temp	8 year old stand	Vermes and Myrold (1992)
Northern hardwood *New Hampshire, U.S.A.*	1.4	1	1 (12)	C_2H_2 block, soil mixed, field temp	clear-felled, 2 year old stand	Melillo *et al.* (1983)

Table 2.1. Estimates of annual denitrification rates in forest soils (continued)

Forest system and location	Rate (kg N ha^{-1} yr^{-1})	Study duration (months)	No. of observation days (replicates per day)	Method	Comments	Reference
Popular plantation *The Netherlands*	18	21 Spring	3 weeks (15)	C_2H_2 block, chamber	formerly a pastoral soil	van Veen *et al.*(1981)
Disturbed tropical forest						
Tropical forest *Costa Rica*	4.5	9	3 (18)	C_2H_2 block, soil cores	clearfelled, rate after first year	Matson *et al.*(1987)
Wastewater-irrigated						
Northern red oak, white oak , red maple *Appalachians, U.S.A.*	11.9	24 May-October	12 (3)	C_2H_2 block, chamber	secondary treated, 16.8 kg N ha^{-1} y^{-1}, 35 cm wastewater $year^{-1}$	Kim and Burger (1997)

Table 2.2. Estimates of annual denitrification rates in agricultural soils

Agricultural system, crop cover and location	Rate ($kg\ N\ ha^{-1}\ yr^{-1}$)	Soil texture	N applied ($kg\ N\ ha^{-1}$)	Study duration (months)	No. of observation days(replicates per day)	Method	Reference
Unfertilised, not irrigated							
Pasture *New Zealand*	5	silt loam	0	36	30 (6)	C_2H_2 block, soil cores, field temp	Ledgard *et al.* (1997)
Tallgrass prairie *Kansas, U.S.A.*	unburned: 6.7 burned: 2.0 burned/grazed1.5	silty-clay	0	6	4	C_2H_2 block, soil cores, laboratory temp	Groffman and Turner (1995)
Grass *Spain*	7.9	clay loam	0	23	28 yr1: Mar-Sept yr2: Mar-April (36)	C_2H_2 block, soil cores, field temp	Estavillo *et al.* (1994)
Pasture *New Zealand*	4.5 (average of 5 sites)	silt loam	0	12	16 (16)	C_2H_2 block, soil cores, field temp	Luo *et al.* (1994)
Ryegrass *Pennsylvania, U.S.A*	17.4 15.1	fine loam coarse loam	0 0	13	23 (23)	C_2H_2 block, soil cores, field temp	Schnabel and Stout (1994)
Ryegrass *Germany*	0.3	sandy loam	0	24	22 (4)	C_2H_2 block, soil cover	Schwarz *et al.* (1994)
Grass-clover Herbal-ley *New Zealand*	3.4 4.4	fine sandy loam	0	21	30 (14)	C_2H_2 block, soil cores, field temp	Ruz-Jerez *et al.* (1994)

Table 2.2. Estimates of annual denitrification rates in agricultural soils (continued)

Agricultural system, crop cover and location	Rate (kg N ha^{-1} yr^{-1})	Soil texture	N applied (kg N ha^{-1})	Study duration (months)	No. of observation days(replicates per day)	Method	Reference
Lucerne *Sweden*	17	loam	0	12	68 (at least 16)	C_2H_2 block, soil cores, 15°C incubation adjusted to field temp	Svensson *et al.* (1991)
Ryegrass (cut) *Ireland*	1.5 0	clay loam clay	0	12	aprox 103 (10)	C_2H_2 block, soil cores, field temp	Jordan (1989)
Ryegrass *England*	1.6	loam	0	14	242	C_2H_2 block, soil cover	Ryden (1983)
Fertilized, not irrigated							
Pasture *New Zealand*	17 25	silt loam	225 360	36	30 (6)	C_2H_2 block, soil cores, field temp	Ledgard *et al.* (1997)
Rye-grass sward *The Netherlands*	5.2 6.9 24.6 19.6 11.4 5.7	sand sand loam loam peat peat	250 400 250 400 75/110 165/220	19 19 19 19 19 19	12 (4) 12 (4) 12 (4) 12 (4) 12 (4) 12 (4)	C_2H_2 block, soil cores, field temp	de Klein and van Logtestijn(1994)
Grass *Spain*	21 29 9.2 15	clay loam	132 265 132 (slurry) 265 (slurry)	23	58 (36)	C_2H_2 block, soil cores, field temp	Estavillo *et al.* (1994)

Table 2.2. Estimates of annual denitrification rates in agricultural soils (continued)

Agricultural system, crop cover and location	Rate ($kg\ N\ ha^{-1}\ yr^{-1}$)	Soil texture	N applied ($kg\ N\ ha^{-1}$)	Study duration (months)	No. of observation days(replicates per day)	Method	Reference
Ryegrass	50.2	fine loam	84	13	23	C_2H_2 block, soil cores, field temp	Schnabel and Stout (1994)
Pennsylvania, U.S.A.	63.9	fine loam	168				
	109.6	fine loam	252				
	27.8	coarse loam	84				
	29	coarse loam	168				
	59.2	coarse loam	252				
Ryegrass	2.2	sandy loam	450	24	22 (4)	C_2H_2 block, soil cover	Schwarz *et al.* (1994)
Germany	0.65		450 (slurry)				
Ryegrass	19.3	fine sandy loam	400	21	30 (14)	C_2H_2 block, soil cores, field temp	Ruz-Jerez *et al.* (1994)
New Zealand							
Barley	4.5	loam	120	24	118	C_2H_2 block, soil cores, 15°C incubation adjusted to field temp	Svensson *et al.* (1991)
Grass ley	9	loam	200		118 (at least 16)		
Sweden							

Table 2.2. Estimates of annual denitrification rates in agricultural soils (continued)

Agricultural system, crop cover and location	Rate (kg N ha^{-1} yr^{-1})	Soil texture	N applied (kg N ha^{-1})	Study duration (months)	No. of observation days(replicates per day)	Method	Reference
Grass sward	18	fine loam/silt	100	21	28 (6-8)	C_2H_2 block, soil cores, field temp	Barraclough *et al.* (1992)
England.	38	fine loam/silt	250				
	62	fine loam/silt	450				
	85	fine loam/silt	750				
	10	loam	100				
	10	loam	250				
	21	loam	450				
	22	loam	750				
	0.5	clay	100				
	2.5	clay	250				
	2	clay	350				
	6	clay	450				
	10	clay	750				
Ryegrass (cut)	7.3	clay loam	100 (CAN[A])	12	aprox 103 (10)	C_2H_2 block, soil cores, field temp	Jordan (1989)
Ireland	23	clay loam	200 (CAN)				
	29	clay loam	300 (CAN)				
	10	clay loam	300 (urea)				
	8.9	clay loam	300(GRANUMS[B])				
	79	clay	300 (CAN)				
	31	clay	300 (urea)				
Winter wheat	1.7	silt loam	180	24	25 (10)	C_2H_2 block, soil cores, field temp	Myrold (1988)
Ryegrass	0.7	silt loam	107				
Oregon, U.S.A							
Wheat	2	clay loam	50	24	not stated	C_2H_2 block, soil cores	Aulakh *et al.* (1983)
Canada							

Table 2.2. Estimates of annual denitrification rates in agricultural soils (continued)

Agricultural system, crop cover and location	Rate (kg N ha^{-1} yr^{-1})	Soil texture	N applied (kg N ha^{-1})	Study duration (months)	No. of observation days(replicates per day)	Method	Reference
Ryegrass	11.1	loam	250	14	242	C_2H_2 block,	Ryden (1983)
England.	29.1		500		yr1: Mar-Sept, yr2: Mar-April	cover	
Fertilised, irrigated							
lettuce/celery/broccoli	156	sandy loam/loam	475	7	50	C_2H_2 block, soil	Ryden and Lund (1980)
cauliflower	204	loam/silt loam	290			cover	
artichoke	114	sandy loam/loam	665				
California, U.S.A.							

[A] CAN, calcium ammonium nitrate

[B] GRANUMS, 30% ammonium nitrate, 30% urea, 30% dolomite

Table 2.3. Summary of annual denitrification rates in forest and agricultural soils.

System	No. of Observations	Geometric mean (kg N ha^{-1} yr^{-1})	Range (kg N ha^{-1} yr^{-1})
Forest	**31**	**1.9**	**<0.1-40**
Undisturbed coniferous	9	0.22	<0.1-2.4
Undisturbed deciduous	8	3.0	0.4-28
Disturbed coniferous	9	2.8	<0.1-40
Disturbed deciduous	2	2.9	1.4-5.4
Agricultural	**66**	**11**	**0-204**
Unfertilised, not irrigated	14	3.2	0-17.4
Fertilised, not irrigated	49	13.4	0.5-110
Fertilised, irrigated	3	153	114-204

Annual denitrification rates tend to be greater in disturbed forest sites, such as sites that have recently been clear-felled or have had nitrogen applied, than undisturbed forest areas (Table 2.3). Studies comparing undisturbed and disturbed coniferous forests have shown up to a ten-fold increase in denitrification in disturbed sites, which have been attributed to increased nitrate availability (Hulm and Killham 1988; Myrold *et al.* 1989; Dutch and Ineson 1990). However, increased denitrification rates after clear-felling and fertilisation may only occur for a relatively short period of time. For example, in Sitka spruce stands in Scotland, denitrification returned to pre-felling rates after four years (Dutch and Ineson 1990) and to pre-fertilisation rates within four months (Hulm and Killham 1988).

In agricultural soils, annual denitrification rates have been measured in grassland and leguminous pasture soils, and in soils cropped with wheat and vegetables (Table 2.2). Most reported studies, however, have been for grassland soils in northern hemisphere regions. Investigations have included the effect of grazing, nitrogen fertilisers, slurry application and irrigation on annual denitrification rates in agricultural soils. In agricultural soils, *in situ* denitrification rates have either been measured using soil cores or covers, both with acetylene block. Jarvis *et al.* (1991) suggested that the soil core method is the most appropriate technique for measuring *in situ* denitrification rates in grazed, grassland soils as the uneven distribution of urine and sheep faeces causes considerable spatial variability.

Annual denitrification rates for agricultural soils range from 0 to 204 kg N ha^{-1} yr^{-1} (Figure 2.1b). The highest denitrification loss reported was from an irrigated cauliflower crop receiving 290 kg N ha^{-1} yr^{-1} and 140 kg H_20 ha^{-1} yr^{-1} (Ryden and Lund 1980). Only 10% of annual denitrification rates for agricultural soils are less than 1 kg N ha^{-1} yr^{-1} (in comparison to 50% for forest soil), while 80% are less than 50 kg N ha^{-1} yr^{-1} (Figure 2.1b) Applying inorganic or organic nitrogen fertiliser generally increases annual denitrification rates in fertilised agricultural soils in comparison to unfertilised soils (Table 2.3). Inorganic nitrogen fertilisers, however, increase annual denitrification rates more than organic nitrogen fertilisers, due to greater nitrate availability (Estavillo *et al.* 1994; Schwarz *et al.* 1994). In a limited number of studies, irrigating fertilised soil has further increased annual denitrification rates. (Ryden and Lund 1980; Table 2.3).

A comparison between annual denitrification rates for forest and agricultural soils shows higher rates can be expected for agricultural soils than forest soils (Figure 2.2). From the studies compiled, average annual denitrification rates for forestry and agricultural soils are 1.9 kg N ha^{-1} yr^{-1} and 11 kg N ha^{-1} yr^{-1}, respectively (Table 2.3). The distribution of annual denitrification rates is also more negatively skewed for agricultural than forest soils, with agricultural soils recording a greater range in annual denitrification rates than forestry soils (Figure 2.1). Greater denitrification rates in agricultural than forest soils have generally been attributed to higher soil nitrate concentrations (Davidson *et al.* 1990).

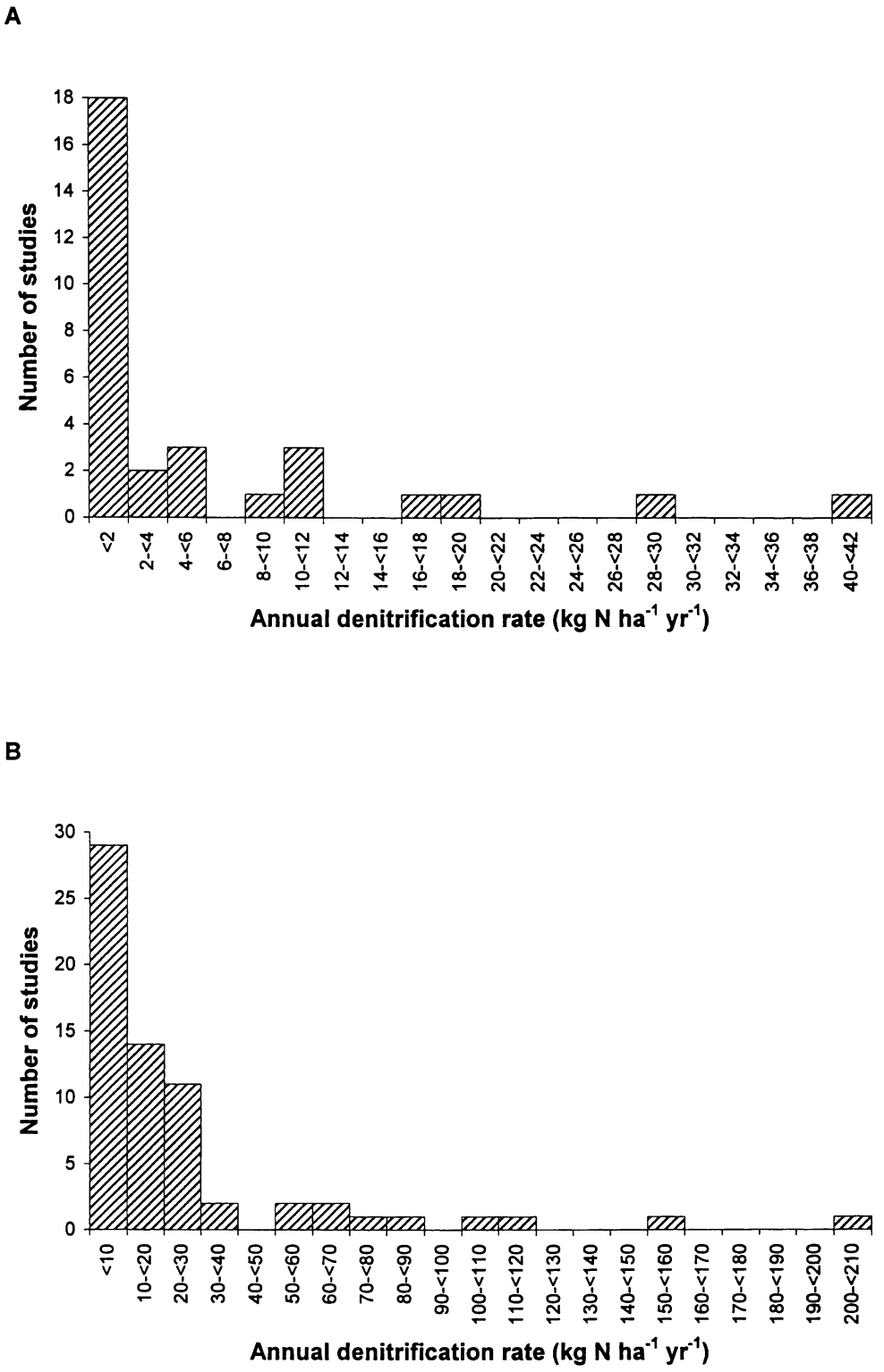

Figure 2.1. The distribution of annual denitrification rates in forest (A) and agricultural (B) soils. Summarised from Table 2.1 and 2.2.

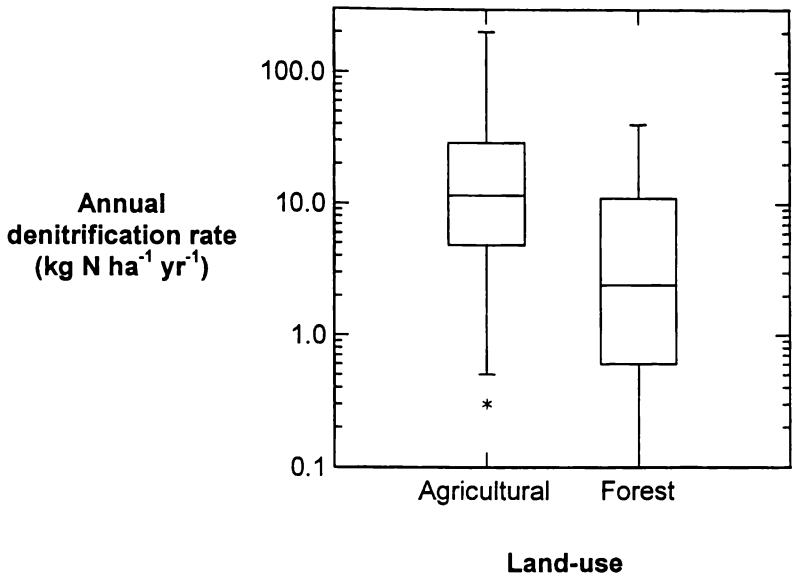

Figure 2.2. Box-plots comparing annual denitrification rates in agricultural and forest soils. The centre vertical line marks the median, the edges of the box (hinges) mark first and third quartiles, and the central 50% of annual rates are within the range of the box. Vertical lines show the range values that fall within 1.5(midrange) of the hinges. Asterisks are values outside inner fences (1.5 (| median-hinge |)).

2.5 REGULATORS OF DENITRIFICATION IN UPLAND SOILS

Denitrifiers are widely distributed throughout a variety of soil environments and, consequently, denitrification is generally not limited by the absence of denitrifiers (Tiedje 1988). Instead, soil oxygen, nitrate and carbon are the main regulators of denitrification and directly affect the activity of denitrifiers ('proximal factors', Groffman *et al.* 1987). In the field, proximal factors are, in turn, affected by various physical and biological factors ('distal factors', Groffman *et al.* 1987; Figure 1.2), making regulation of *in situ* denitrification very complex.

Groffman *et al.* (1987) suggested that as the scale of the study increases from the cellular- to the global-scale, it is necessary to concentrate more on distal factors regulating denitrification, than proximal factors. Hence the importance of the distal

factors is dependent on the scope or scale of the study. To date, studies of denitrification have generally been confined to the organism-, field-, and to a lesser extent the landscape-scale. Factors that regulate denitrification at the cellular-scale have been reviewed extensively elsewhere (e.g. Groffman *et al.* 1987; Tiedje 1988; Davidson *et al.* 1990) and the reader is referred to those papers for further discussion. At the field-scale, oxygen availability, nitrate and carbon availability are thought to be the main factors which control denitrification (Groffman *et al.* 1987).

2.5.1 Soil water and oxygen

Soil oxygen is the factor which most commonly limits denitrification in upland agricultural (e.g. Smith and Tiedje 1979; Mosier *et al.* 1986) and forest soils (e.g. Robertson and Tiedje 1984; Groffman and Tiedje 1989b; Henrich and Haselwandter 1997). In the field, restricting soil aeration by increasing the incidence of high soil moisture contents (Ryden and Lund 1980) generally increases annual denitrification rates. Threshold values of soil water content (Table 2.4) above which denitrification rates have increased in the field have been reported for various soils. The threshold soil water content, expressed as water-filled porosity (WFP), differs according to soil type, but is generally greater for coarse-textured than fine-textured soils (Table 2.4). For example, threshold soil water contents range from 82% to 83% WFP in sandy and sandy loam soils, from 62 to 83% WFP in loam soils, and from 50 to 74% WFP in clay loam soils (Table 2.4). Groffman and Tiedje (1991) attributed greater threshold WFP with soil coarseness to the effect of soil texture on oxygen availability. Finer-textured soils have smaller pores that lead to greater water retention and a greater opportunity for creating anaerobic microsites than coarser-textured soils. Consequently, in finer-textured soils, anaerobic microsites are more likely to be present at lower WFP than in coarser soils. De Klein and van Logtestijn (1996) proposed that in many cases, the critical WFP for many soils is equivalent to field capacity or above.

In unirrigated soil, the incidence of high soil moisture contents is often, but not always, related to rainfall. If nitrate and carbon are not limiting, the effect of rainfall on denitrification rates will depend on soil texture and drainage. Higher denitrification rates occur for longer periods in poorly drained or finely textured agricultural and forest soils than free draining coarsely textured soils (Sexstone *et al.* 1985; Bijay-Singh *et al.* 1989; Groffman and Tiedje 1989a; de Klein and van Logtestijn 1994). In a study of

nine northern hardwood forests, annual denitrification rates ranged from less than 1 kg N ha^{-1} yr^{-1} in a well-drained sandy soil to 40 kg N ha^{-1} yr^{-1} in a poorly drained clay loam (Groffman and Tiedje 1989a). Groffman and Tiedje (1989b) suggested that soil drainage and soil texture were the most important factors affecting annual denitrification rates in the hardwood forests.

Table 2.4. Threshold values of water-filled porosity above which *in situ* denitrification rates increase when using intact soil cores or soil cover methods.

Soil texture	Water-filled porosity (%)	Reference
sand	>82	de Klein and van Logtestijn 1996
sandy loam	linear	Sexstone *et al.* 1988
fine sandy loam	>83	Ruz-Jerez *et al.* 1994
loam	>70	Bergstrom and Beauchamp 1993
loam	>83	de Klein and van Logtestijn 1996
loam	>62	Grundman and Rolsten 1987
loam	>57	Johnsson *et al.* 1991
loam	100% WHC[A]	Nommik and Larsson 1989
loam	>62	Ryden 1983
loam	>80	Ryden and Lund 1980
clay loam	>50	Nelson and Terry 1996
clay loam	>74	Estavillo *et al.* 1994
clay loam	>70	Jordan 1989
clay loam	>60	Sexstone *et al.* 1988
silty clay	>70	Jordan 1989
clay	100% WHC	Nommik and Larsson 1989
peat	>71	de Klein and van Logtestijn 1996

[A] water holding capacity

Soil oxygen availability primarily limits denitrification rates in nitrogen fertilised grassland soils, where nitrate and carbon availability are considered adequate for denitrification (Groffman *et al.* 1987; Jarvis *et al.* 1991). Consequently, the effects of soil texture on annual denitrification rates were investigated by plotting the annual denitrification rates in fertilised grassland soils by soil texture (Figure 2.3). Overall, annual denitrification losses were greater in loam soils than sandy or clay textured soils. Annual rates greater than 10 kg N ha^{-1} yr^{-1} in either sandy or clay textured soils were not recorded, despite application of up to 750 kg N ha^{-1} yr^{-1}. In comparison, annual denitrification rates as high as 110 kg N ha^{-1} yr^{-1} were reported for fertilised loam soils. Denitrification may be greater in fertilised loam soils as these heavier textured soils become anaerobic easily and do not restrict the diffusion of carbon substrates to

denitrifying microsites. In soils other than loams, soil texture may limit denitrification because the soil is well aerated (sand) (Groffman and Tiedje 1991) or carbon diffusion is limited (clay) (Myrold and Tiedje 1985a).

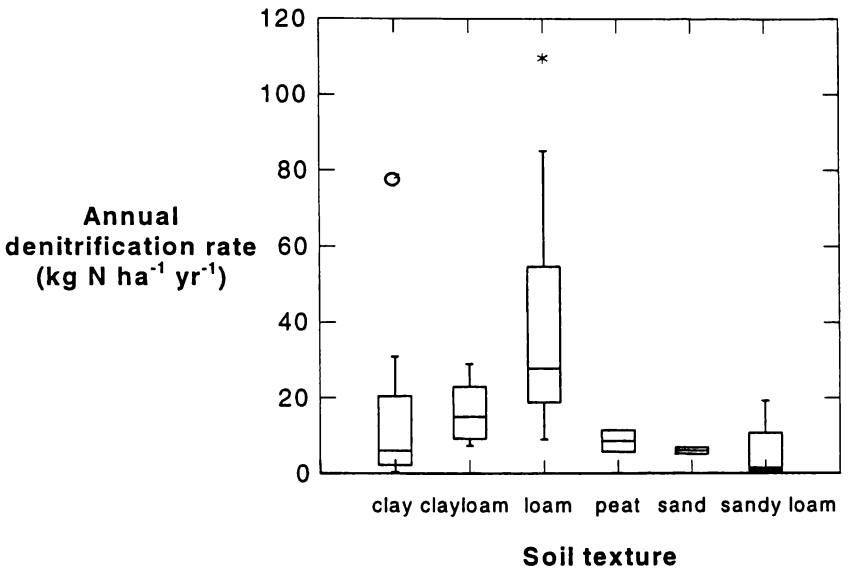

Figure 2.3. The distribution of denitrification rates according to soil texture in fertilised grassland soils, as summarised from Table 2.2. The centre vertical line marks the median, the edges of the box (hinges) mark first and third quartiles, and the central 50% of annual rates are within the range of the box. Vertical lines show the range values that fall within 1.5(midrange) of the hinges. Asterisks are values outside inner fences (1.5 (| median-hinge |)), and outliers are marked with an empty circle.

2.5.2 Soil nitrate

Soil nitrate concentrations that limit denitrification activity vary amongst studies, but concentrations generally range from less than 5 to 10 mg NO_3-N kg dry $soil^{-1}$ (Table 2.5). Soil nitrate availability, in addition to soil aeration, will generally limit denitrification in unfertilised grassland soils (Groffman *et al.* 1993; Tenuta and Beauchamp 1995) and unfertilised forest soils, including both coniferous and deciduous forest soils (Roberston and Tiedje 1984; Groffman and Tiedje 1989a; Merrill and Zak

1992; Vermes and Myrold 1992; Henrich and Haselwander 1997). Soil nitrate availability may also limit some fertilised grassland soils when carbon availability is especially high (Jordan 1989; Colbourn 1993).

Table 2.5. Threshold values of soil nitrate above which *in situ* denitrification rates increase when using intact soil cores or soil cover methods.

Soil texture	Soil nitrate ($mg\ NO_3\text{-}N\ kg\ soil^{-1}$)	Reference
loam	>5	Ryden 1983
loam	>10-15	Ryden and Lund 1980
clay loam	>1-2	Estavillo *et al.* 1994
clay loam	>2	Jordan 1989
silty clay	>2	Jordan 1989

2.5.3 Soil carbon

Unlike soil water content and soil nitrate, the amount of carbon required for denitrification has not been regularly cited in the literature. Reasons for this probably include the difficulties associated with assessing soil carbon availability, plus the variety of techniques used to measure carbon availability in the field. Soil carbon, in addition to soil aeration, has been found to limit denitrification in nitrogen-fertilised agricultural soils (Bergstrom and Beauchamp, 1993; Weier *et al.* 1993; Myrold, 1988; Parsons *et al.* 1991; Myrold and Tiedje 1985b), nitrogen-fertilised forest soils (Hulm and Killham 1988), and leguminous pasture soils (Limmer and Steele 1982). In an irrigated, fertilised agricultural soil, Rolston *et al.* (1982) reported that denitrification rates were three times greater at a site amended with 10 t crop residue ha^{-1} than an unamended site. In agricultural soils of different porosity, Bijay-Singh *et al.* (1989) related denitrification rates to carbon availability when soil water content did not differ between soil types. In only a few reported examples has carbon availability also limited denitrification in forest soil. For example, low carbon availability has restricted denitrification activity in the sub-surface soils of a coniferous forest (Henrich and Haselwandter 1997), and at certain times of the year (e.g. immediately before leaf fall) in a deciduous forest (Groffman and Tiedje 1989b).

The factors limiting denitrification can change as a result of management practices. In a tropical forest soil, normally limited by nitrate availability, clear-felling increased nitrate availability such that carbon availability limited denitrification (Matson *et al.* 1987). In

a grassland soil, applying herbicide increased nitrate availability to an extent that denitrification became limited by carbon availability (Tenuta and Beauchamp 1995).

2.6 PREDICTING UPLAND DENITRIFICATION RATES FROM SOIL PROPERTIES

At the field-scale, denitrification rates have been related to regulating factors with varying success. Soil moisture content (e.g. Myrold 1988; Sexstone *et al.* 1985; Bergstrom and Beauchamp 1993; Robertson and Klemdtsson 1996), soil nitrate (e.g. Robertson and Tiedje 1984; Davidson and Swank 1986; Myrold 1988) and available carbon (e.g. Myrold 1988; Bergstrom and Beauchamp 1993; Robertson and Klemdtsson 1996) have all been related to denitrification, however none of these factors have singly been able explain more than 55% of the variation in denitrification rates.

Multiple regression analysis has successfully related denitrification rates to soil and environmental factors in some field studies (Table 2.6). However, in many field-scale studies, multiple regression has not explained more than 50% of the variation in denitrification rates. In some studies, increasing the scale of the study either spatially or temporally has improved the relationship between denitrification rates and soil and environmental factors. (Groffman and Tiedje 1989b; Ambus and Christensen 1993; Schipper *et al.* 1993). In a landscape-scale study, multiple regression analysis explained 80% of the variability in annual denitrification rates using soil drainage class and percentage sand (Groffman and Tiedje 1989b). In another landscape-scale study, Corre *et al.* (1996) related denitrification rates to soil type and land use.

Measuring denitrification rates and developing models based on soil properties requires intensive sampling strategies over an extended period of time. Groffman and Tiedje (1989b) first showed that measurements of denitrification enzyme activity (DEA) may provide a simple soil measure to estimate annual denitrification rates. Soil DEA, or phase 1 assay, measures the concentration of functional denitrifying enzymes in a sample of soil (Smith and Tiedje 1979). By optimising all the requirements for denitrification (i.e. non-limiting concentrations of nitrate, oxygen and carbon in an

acetylene atmosphere) the rate of nitrous oxide production is related to the denitrifying enzyme content of the soil sample.

Table 2.6. Variables included in multiple regression equations relating *in situ* denitrification rates to soil properties in different scaled studies

Variables	R^2	Reference
Field-scale		
Soil pH, soil temperature	0.41	Roberston and Klemedtsson 1996
NO_3^-, soil moisture content	0.4	Estavillo *et al.* 1994
Soil temperature, gravimetric water content, NO_3^-	0.19-0.33	Jarvis *et al.* 1994
WFP, NO_3^-, NH_4^+	0.41	de Klein and van Logtestijn 1994
WFP, NO_3^-, NH_4^+, rain	0.51	de Klein and van Logtestijn 1994
COD[B], NO3-, soil temperature	0.4	Ambus and Christensenn 1993
Respiration, WFP, NO_3^-, MPN[A], soil temperature	0.71-0.91	Parsons *et al.* 1991
Volumetric water content, NO_3^-, water soluble C	0.37	Grundmann *et al.* 1988
Soil moisture content, respiration, NO_3^-, soil temperature	0.43	Myrold 1988
NO_3^- production, CO_2 production, % water	0.53	Roberston and Tiedje 1984
Landscape-scale		
Drainage index, % sand	0.86	Groffman and Tiedje 1989b

[A] most probable number
[B] chemical oxygen demand

In field studies, soil DEA only accurately predicts annual rates when DEA is measured across a range of treatments which cause *in situ* denitrification rates to vary significantly. For example, DEA has successfully predicted annual denitrification rates in upland soils at the landscape level (r^2 = 0.79; Groffman and Tiedje 1989b) and in a field study where different rates of nitrogen were applied (r^2= 0.91; Bailey 1997). However, in none of these studies has the variation in the relationship between DEA and annual rates from year-to-year been examined. Soil DEA has not been strongly related to denitrification rates in field-scale studies where soil DEA has not varied, or when DEA has been compared with hourly denitrification rates (e.g. Parsons *et al.* 1991; Bergstrom and Beauchamp 1993). From the limited number of studies in which annual denitrification rates and soil DEA have been measured, DEA is not strongly related to annual denitrification rates when the data is pooled (r^2= 0.47; Figure 2.4). Instead, the relationship between DEA and annual rates appears to vary between individual studies.

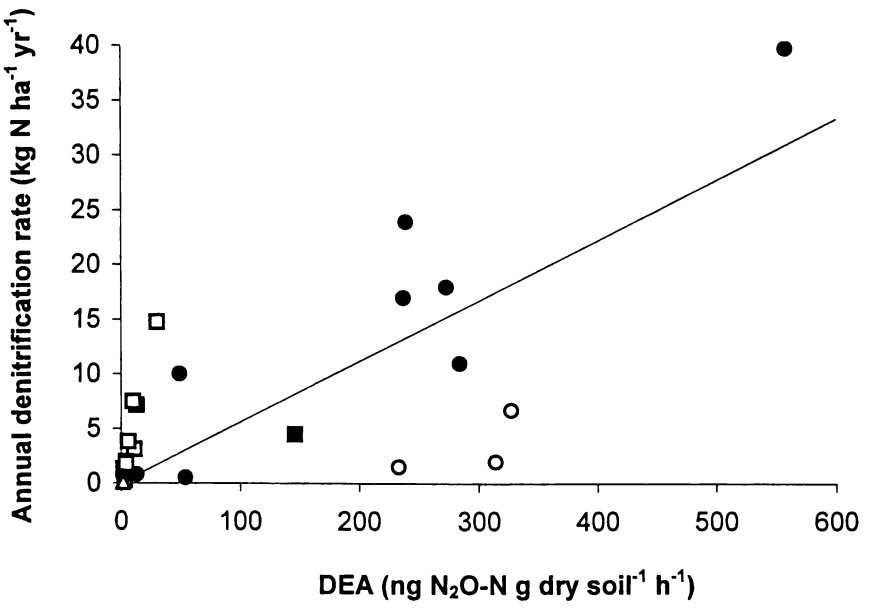

Figure 2.4. Annual denitrification rates versus DEA in five studies. Studies are Bailey (1997, □) , Luo *et al.* (1994; ■), Groffman *et al.* 1993; ○), Groffman and Tiedje (1989b; ●) and Strauss and Firestone (1982; △).

2.7 FUTURE RESEARCH DIRECTIONS

Our understanding of upland denitrification, and its contribution to nitrogen removal, is largely based on field-scale studies of unfertilised forest and fertilised grassland soils. These studies have shown upland denitrification rates vary from less than 0.1 to 110 kg N ha^{-1} yr^{-1}. Limited research, however, suggests annual denitrification rates may be as great as 204 kg N ha^{-1} yr^{-1} in irrigated, fertilised loam soils. Clearly, our understanding of contribution of denitrification to nitrate removal across a range of upland soil types remains limited. Our understanding of upland denitrification, and factors controlling denitrification, could be improved by studying denitrification losses from a wider variety of land uses and climatic regions. For example, other land uses could include intensive agricultural systems (e.g. vegetable crops), fertilised cereal crops, cereal crops grown in rotation with legumes, and agricultural soils used for the land treatment of wastes. Other climatic regions could include tropical, sub-tropical and southern

temperate climates where wet, warm soil conditions may enhance denitrification rates in fertilised soils.

Previous studies of denitrification in temperate forests have focused mainly on non-commercial, unfertilised forest soils. In temperate forest soils, nitrate often limits denitrification; consequently, in a limited number of studies, increasing nitrate availability (by adding nitrogen fertiliser or clear-felling) has increased annual denitrification rates in forest soils. The effect of applying nitrogen fertiliser to forest soils on denitrification rates needs further examination. In grassland soils, and a single study in an undisturbed forest soil, the distribution of denitrification rates suggests denitrification losses will be greater in finer-textured, than coarse-textured fertilised forest soils. Therefore, in fertilised forest soils, it would also be interesting to see how denitrification rates vary with soil texture.

The relationship between DEA and annual denitrification rates warrants continued attention. Currently, using *in situ* measurements to estimate annual denitrification rates is labour intensive and costly. Measuring DEA may provide a cost effective, and realistic alternative to estimating annual denitrification rates under particular circumstances. In ecosystems where DEA is found to be strongly related to annual denitrification rates, the yearly variation in the relationship needs to be assessed to determine if the same relationship can be used from one year to the next. Soil DEA will only be a useful predictive tool if the relationship does not vary yearly. Furthermore, information on how soil DEA varies seasonally and spatially is essential for establishing protocols for measuring annual DEA. To establish if there is a universal relationship between soil DEA and annual denitrification rates across a number of soil types, researchers will need to collaborate by: i) incorporating measurements of DEA in research programmes measuring *in situ* annual denitrification rates; ii) using a universally accepted DEA method (e.g. Tiedje *et al.* 1989); and iii) allowing data to be pooled.

2.8 CONCLUSIONS

The contribution of denitrification to nitrogen cycling varies greatly in upland soils. Reported annual rates have ranged from less than 0.1 to 204 kg N ha^{-1} yr^{-1}. Approximately 60% of these rates were less than 10 kg N ha^{-1} yr^{-1}, and 40% were less than 5 kg N ha^{-1} yr^{-1}. Upland denitrification removed more nitrogen, on a per hectare basis, from agricultural than unfertilised forest soils. Annual denitrification rates averaged 1.9 kg N ha^{-1} yr^{-1} in forest soils and 12 kg N ha^{-1} yr^{-1} in agricultural soils. Greater denitrification in agricultural soils than forest soils has been attributed to greater nitrogen inputs, and finer soil textures associated with grassland soils.

Factors limiting upland denitrification varied depending upon land use. In temperate forest soils, nitrate and soil aeration were factors reported to limit denitrification, whereas in fertilised grassland soil, denitrification has generally been limited by soil aeration. Consequently, in grassland soils, the distribution of denitrification rates with soil texture has shown denitrification to be greater in finer-textured, than coarse-textured soils. In only a limited number of studies have both the effects of forest soil texture, and the effect of applying nitrogen on denitrification rates been investigated.

Annual denitrification rates reported in this review suggest the greatest denitrification activity should occur in nitrogen fertilised soils, or in soil where site management increases nitrate availability. In soil where denitrification is not nitrate limited, largest denitrification rates would be expected to occur in loam textured soils. In addition, irrigating fertilised soils should further increase denitrification rates. Consequently, annual denitrification rates should be greater in intensive agriculture, such as horticulture, where considerable amounts of nitrogen and water are being applied, than conventional agricultural soils. Furthermore, in forested land treatment systems applying nitrate-containing wastewater would be expected to increase denitrification rates, especially in the soils that are not too coarse textured.

Current knowledge of upland denitrification and its contribution to nitrate removal in soils comes mainly from studies in temperate forest and agricultural soils. If we are to fully understand, and therefore manage nitrogen fluxes, annual denitrification rates and the factors controlling denitrification need to be investigated in a greater variety of land uses and climatic regions.

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CHAPTER 3

PROCEDURES FOR CHARACTERISING DENITRIFICATION RATES IN A WASTEWATER-IRRIGATED FOREST SOIL

To accurately estimate annual denitrification rates, the scientific literature indicates that it is necessary to account for the spatial and temporal variability often associated with *in situ* denitrification rates. To establish a suitable strategy for measuring *in situ* denitrification rates in a forested land treatment system, the following preliminary study investigated the importance of spatial and temporal variability in the Rotorua Land Treatment System.

CHAPTER 3

PROCEDURES FOR CHARACTERISING DENITRIFICATION RATES IN A WASTEWATER-IRRIGATED FOREST SOIL

3.1 ABSTRACT

In land-based wastewater treatment systems, soil denitrification can be an important process that decreases water pollution by biologically reducing nitrate to gaseous nitrogen end-products. To establish a suitable sampling strategy for measuring *in situ* denitrification rates, the importance of spatial and temporal variability of denitrification was investigated in forest soil regularly irrigated with wastewater. To determine appropriate sampling depths and locations in a catchment, denitrifying enzyme activity (DEA) was measured at five sampling depths (litter, 0-5, 5-10, 10-20 and 20-40 cm), and three topographic positions (ridge, midslope, and toeslope) in irrigated and unirrigated sites. To determine appropriate times for sampling after irrigation, *in situ* denitrification rates were measured at time intervals before and after irrigation for one week, using soil cores and acetylene inhibition.

DEA was found to be greatest in the litter layer and decreased with depth. In irrigated soils, DEA was greater than zero in the upper 20 cm of toeslopes, and the upper 10 cm of midslopes and ridge positions. *In situ* denitrification rates increased immediately after wastewater-irrigation, peaking at 24 h and then decreased to pre-irrigation rates after 3 days unless rain fell. It was concluded that soil cores need to be collected from at least the upper 10 cm soil (including the litter layer), and on a daily basis between irrigation events, to quantify denitrification losses from soil regularly irrigated with wastewater.

3.2 INTRODUCTION

Annual denitrification rates are often calculated by integrating fluxes of gaseous denitrification products with time. Quantifying annual denitrification losses, therefore, requires a sampling strategy which accounts for the large spatial and temporal variability

often associated with *in situ* denitrification rates. Large spatial variation is generally caused by 'hot spots' of denitrifier activity, resulting from non-homogenous soil distribution of available carbon (Parkin 1987) and other factors which regulate soil denitrification, such as nitrate and soil aeration (e.g. Groffman and Tiedje 1989b). Denitrification rates vary seasonally with changes in soil temperature and the distribution of soil moisture, carbon and nitrate (Myrold 1988; Groffman and Tiedje 1989b). Daily denitrification rates can also change in response to irrigation (e.g. Rolsten *et al.* 1982), rainfall (e.g. Jarvis *et al.* 1991) or the application of nitrogen fertiliser (e.g. Hulm and Killham 1988). To overcome difficulties associated with large amounts of spatial and temporal variability, intensive sampling at one point in time in combination with repeated sampling throughout the year has been recommended when determining annual rates of denitrification (Tiedje *et al.* 1989).

Annual denitrification rates have not been widely reported for land treatment systems and, consequently, recommendations for measuring annual denitrification rates in land treatment systems have not been published. In a land treatment system, daily denitrification rates would be expected to vary considerably both temporally (between seasons and also over shorter periods in response to wastewater irrigation) and spatially, due to inherent variability and to uneven distribution of wastewater (Tomer, pers. comm.). In unirrigated soils, the literature suggests soil cores with acetylene inhibition is an appropriate method for measuring denitrification when *in situ* rates are expected to be highly variable (Jarvis *et al.* 1991; de Klein and van Logtestijn 1994). The method compares favourably with other techniques (e.g. Ryden *et al.* 1987; Tiedje *et al.* 1989) and enables a greater number of samples to be analysed over a shorter period of time (Tiedje *et al.* 1989). However, it is not known to what soil depth cores should be taken and how often measurements should be made to quantify denitrification rates after wastewater irrigation.

The depth to which the soil cores are taken in unirrigated soils varies amongst workers, but is often restricted to the surface of the soil (Tiedje *et al.* 1989). Beyond this depth, soil organic matter and nitrifying bacteria (if nitrification is the source of soil nitrate) are thought to be insufficient to support denitrification activity (Weier *et al.* 1993; Sotomayor and Rice 1996). However in a forested land treatment system, application of water, nitrate and carbon may increase soil nitrate and organic carbon in sub-surface

soils. For example, in sub-surface grassland soils, greater potential denitrification rates have occurred in nitrate fertilised swards than unfertilised swards due to the movement of nitrate to lower parts of the profile (Jarvis and Hatch 1994). Regular application of nitrate and water to the soil may, therefore, increase denitrification activity in sub-surface soils of a land treatment system.

To characterise denitrification losses after an irrigation event, frequent measurements of denitrification are often made for the first few days after irrigation, and then less frequently until the next irrigation event (Ryden and Lund 1980; Rolsten *et al.* 1982). In the past, this approach has been taken because denitrification rates were generally greatest immediately after irrigation or rainfall, and then rapidly decreased as soil water redistributes and the soil becomes less anaerobic (Rolsten *et al.* 1982; Sexstone *et al.* 1985). Although studies have reported denitrification responses after irrigation, no study appears to have reported the rationale for the sampling periodicity when the aim is to quantify, rather than characterise, denitrification losses after irrigation.

The objective of this part of the study was to develop a sampling strategy for measuring annual denitrification rates in upland soils of the Rotorua Land Treatment System (RLTS). Specifically, the study aimed to determine: i) the soil depth required to measure denitrification rates; ii) the importance of sampling across topographic positions in permeable, well-aerated pumice soils; and iii) the amount of temporal variation that must be accounted for when sampling in a land treatment system. To achieve the first two aims, the size of the denitrifying population at different depths was assessed by measuring denitrification enzyme activity (DEA; Smith and Tiedje 1979). To quantify the denitrification response to wastewater-irrigation, *in situ* denitrification rates were measured before and after irrigation.

3.3 MATERIALS AND METHODS

3.3.1 Soil and site details

The RLTS is located in Whakarewarewa Forest, New Zealand (38°10'S, 176°16'E) and has been in operation since October 1991 (Tomer *et al.* 1997). The upland soils

(i.e. soils not influenced by groundwater levels) form part of a commercial *Pinus radiata* forest (242 ha) irrigated with tertiary treated wastewater for 12 h (5 mm h^{-1}), on a weekly basis, and contains an average nitrogen concentration of 11 mg N L^{-1} (Table 3.1). Soils are pumiceous sandy loams, which are classified as Vitric Orthic Allophanic Soils (New Zealand Soil Classification System; Hewitt 1993) (Plate 3.1). Selected soil properties are given in Table 3.2. In the study area, the topography was mainly moderately steep slopes (12-23°), and supported a *Pinus radiata* stand planted in 1970. Study sites were located in irrigated and unirrigated areas within the same forest stand and, during the study, wastewater irrigation was similar to the greater land treatment system.

Table 3.1. Selected properties and mean loadings of major constituents in tertiary-treated wastewater applied to the RLTS

Constituent	Concentration (mg L^{-1})	Loading (kg ha^{-1} yr^{-1})
Biological oxygen demand [A]	5.4 (1.2)	150
Chloride[A]	42 (4.3)	1140
Total nitrogen[A]	11 (2.4)	298
Organic nitrogen[A]	1.7 (0.8)	43
Ammonium[A]	3.9 (3.0)	105
Nitrate + nitrite[A]	5.6 (2.4)	150
Total phosphorous[A]	2.8 (1.8)	76
pH[B]	7.2 (0.2)	

[A]Values are means (and standard deviations) of monthly observations (Rotorua District Council, unpublished data).
[B]Values are means of 308 observations from August 1993 to November 1996

3.3.2 Variation in DEA with soil depth and topographic position

In September 1995, soils were collected for DEA analysis both immediately before and after irrigation at irrigated and unirrigated sites. At both sites, four transects were located 20 m apart, and at right angles to a drainage line. Two soil cores (40 cm in depth) were taken at toeslope, midslope and ridge positions along the transects. Cores were divided into five depths (litter, 0-5, 5-10, 10-20, 20-40 cm), placed in polyethylene bags and refrigerated (4 °C) on return to the laboratory. The DEA of all soils was determined within three days of soil collection.

Plate 3.1 Profile of a Vitric Orthic Allophanic Soil. (Photo J. Barran).

Table 3.2. Properties of soil from irrigated and unirrigated sites (0-15 cm)

	Nitrate (μg g dry soil^{-1})	Total N (%)	Total C (%)	Bulk density (g cm^{-3})	Total porosity (%)	pH[A]
Irrigated	5.7	0.31	5.5	0.51	76	6.8
Unirrigated	0.5	0.30	5.8	0.48	78	5.8

[A]1:2.5 soil:water ratio

To measure DEA (Smith and Tiedje 1979), 10 g of fresh soil was weighed into glass screw-top jars (120 mL) with lids fitted with rubber septums. Each jar was flushed with oxygen-free nitrogen gas, and 20 mL of 1 mM glucose and 1 mM potassium nitrate added, followed by acetylene gas (10 mL) to block conversion of N_2O to N_2 (Yoshinari *et al.* 1977). Each jar was then shaken at 25 °C and 5 mL of head-space was transferred to 3 mL evacuated Vacutainers after 15 and 75 minutes. The N_2O concentration of the gas samples was measured using a Hewlett Packard gas chromatograph, fitted with an electron capture detector (350 °C). Gases were separated using a porous packed column (HaySep Q) at 70 °C and at an injector port temperature of 140 °C. The carrier gas (argon with 10 % methane (v/v)) had a flow rate of 30 mL min^{-1} and was passed through a molecular sieve (5A 45/60 mesh).

3.3.3 Temporal response of denitrification to wastewater irrigation

In October 1995, *in situ* denitrification was measured in midslope positions, at various time intervals at irrigated and unirrigated sites. At each site, an experimental plot (30 m x 20 m) was surveyed and further divided into 10 subplots (6 x 10 m). Denitrification rates were measured 20 h prior to irrigation, and then 0, 5, 10, 24, 34, 48, 72 and 168 h following wastewater irrigation. At each time interval, 20 cores were collected (two randomly from each sub-plot). The experiment was conducted on two occasions, two weeks apart.

Denitrification rates were measured using a core incubation method (Ryden *et al.* 1987). Two intact soil cores (3.75 cm in diameter, 14 cm in length, in a PVC sleeve) were placed in a 1 L glass jar, sealed with an airtight screw-lid fitted with a septum, and acetylene (60 mL, 10% v/v) added. Another hypodermic needle inserted in the septum maintained atmospheric pressure during acetylene addition. To aid diffusion of the acetylene throughout the core, holes had been drilled into the PVC sleeve. The soil

cores were incubated in the jar, which was then placed in a covered hole adjacent to the field plot for 24 h. After 24 h, the head-space of the jars was sampled (12 mL) and stored in a 10 mL evacuated Vacutainer for N_2O analysis.

Cores were incubated in the field for 24 h to allow soils to be exposed to the diurnal nature of *in situ* soil temperatures. Incubating soils for less time, or at constant temperature, can overestimate rates (Jarvis *et al.* 1994). Incubating soil cores for 24 h also allows time for acetylene to diffuse through the soil core (Ineson *et al.* 1991), but not so much time that it becomes a substrate source (Smith *et al.* 1978).

Rainfall occurred during both weeks of experimentation. In the first study week, rain fell on day five after irrigation, and in the second week, rain fell throughout the irrigation period and intermittently for the following three days. The total hydraulic loading (rainfall + irrigation) in the second week (130 mm) was double that of the first week (70 mm).

3.3.4 Data analysis

Daily denitrification rates were calculated for each of the sampling dates, and were corrected for the N_2O dissolved in the soil solution using the Bunsen coefficient (Tiedje 1982). Cumulative denitrification losses for each study week were calculated by extrapolation and summation of daily denitrification rates. The distribution of the denitrification rates was negatively skewed and consequently transformed using natural log to normalise data prior to statistical analyses. Analysis of variance was performed using SAS (SAS Institute Inc. 1989) to determine the effects of time, soil depth and topographic position on DEA. Reported differences were significant at the 5% level, unless reported otherwise.

3.4 RESULTS

3.4.1 Variation in DEA with soil depth and topographic position

Soil DEA was not different between samples which were collected before irrigation and after irrigation. Subsequent data analysis, therefore, combined soil DEA measurements from both sample dates.

Soil DEA was greater in irrigated soils than unirrigated soils ($p < 0.001$). In the irrigated soil, DEA averaged 2.25 ng N_2O-N g dry soil^{-1} h^{-1} across all soil depths, whereas in the unirrigated soil DEA averaged 0.95 ng N_2O-N g dry soil^{-1} h^{-1}. On average, soil DEA was greatest in the litter and then decreased with depth in both irrigated and unirrigated soils (Table 3.3). However in the irrigated soil, litter DEA was at least 18 times greater than subsequent soil depths, whereas in the unirrigated soil, litter DEA was only three times greater than other soil depths. Consequently, DEA was greater in the upper 5 cm of the irrigated soil than the unirrigated soil, but beyond 5 cm there was no difference in DEA between irrigated and unirrigated soils (Table 3.3).

Table 3.3. Changes in DEA with soil depth in irrigated and unirrigated soil

Means (averaged across all topographic positions) and standard errors are given (n = 24). Means with different letters are significantly different ($p < 0.05$).

Depth (cm)	Irrigated soil (ng N_2O-N g fresh^{-1} soil h^{-1})	Unirrigated soil (ng N_2O-N g fresh^{-1} soil h^{-1})
Litter	18.7 (0.40)^{a}	4.13 (0.22)^{b}
0-5	3.09 (0.40)^{b}	1.33 (0.21)^{c}
5-10	1.06 (0.25)^{cd}	0.64 (0.21)^{cd}
10-20	0.51 (0.16)^{cd}	0.20 (0.06)^{d}
20-40	0.46 (0.14)^{cd}	0.19 (0.05)^{d}

Soil DEA was greater ($p<0.001$) in toeslope than midslope and ridge soils for both irrigated and unirrigated soils (Table 3.4). At each topographic position, soil DEA was greatest in the litter layer and decreased with depth (Figure 3.1). However, at each topographic position DEA was greater in the irrigated than the unirrigated soil. Wastewater irrigation increased DEA in the upper 20 cm of the toeslope soils, but only in the litter layers of the ridge and midslope soils. In irrigated soils, DEA was greater than zero in the upper 20 cm of toeslopes, and greater than zero in the upper 10 cm of midslopes and ridge positions. When results were expressed on a volumetric basis, similar findings were obtained (data not shown).

Table 3.4. Effect of topographic position on DEA in irrigated and unirrigated soils. Means (averaged across all soil depths) and standard errors are given (n = 40). Means with different letters are significantly different ($p < 0.05$).

Topographic position	Irrigated soil (ng N_2O-N g fresh^{-1} soil h^{-1})	Unirrigated soil (ng N_2O-N g fresh^{-1} soil h^{-1})
Ridge	1.09 (0.16)^{c}	0.56 (0.09)^{a}
Midslope	1.22 (0.22)^{c}	0.61 (0.10)^{a}
Toeslope	6.46 (0.36)^{d}	1.65 (0.22)^{b}

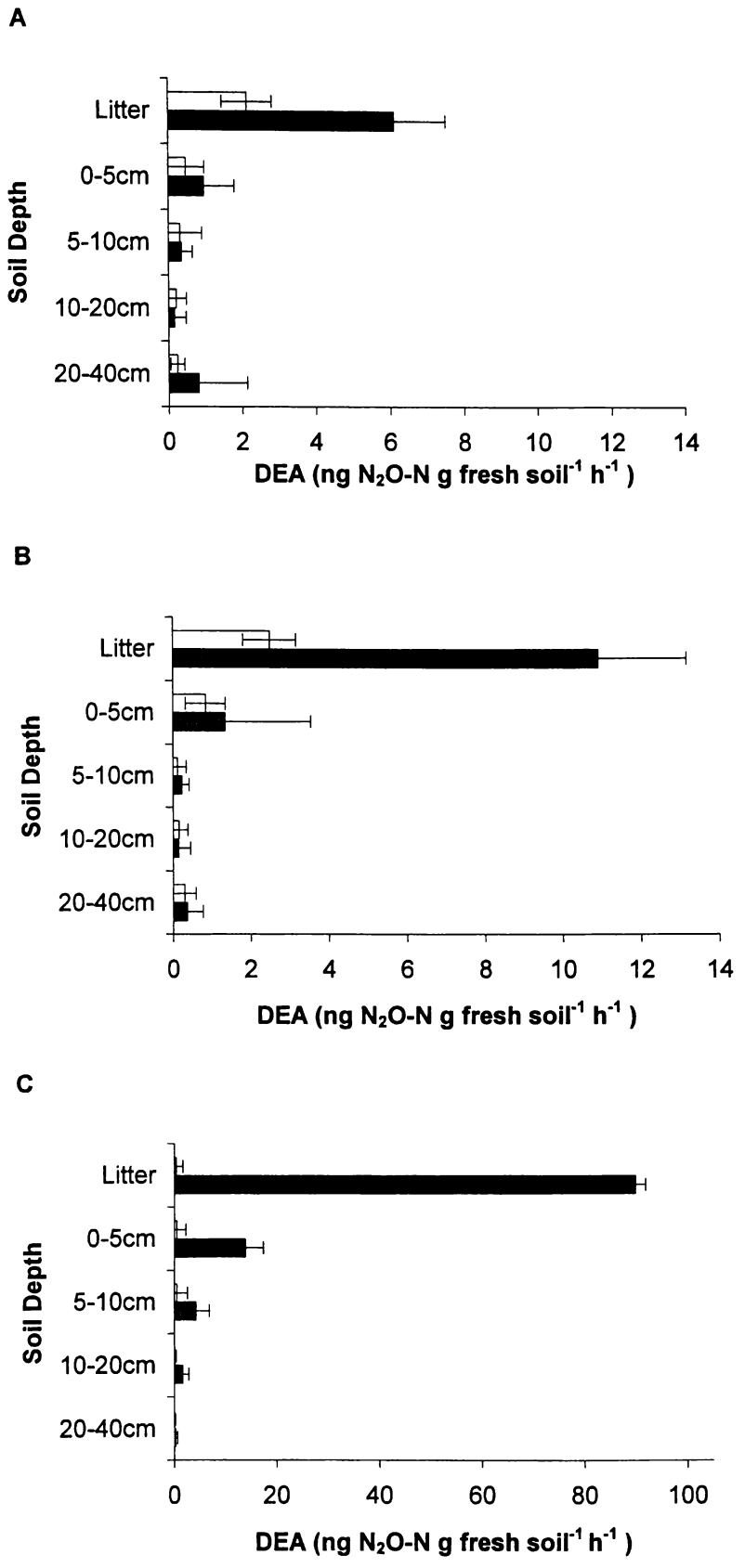

Figure 3.1. Variation in DEA (ng N_2O g fresh $soil^{-1}$ h^{-1}; mean $\pm$ CI, n = 8, $p < 0.05$) with soil depth at ridge (A), midslope (B) and toeslope (C) topographic positions in irrigated (■)and unirrigated sites (□). Note different x-axis scale.

3.4.2 Temporal response of soil denitrification to wastewater irrigation

In situ denitrification rates varied throughout the week at the irrigated and unirrigated sites (Figure 3.2). At the unirrigated site, denitrification rates remained relatively unchanged during the first study week, but responded to rainfall during the second week. In the second study week in the unirrigated site, daily rates approached those recorded at the irrigated site. At the irrigated site, denitrification decreased immediately after irrigation and then increased, peaking at 24 h in both weeks. However, the pattern of denitrification after irrigation differed between the two study weeks. In the first study week, denitrification rates increased 5 h after irrigation stopped, peaked at 24 h, and then decreased to denitrification rates recorded prior to irrigation by day three. In the second week, denitrification again peaked at 24 h after irrigation, but did not decrease to pre-irrigation rates.

Coefficients of variation (CV) were calculated for the denitrification rates at each of the time intervals (Table 3.5). Although CV varied from 1.5 to 98% in irrigated and unirrigated soils, in only seven of the 36 interval measurements were CVs greater than 50%. *In situ* denitrification rates were more variable in the irrigated than the unirrigated soils, and CVs were greater in the first sample week than the second sample week. Rainfall in the second week may have decreased variability by redistributing carbon and nitrate to denitrifying soil microsites. Denitrification rates may have been more variable in the irrigated than the unirrigated soils due to the uneven distribution of the wastewater (Tomer, pers comm.).

Table 3.5. Coefficients of variation (%) for denitrification rates measured at different time intervals before and after wastewater irrigation.

Time after irrigation (h)	Irrigated		Unirrigated	
	Week 1	Week 2	Week 1	Week 2
-20	66	140	13	1.5
0	41	14	3.9	56
5	52	5.8	60	7.3
10	42	34	14	17
24	34	4.2	35	30
34	44	3.2	11	1.0
48	52	8.4	76	3.4
72	98	7.2	11	7.9
168	52	21	58	7.2

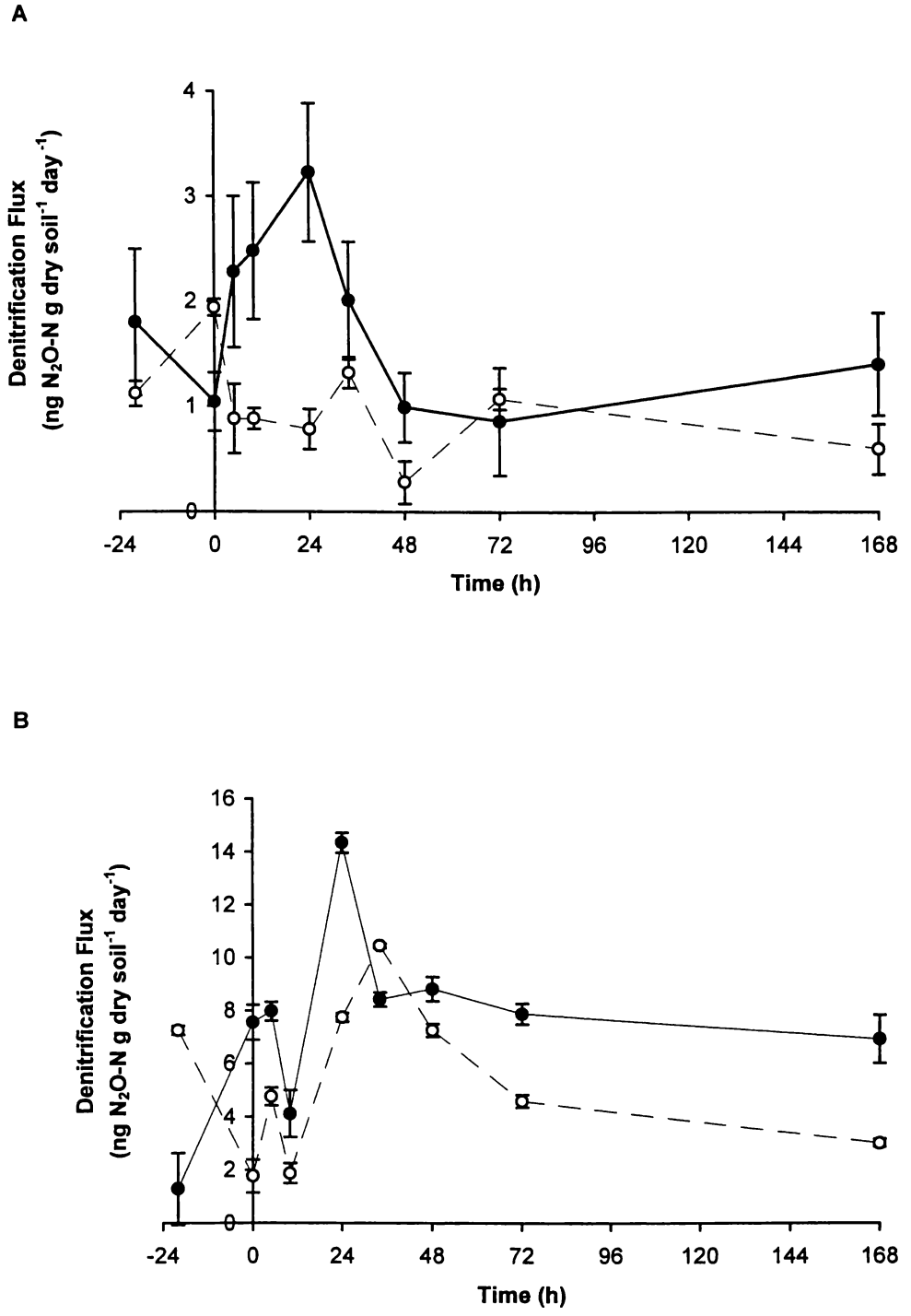

Figure 3.2. Variation of mean daily denitrification rates (bars represent 95% CI, n=10) with time (h) during week one(A) and two (B) (● irrigated site,o unirrigated site).

Weekly denitrification rates were generally greater in the irrigated than the unirrigated plots (Table 3.6). Denitrification in the second week was approximately five and eight times higher than the first week in the unirrigated and irrigated sites, respectively, and presumably due to rainfall. The proportion of weekly denitrification that occurred within the first 24 h after irrigation, varied from 15 to 25%, and was less in the second study week than the first week (Table 3.6). Decreasing the number of time intervals, to determine cumulative denitrification after irrigation, from eight to five gave a similar weekly denitrification rate as when all eight intervals were used (Table 3.7). However, decreasing the number of intervals from eight to three underestimated denitrification losses in the irrigated site.

Table 3.6. Weekly denitrification rates and the proportion of the weekly rate after one, two, three and seven days in irrigated and unirrigated sites.

		Total denitrification (g N_2O-N ha^{-1} $week^{-1}$)	Proportion of total denitrification (%)			
			1 d	2 d	3 d	7 d
Unirrigated	week 1	6.0	16	32	43	100
	week 2	36	12	38	56	100
Irrigated	week 1	10	25	45	54	100
	week 2	59	15	32	47	100

Table 3.7. Weekly denitrification rates (g N_2O-N ha^{-1} $week^{-1}$) estimated using different numbers of measurements.

Numbers in brackets are proportion (%) of weekly denitrification rate estimated from all measurements.

		n = 8[A]	n = 5[B]	n = 3[C]
Unirrigated	Week 1	6.0	6 (100)	8.5 (142)
	Week 2	36	35 (97)	38 (106)
Irrigated	Week 1	10	10 (100)	8.4 (84)
	Week 2	59	64 (108)	58 (99)

[A]0, 5, 10, 24, 34, 48, 72 and 168 h after irrigation.
[B]0, 24, 48, 72 and 168 h after irrigation.
[C]0, 48 and 168 h after irrigation.

3.5 DISCUSSION

Estimates of denitrification rates are often made by integrating the flux of denitrification gaseous end-products with time (Rolsten *et al.* 1982). Therefore, to obtain a good estimate of denitrification after an irrigation event, the sampling strategy must characterise the variation in denitrification rates after irrigation, and sample to a soil depth which includes most of the denitrifying population. In the RLTS, soil DEA was not significantly different from zero beyond the surface 20 cm of the soil. Daily denitrification rates varied from day-to-day within each sample week, and quantifying denitrification losses after an irrigation event required measurements to be taken each day. Furthermore, the pattern of denitrification after irrigation varied between each sample week, indicating that the denitrification response to irrigation needs to be determined more than once. For a forested land treatment system which is irrigated weekly, it is recommended that weekly denitrification losses be estimated using soil cores sampled at least three times, throughout the week; and repeated during the year if the annual denitrification rate is to be determined accurately. However, more accurate measurements may be obtained by using daily measurements, especially if unpredictable weather patterns occur.

Four years of wastewater irrigation has not affected the distribution of denitrifying population through the soil profile, and most denitrification activity remained near the surface in the irrigated site. Soil DEA increased below the upper 10 cm only in the toeslope positions of the irrigated site. In past studies, small denitrification potentials at depths greater than 10 to 20 cm have been attributed to insufficient soil organic matter at depth (e.g. Weier *et al.* 1993). Greater carbon availability at depth, or sustained moisture contents after irrigation may have enabled the denitrifying population to increase to depths greater than 10 cm in the toeslopes in the RLTS, as plant debris and water often accumulated in the toeslope positions. Slightly different sampling depths may be required for different landscape positions, although this study indicated the uppermost 10 cm was generally most important in a forested land treatment system.

Daily and weekly denitrification rates were influenced by irrigation and rainfall. Overall, denitrification increased immediately in response to irrigation, peaking 24 h after irrigation ceased and then either decreased to pre-irrigation rates or remained

elevated in comparison to pre-irrigation denitrification. This pattern was similar to previous observed responses to irrigation (Ryden and Lund 1980; Rolsten *et al.* 1982). Lag periods prior to increased denitrification rates have been observed in some studies using static gas incubation (Ryden and Lund 1980; Rice and Smith 1982) and attributed to large soil moisture contents limiting the diffusion of N_2O from the profile as a result of saturated conditions at the surface. Extended periods of enhanced denitrification rates following an irrigation event have usually been reported to occur in finer textured soils or, as was the case in week two of this study, when rainfall occurred (Sexstone *et al.* 1985; Bijay-Singh *et al.* 1989). Overall, rainfall increased weekly denitrification losses approximately six-fold at both the irrigated and unirrigated sites, and decreased the proportion of weekly denitrification which occurred in the first day after irrigation. Consequently, characterising the denitrification response to irrigation for only a few days after irrigation does not adequately explain total denitrification losses between irrigation events in land treatment systems, especially if rainfall occurs. Instead, sampling daily between irrigation events is recommended.

In the RLTS, collecting 10 cores per time interval in the irrigated site resulted in coefficients of variation less than values generally associated with *in situ* measures of denitrification (i.e. < 100 %) (Burton and Beauchamp 1985; Parkin *et al.* 1987; Groffman and Tiedje 1989a). Using ten cores at a sampling density of 1 core per 60 m^2 also enabled differences between daily denitrification rates in the irrigated and unirrigated site to be detected, as well as differences in daily denitrification rates within the same sites. In the RLTS, therefore, increasing the number of sample days, rather than the number of soil cores collected each day, is recommended.

In conclusion, if the aim of a field study is to quantify annual or seasonal denitrification losses from a forested land treatment system, the sampling approach needs to measure denitrification rates with an intensity and frequency which adequately quantifies denitrification losses between irrigation events, and at a sampling depth which includes a significant portion of the denitrifying population. To estimate annual denitrification rates in an irrigated forested land treatment system, it is suggested that it is necessary to collect cores from at least the uppermost 10 cm soil (including the litter layer), on a frequent basis (at least three times per week, but preferably daily) between irrigation events, and repeatedly throughout the year.

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CHAPTER 4

DENITRIFICATION RATES IN A WASTEWATER-IRRIGATED FOREST SOIL

A literature search has revealed that rates and patterns of denitrification, and the factors regulating denitrification have not been well characterised in forested land treatment systems. Applying nitrogen and increasing the incidence of anerobicity by regularly irrigating the soil, however, would be expected to enhance denitrification. A 12 month field study was therefore conducted to investigated the spatial and temporal variability of *in situ* denitrification rates in the Rotorua Land Treatment System, using a survey procedure outlined in Chapter 3. In addition, *in situ* denitrification rates were related to soil and environmental factors which have previously been suggested to regulate denitrification.

The subject matter of the following chapter is to be presented at the "Australian Society of Soil Science National Conference" in Brisbane, April 1998 and at the "World Congress of Soil Science", to be held in Montpellier, France, in August 1998.

CHAPTER 4

DENITRIFICATION IN A WASTEWATER-IRRIGATED FOREST SOIL

4.1 ABSTRACT

Land application of wastewater is thought to enhance ecosystem nitrogen removal by denitrification, however *in situ* denitrification rates have rarely been measured in land treatment systems. To determine the contribution of denitrification to nitrogen removal in a land treatment system, we measured denitrification rates for 12 months in a forest irrigated with tertiary-treated wastewater. We investigated spatial variability in denitrification rates, using a nested field design that divided the land treatment system into four stages (irrigation block, topographic position, field site and sample point). Denitrification was measured using undisturbed cores collected daily, and incubated for 24 h in the field with acetylene, for six consecutive days on 21 occasions throughout the year. Soil moisture content, nitrate concentration, respiration, denitrifying enzyme activity and temperature were also measured to determine their effects on *in situ* denitrification. In the laboratory, the relationship between soil moisture content and denitrification rates was examined.

Annual denitrification rates were 2.4 kg N ha^{-1} yr^{-1} in the irrigated soil and 1.7 kg N ha^{-1} yr^{-1} in the unirrigated soil. Denitrification rates varied considerably, both spatially and temporally. Spatially, differences in denitrification rates between irrigation blocks contributed more to variance than differences between or within topographic positions. However, over 12 months, temporal effects (e.g. seasonal and day-to-day variation) contributed more than spatial effects to the overall variation in denitrification rates. Multiple regression analysis showed that soil factors could only explain 29% of the variation in denitrification rates. Water-filled porosity was low, and less than the critical threshold value (80% water-filled porosity). It is proposed that denitrification in the land treatment system studied was limited by highly aerated, permeable soils. The results suggest if nitrogen removal by denitrification is to be important in a land treatment system, wastewater should be applied to soils which are not as excessively drained as the soils in the land treatment system which was studied.

4.2 INTRODUCTION

In land-based wastewater treatment systems, denitrification can decrease water pollution by biologically reducing nitrate to gaseous nitrogen (N) end-products (mainly N_2 and N_2O; Tiedje 1988). Denitrification only occurs in soil when oxygen is absent, and carbon and nitrate are present (Tiedje 1988). In a land treatment system, applying nitrogen and increasing the incidence of anaerobicity by regularly irrigating the soil with nitrate-containing wastewater, would be expected to enhance denitrification.

In New Zealand, tertiary-treated municipal wastewater from Rotorua City has been spray-irrigated onto upland soils (i.e. soil profile not influenced by groundwater) in a commercial *Pinus radiata* forest since October 1991. The land treatment system was designed on the basis that nitrogen applied to upland forest soils would partially be utilised by trees and denitrified in the soil. However, the annual contribution of denitrification to nitrogen renovation in the upland soils of the Rotorua Land Treatment System (RLTS) is unknown. Annual denitrification rates have not been widely reported for land treatment system, or for soils in the Southern Hemisphere. The objectives of this field study were to use the static core and acetylene-inhibition method to intensively study denitrification in the upland soils of the RLTS by: i) determining the annual contribution of denitrification to nitrate removal; ii) investigating the temporal and spatial variability of denitrification; and iii) establishing whether any relationships exist between denitrification rates and other soil or environmental factors. In a laboratory experiment, the relationship between soil moisture content and denitrification rates was further examined.

4.3 MATERIALS AND METHODS

4.3.1 Soil and site details

The RLTS is located in Whakarewarewa Forest, New Zealand (38°10'S, 176°16'E). Soils are pumiceous sandy loams, which are classified as Vitric Orthic Allophanic Soils (New Zealand Soil Classification System; Hewitt 1993). In the study area, the slopes are mainly moderately steep (12-23°). The climate in the region is temperate, with an annual rainfall of 1500 mm and mean annual temperature of 12.7 °C. Long-term

monthly averages range from a maximum of 17.7 °C in February to a minimum of 7.5 °C in July.

The system covers 350 ha and has been in operation since October 1991 (Tomer *et al.* 1997). Tertiary-treated wastewater is irrigated, using a sprinkler system, onto upland soils which have a total area of 242 ha. The remaining area comprises wetlands plus reserve and buffer zones. The upland soils form part of a commercial forest, mainly managed for *Pinus radiata*. The wastewater-irrigated area is divided into 14 irrigated blocks, with stands of various ages. In this study, sites were located on similar soil types in three irrigation blocks (13, 14 and 11), of similarly aged, mature stands (planted in 1975, 1970 and 1974, respectively). During irrigation, wastewater is applied for 12 h at a rate of 5 mm h^{-1}, once a week in each block. Over the past six years, the wastewater has contained an average nitrogen concentration of 11 mg N L^{-1}, resulting in a nitrogen loading of approximately 300 kg N ha^{-1} yr^{-1} (Table 4.1).

Table 4.1. Selected properties and mean loadings of major constituents in tertiary-treated wastewater applied to the RLTS during the past six years of operation.

Constituent	Concentration (mg L^{-1})	Loading (kg ha^{-1} yr^{-1})
Biological oxygen demand [A]	5.4 (1.2)	150
Chloride[A]	42 (4.3)	1140
Total nitrogen[A]	11 (2.4)	298
Organic nitrogen[A]	1.7 (0.8)	43
Ammonium[A]	3.9 (3.0)	105
Nitrate + nitrite[A]	5.6 (2.4)	150
Total phosphorous[A]	2.8 (1.8)	76
pH[B]	7.2 (0.2)	

[A]Values are means (and standard deviations) of monthly observations (Rotorua District Council, unpublished data).
[B]Values are means of 308 observations from August 1993 to November 1996.

4.3.2 Field study

4.3.2.1 Experimental design

A nested field design (Webster and Oliver 1990) was chosen to quantify and characterise denitrification rates. The design divided the RLTS into four stages: irrigation block, topographic position, field site, and sampling point. The topography of the land treatment system was divided into ridge, midslope and toeslope (Plate 4.1). Two replicate plots (5 m x 40 m) were located on each topographic unit, in each of the

Plate 4.1. The RLTS landscape. Picture taken in irrigation block 13 of this study. (Photo J. Barran).

three irrigation blocks, and are referred to as 'field sites'. The field sites were positioned along the contour, and further divided into four 5 m x 10 m sampling plots referred to as 'sample points'. The design provided a total of 72 sample points in the wastewater-irrigated area.

Unirrigated control sites were located adjacent to irrigated areas and divided into topographic position, field site and sample point, as above. However, in the unirrigated areas, only one field site was located in each topographic position per irrigation block, thus providing 36 sample points in unirrigated areas.

Denitrification rates were measured daily, at each of the sample points, for six days subsequent to irrigation, on seven separate occasions (referred to as 'sample period') during the year. Irrigation occurred on different days for each of the blocks, and therefore weekly denitrification rates were measured in the RLTS on 21 occasions during the course of the year. At the same time, daily denitrification rates were measured in irrigated areas, rates were also measured in adjacent unirrigated areas.

4.3.2.2 Denitrification measurement

Denitrification rates were measured using a core incubation method (Ryden *et al.* 1987). Two minimally disturbed soil cores (3.75 cm in diameter, 15 cm in length, contained in a PVC sleeve) were taken from each sample point and put in a 1 L glass jar, which was sealed with an air-tight screw-top fitted with a septum. Acetylene (60 mL, 10% v/v) was injected into the jar to inhibit the reduction of N_2O to N_2 during denitrification (Yoshinari *et al.* 1977). To maintain the atmospheric pressure, another hypodermic needle inserted in the septum acted as a vent during acetylene addition. To aid diffusion of the acetylene throughout the soil core, the PVC sleeve had holes drilled into the sides. In the field study, the soil cores were incubated at field temperature, for 24 h, by placing jars in soil bunkers located near field sites. In the laboratory study, soil cores were incubated at 15 °C for 24 h.

After incubation, the head space of the jars was sampled (12 mL) and stored in 10 mL evacuated Vacutainers until N_2O analysis. The N_2O concentration of the gas samples was measured using a Hewlett Packard gas chromatograph, fitted with an electron capture detector at an operating temperature of 350 °C. Gases were separated using a

porous packed column (HaySep Q) at 7O °C and at an injector port temperature of 140 °C. The carrier gas (argon with 10 % methane (v/v)) had a flow rate of 30 mL min^{-1} and passed through a molecular sieve (5A 45/60 mesh).

4.3.2.3 Soil analysis

Soil nitrate and soil moisture content were determined daily. After soil cores had been incubated to measure denitrification, and head-space in the jar sampled, soil cores from each jar were removed and thoroughly mixed. Soil water content at each sample point was determined gravimetrically after drying sub-samples at 104 °C for 24 h. To measure soil nitrate, sub-samples from each sample point in the same field site were bulked, and measurements made on duplicate samples. Soil nitrate was determined by adding 100 mL of 2 M potassium chloride to 10 g of field moist soil and extracting for one hour. The filtered solution was later stored (4 °C) until analysed for nitrate using a modified hydrazine reduction method (Downs 1978).

Denitrifying enzyme activity (DEA) (Smith and Tiedje 1979), soil respiration and soil pH were measured at the commencement of each sample week. An additional soil sample (0-15 cm) was collected from each sample point and returned to the laboratory on the same day. In the laboratory, the field moist soil was sieved (<4 mm) to remove roots, macro-fauna and stones. To measure the DEA, 10 g of fresh soil was weighed into glass screw-top jars (120 mL) with lids fitted with a rubber septum. The jars were flushed with oxygen-free nitrogen gas and 20 mL of solution containing 1 mM glucose and 1 mM potassium nitrate was added, followed by 10 mL of acetylene gas. Each jar was shaken at 25 °C for 15 minutes before 5 mL of head space was removed and stored in a 3 mL evacuated vacutainer. One hour later, another 5 mL of head space was removed and stored as before, until N_2O analysis.

Soil respiration was measured to assess soil carbon availability. Soil respiration was measured by incubating 50 g of fresh soil in a 1 L air-tight vessel at 25 °C for seven days. To maintain a humid environment, 5 mL of water was placed in the vessel. Gas samples (12 mL) were collected at the end of incubation and stored in 10 mL evacuated vacutainers for analysis of CO_2 using an isothermal gas chromatograph (Carle Instruments Inc) fitted with a thermal conductivity detector. Soil DEA and respiration results were corrected to an oven-dry basis.

Soil pH by determined by extracting air-dry soil (<2 mm) with de-ionised water using an extraction ratio of 1:2.5. Soil temperature and rainfall were measured daily during each of the sample weeks throughout the study. Soil temperature was measured at each field site using a temperature probe inserted in the soil to a depth of 7.5 cm. Rain gauges were used to record the amount of rainfall.

4.3.3 Laboratory study

To establish the relationship between soil moisture content and denitrification rates for irrigated soils in the RLTS, the moisture content of 36 soil cores were experimentally adjusted and denitrification rates measured. Intact soil cores (15 cm in diameter, 20 cm in depth) were collected from the RLTS by gently hammering rings to a soil depth of 15 cm, before being removed and taken to the laboratory. Smaller cores were also collected next to soil rings to determine initial soil moisture content. The moisture content of each soil core was then was adjusted to between 0.65 and 2.3 g H_2O g $soil^{-1}$, using de-ionised water, and incubated for seven days at 15 °C. Nitrate (50 kg N ha^{-1}) was added in solution at the beginning of the experiment to prevent denitrification being limited by nitrate in the higher moisture content treatments. To maintain soil moisture contents at the higher rates, soil rings were capped at the bottom to prevent the water from draining. At the end of the incubation, denitrification rates and soil moisture contents were measured as described above.

4.3.4 Data analysis

Daily denitrification rates were calculated for each of the sampling dates, and were corrected for the N_2O dissolved in the soil solution using the Bunsen coefficient (Tiedje 1982). The distribution of the denitrification rates was negatively skewed and consequently transformed using natural log (ln) to normalise variance prior to analysis. Weekly estimates were calculated by summing daily rates measured for that week. Annual *in situ* denitrification rates and annual potential denitrification rates were calculated for the irrigated and unirrigated area by extrapolation and summation of weekly denitrification rates and hourly DEA, respectively.

All data were statistically analysed using SAS (SAS Institute Inc. 1989). The Nested Random Effects Procedure calculated the contribution of each stage (in the order

irrigation block, topographic position, field site and sample point) of the nested design to total variation. Before conducting spatial analysis, temporal effects were removed by averaging daily denitrification rates for each of the sampling points over the year. The contribution of each of the stages to total variation was expressed as a percentage of the total variation. The F statistic was used to determine if variances between the different stages were statistically different.

The General Linear Model Procedure was used for analysis of variance and multiple regression. To relate denitrification rates to soil and environmental factors in the irrigated soils, multiple linear regression using a backwards elimination procedure was completed for all sample points. Skewness of denitrification rates, soil respiration and DEA were corrected for by natural log transformation. Prior to conducting regression analysis, Pearsons correlation coefficients were calculated between all of the soil variables to determine if any of the variables were highly correlated and could therefore cause problems with multicollinearity (highly correlated variables) during multiple regression. Seasonal patterns in denitrification were compared with seasonal variation in soil factors in each of the irrigation blocks by averaging denitrification rates at each sample point and then relating these to averaged soil properties (n=24). Reported differences were significant at the 5% level unless reported otherwise.

In the laboratory experiment, soil moisture content was related to logarithmic transformed denitrification rates by using a non-linear regression procedure to fit a segmented linear function.

4.4 RESULTS

4.4.1 Effects of wastewater irrigation on denitrification

Wastewater irrigation increased the magnitude ($p < 0.001$) and variability of daily denitrification rates (Table 4.2). In the irrigated soils, daily denitrification rates ranged from 0 to 850 g N_2O-N ha^{-1} day^{-1} and in the unirrigated soils, daily denitrification rates varied from 0 to 100 g N_2O-N ha^{-1} day^{-1}. Mean daily denitrification rates were almost twice as great in the irrigated soils than in the unirrigated soils. Daily denitrification rates were highly variable in both irrigated and unirrigated sites, however variability

appears to have been enhanced as a consequence of wastewater irrigation. For example, the coefficients of variation (CV) for daily denitrification rates were approximately 2.5 times greater in the irrigated sites than the unirrigated sites (Table 4.2).

Table 4.2. Descriptive statistics for daily denitrification rates and soil properties of irrigated and unirrigated soils.

Soil parameter	n	Mean	Median	Range	CV[A]
Irrigated soil					
Denitrification (g N_2O-N ha^{-1} day^{-1})	4527	9.2	5.4	0-850	230
Soil moisture content (g H_2O g^{-1})	4527	0.9	0.9	0.03-3.0	30
Soil nitrate (μg g^{-1})	756	8.5	8.0	0.2-24	40
Soil respiration (μg C g^{-1} h^{-1})	530	1.5	1.4	0-6.4	53
DEA (ng N_2O-N g^{-1} h^{-1})	535	3.6	0.8	0-230	390
Soil pH	252	6.6	6.6	4.9-7.1	4.3
Soil temperature (°C)	737	11	11	2.6-19	31
Unirrigated soil					
Denitrification (g N_2O-N ha^{-1} day^{-1})	1506	5.0	4.5	0-98	90
Soil moisture content (g H_2O g^{-1})	1506	0.75	0.73	0.17-2.5	31
Soil nitrate (μg g^{-1})	379	2.4	0.84	0-35	170
Soil respiration (μg C g^{-1} h^{-1})	251	1.8	1.7	0.1-8.3	54
DEA (ng N_2O-N g^{-1} h^{-1})	251	4.4	1.2	0-140	300
Soil pH	125	5.6	5.6	4.9-6.6	5.5
Soil temperature (°C)	379	11	11	3-19	29

[A] Coefficient of variation (%)

Daily denitrification rates varied spatially. Denitrification rates varied between irrigation blocks, in both irrigated and unirrigated sites. Weekly denitrification was greater in irrigation blocks 11 and 13 than irrigation block 14 (Figure 4.1). Within irrigation blocks, daily denitrification rates varied with topographic positions, although differently between irrigated and unirrigated soils. In irrigated and unirrigated soils, the effect of topographic position on denitrification rates was not consistent. For example, in the irrigated soils mean daily denitrification rates varied in the order midslope > ridge > toeslope (Block 13); ridge = midslope < toeslope (Block 14); and ridge = midslope > toeslope (Block 11) (Figure 4.2). For each sample period, denitrification rates were highly variable in the irrigated soils (Table 4.3). In the unirrigated soils, denitrification rates were less variable in autumn than spring (Table 4.3). For example, in the unirrigated soils, the CV was 51% and 144% in April and November, respectively.

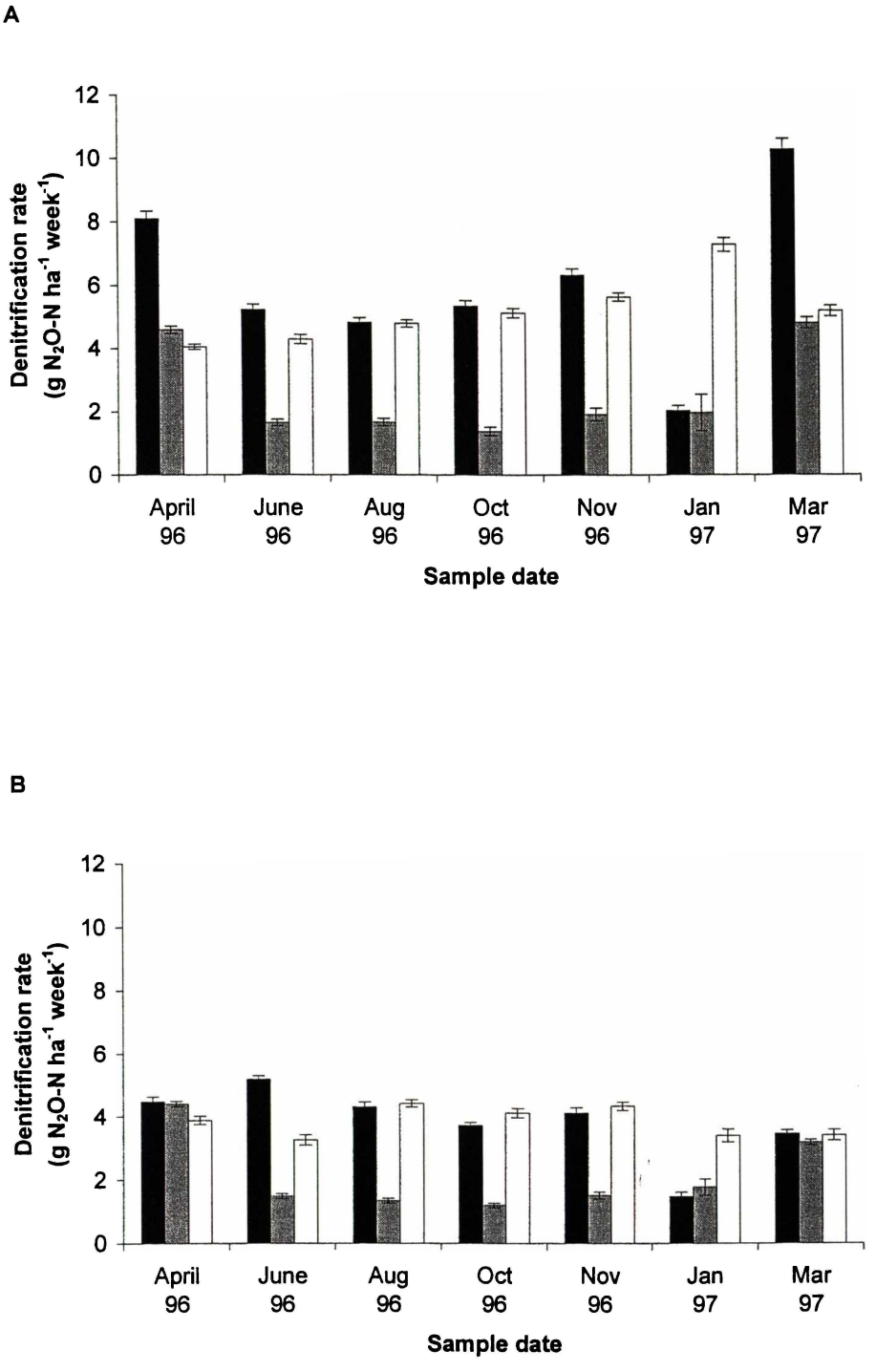

Figure 4.1. Variation in weekly denitrification rates with irrigation block in irrigated (A) and unirrigated (B) sites. Irrigation block 13(■), 14(■) and 11(□).

Table 4.3. Descriptive statistics for daily denitrification rates for each sample period in irrigated and unirrigated soils.

Sample period	Median sample date	Mean (g N_2O-N ha^{-1} day^{-1})	CV (%)
Irrigated			
1	26 April 1996	10	150
2	22 June 1996	6.5	138
3	17 August 1996	6.4	115
4	11 October 1996	7.6	163
5	30 November 1996	8.9	143
6	30 January 1997	8.1	203
7	13 March 1997	17	280
Unirrigated			
1	26 April 1996	6.4	51
2	22 June 1996	5.0	71
3	17 August 1996	5.1	69
4	11 October 1996	4.2	62
5	30 November 1996	5.5	144
6	30 January 1997	3.7	143
7	13 March 1997	5.1	55

Daily denitrification rates also varied temporally. Mean daily denitrification rates varied from day-to-day after irrigation, with values ranging from 1.7 to 21 g N_2O-N ha^{-1} day^{-1} (Figure 4.3). The pattern of denitrification after irrigation, however, changed throughout the year, and, consequently, there was no effect of day after irrigation on denitrification rate. Daily denitrification rates varied seasonally ($p<0.001$), and in a similar way in both irrigated and unirrigated sites. Larger denitrification rates were observed in March (autumn) relative to other times of the year (Figure 4.4).

The annual rate of denitrification for irrigated and unirrigated soils was 2.4 and 1.7 kg N ha^{-1} yr^{-1}, respectively. An estimate of the variance in the annual N loss cannot be determined from only one year of data. Upland denitrification accounted for less than 1% of total N applied annually. Even under optimum laboratory conditions, potential denitrification rates at 25 °C (i.e. DEA) were 13.4 kg N ha^{-1} yr^{-1} in the irrigated soils and 17.8 kg N ha^{-1} yr^{-1} in the unirrigated soils. Potential rates would be expected to be less at 11 °C (average field temperature; Table 4.2) than 25 °C.

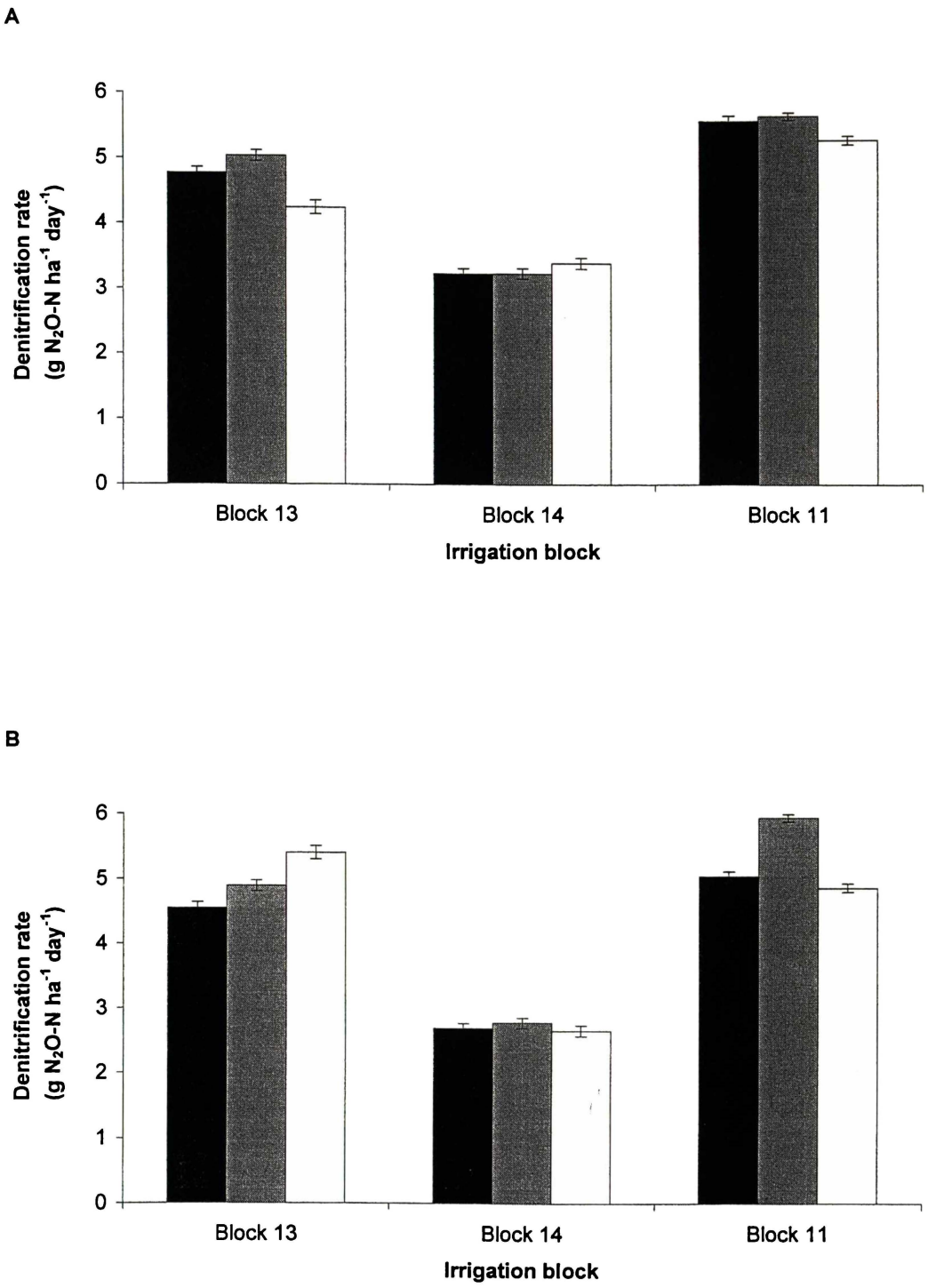

Figure 4.2. Variation in mean daily denitrification rates with topographic position in irrigated (A) and unirrigated (B) sites. Ridge (■), midslope (▩) and toeslope (□).

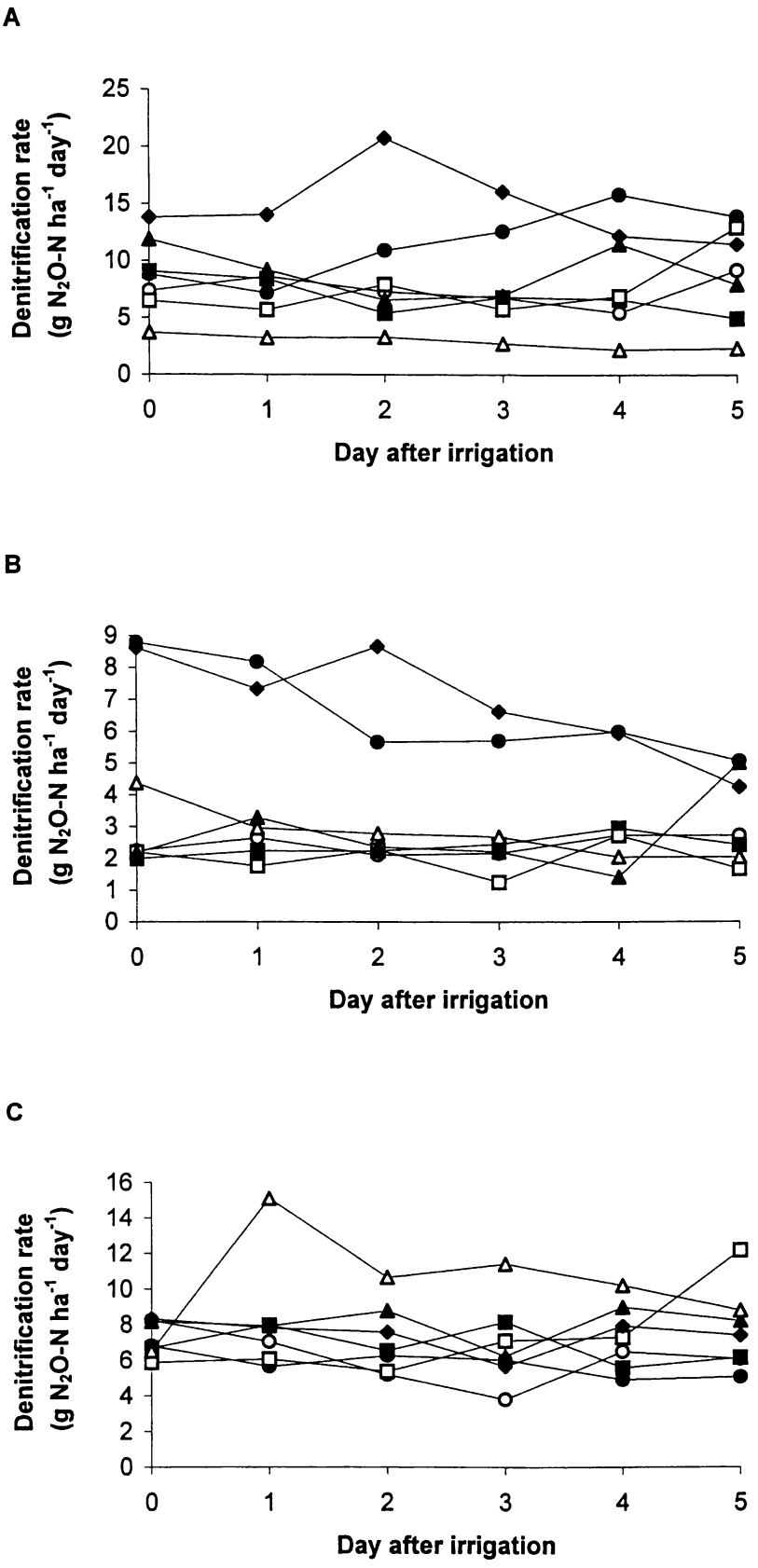

Figure 4.3. Variation in daily denitrification rates with day after irrigation in irrigation block 13 (A), 14 (B) and 11 (C) for each sample period. Sample period one (●), two (○), three (■), four (□), five (▲), six (△) and seven (◆).

4.4.2 Contributions of different sources to spatial and temporal variability of denitrification

Irrigation block was the greatest source of total spatial variation of denitrification rates in the land treatment system ($p < 0.01$; Table 4.4). Topographic position contributed to only 5% of the total variation, which was significantly less than the 9% due to differences between sample points. Differences in denitrification rates in field sites (which is equal to differences between the same topographic positions) contributed to less than 1% of the total variation. Statistical analyses showed that all of the variation due to irrigation block was due to block 14. Excluding block 14 from the analysis decreased the contribution of irrigation block from 86% to zero (Table 4.4). In addition, the variation between sample points became the greatest source of spatial variation (55%), followed by differences between topographic positions (45%).

Table 4.4. The contribution of different sources to the spatial variation of denitrification rates in irrigated soils for a year.

Irrigation block	Source of variation	% of total variance
All	Irrigation block	85.9***
	Topographic position	4.9*
	Field site	0.0
	Sample point	9.2
13&11	Irrigation block	0.0
	Topographic position	44.6*
	Field site	0.5
	Sample point	55.1

*** $p < 0.001$, * $p < 0.05$.

The main source of spatial variation in denitrification rates did not differ substantially with season (Table 4.5). Instead, irrigation block remained the most significant source of variability for all seasons. Seasonally, the contribution of irrigation block and sample point to total variation changed between winter and spring and late summer-autumn. In late winter, spring, and the late summer-autumn periods the contribution of irrigation block to total variation was >80% and 54%, respectively, while variation between sample point changed from <12% in late winter and spring to 36% in late summer-autumn, respectively.

Table 4.5. The contribution of different sources to the spatial variation of denitrification rates in irrigated soils for different seasons

Source of variation	% of total variance
Winter	
Irrigation block	87***
Topographic position	1
Field site	1
Sample point	11
Spring	
Irrigation block	84***
Topographic position	4
Field site	0
Sample point	12
Late summer-autumn	
Irrigation block	4***
Topographic position	7
Field site	2
Sample point	36

*** $p < 0.001$

By using the "Nested model" in SAS, temporal sources of variation (such as sample period and sample day) were included in the analysis (Table 4.6). The results show that temporal effects contributed more than spatial effects to total variation in denitrification rates. The variation due to sample day and sample period accounted for approximately 76% of the total variation in denitrification rates. Repeating the analysis, but without block 14, removed the contribution of irrigation block to total variation (Table 4.6) and resulted in 97% of the variation in denitrification rates being attributed solely to temporal effects.

Table 4.6. The contribution of spatial and temporal sources to the variation of denitrification rates in irrigated soils for one year.

Irrigation Block	Source of variation	% of total variance
All	Irrigation block	22.8*
	Topographic position	1.3*
	Field site	0.0
	Sample point	0.0
	Sample period	19.8***
	Sample day	56.1
13&11	Irrigation block	0.0
	Topographic position	2.5
	Field site	0.1
	Sample point	0.0
	Sample period	22.3*
	Sample day	75.1

*** $p < 0.001$, * $p < 0.05$

4.4.3 Effects of wastewater irrigation on other selected soil properties

Mean values for soil and environmental properties measured in irrigated and unirrigated soils during the field study are presented in Table 4.2. Wastewater irrigation changed both the magnitude and variability of most of the soil properties measured. Daily measurements of soil moisture and soil nitrate, and monthly measurements of soil pH were higher in the irrigated sites than the unirrigated sites ($p < 0.001$), while weekly DEA and daily soil temperature remained unchanged after six years of irrigation. Application of wastewater significantly decreased soil respiration.

Wastewater irrigation affected the variability of soil properties differently. Variability of soil DEA was higher in irrigated than unirrigated soils, although both sites were highly variable (Table 4.2). The variability of soil nitrate, in contrast, was less in irrigated than unirrigated sites. Irrigation of wastewater appeared to have no appreciable affect on the distribution of soil moisture, soil respiration or soil temperature.

Soil moisture content, nitrate and respiration rate varied spatially. For example, soil moisture contents were greater in irrigation block 14 than irrigation blocks 11 and 13. In contrast, soil nitrate was greater in irrigation block 13, followed by irrigation block 14 and irrigation block 11; while respiration rates were greater in irrigation block 11, followed by irrigation block 13 and irrigation block 14. Soil moisture content was greater in toeslopes than ridge or midslope positions. Soil nitrate and respiration, however, did not vary with topographic position.

Soil nitrate, respiration, DEA and temperature all varied seasonally ($p<0.001$), and were greatest between January and March (i.e. summer-autumn; Figure 4.4). Except in early October, soil moisture content did not vary seasonally. Soil moisture content also changed from day-to-day after irrigation, and was greatest for the first two days after irrigation.

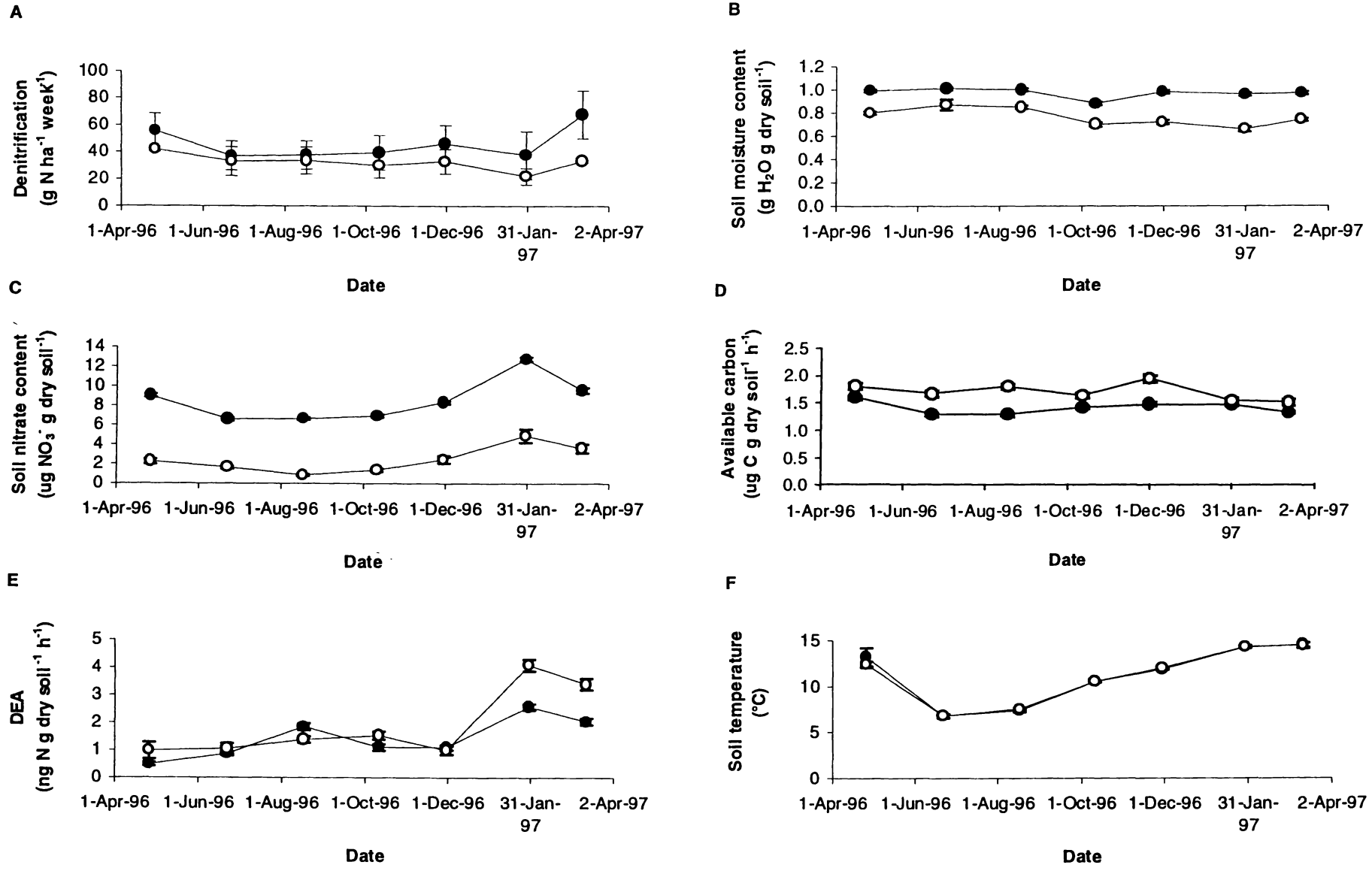

Figure 4.4 Seasonal variation in weekly denitrification rates (A) and soil properties (B-F) in irrigated (●) and unirrigated (○) soils. Means (± SE) are averages of all sample points (Table 4.2) .

4.4.4 Relationship between denitrification rates and soil factors

Daily denitrification rates were poorly correlated with other individual soil and environmental properties, which could not explain more than 29% of the variation in daily denitrification rates in the irrigated soils (Table 4.7). Correlation coefficients between daily denitrification rates, from each sample point, and soil properties were positive and greatest for soil temperature (r = 0.23). The correlation between daily denitrification rates and soil properties was slightly improved when properties from each sample points was averaged across the year (Table 4.7); all correlations were positive and significant for soil nitrate in all blocks (r= 0.39-0.54), and soil moisture in block 14 (r = 0.47).

Table 4.7. Pearsons correlation coefficients for relationships between denitrification rates and soil properties in irrigated soils.

Variable	Individual cores	Cores averaged across the year		
		Block 13	Block 14	Block 11
Moisture content	0.16**	0.33n.s.	0.47*	0.06n.s.
Soil nitrate	0.13**	0.43*	0.39**	0.54*
ln(Basal respiration)	0.13**	0.33n.s.	0.14n.s.	0.22n.s.
Ln (DEA)	0.08**	0.10	0.02n.s.	0.14 n.s.
Soil temperature	0.23**	0.06n.s.	0.23n.s.	0.45**

** $p < 0.01$;* $p < 0.05$; n.s., not significant

Multiple regression between daily denitrification rates, from each sample point (n=3024), and soil properties did not successfully explain the variation of denitrification rates. Soil moisture content, soil respiration, soil temperature and rainfall were all significant variables in the equation, but only explained 11% of the variability:

$$\ln(\text{denitrification} + 1) = 0.65 + 0.40\,MC + 0.26\,Resp + 0.05\,Temp + 0.01R$$

where MC = soil moisture content, Resp = soil respiration, R= rainfall and Temp = soil temperature.

Seasonal patterns between denitrification rates and other soil properties in the irrigated soils were not highly related. Soil nitrate explained 19 and 29% of the seasonal

variability of denitrification rates in blocks 13 and 11, whereas soil moisture content explained 22% of denitrification rates in block 14.

Soil DEA was poorly related to daily denitrification rates in the irrigated soils (r^2= 0.08), and not significantly related to denitrification rates when averaged across the year (Table 4.7). Comparing annual denitrification rates for each of sample points with the average DEA for each sample point did not improve the relationship (r^2= 0.1) (data not shown) between DEA and *in situ* denitrification rates in this study.

4.4.5 Relationship between denitrification rates and soil moisture content

In the laboratory experiment, denitrification increased with increasing soil moisture content (Figure 4.5). Denitrification rates were limited at soil moisture contents less than 1.2 g H_2O g dry soil^{-1}. This value corresponds with 61% volumetric water content or 80% water-filled porosity (WFP) and is near saturation. In the field, soil moisture contents averaged 0.9 g H_2O g dry soil^{-1} (60% WFP), with only 16% (n= 4527) of the samples exceeding 1.2 g H_2O g dry soil^{-1}. Furthermore, soil moisture contents averaged 1.05 g H_2O g dry soil^{-1} (70% WFP) immediately after irrigation and decreased to an average of 0.94 g H_2O g dry soil^{-1} (63% WFP) by day four.

The relationship between denitrification (ln) and soil moisture was best described using a segmented linear curve (r^2 =0.70; Figure 4.6). Logarithmic transformed denitrification rates increased linearly between soil moisture contents greater than 0.51 g H_2O g dry soil^{-1} but less than 1.4 g H_2O g dry soil^{-1} (i.e. ln (denitrification)= 8.7 soil moisture content - 4.5). At soil moisture contents greater than 1.4 g H_20 g dry soil^{-1} soil denitrification rates (ln) did not increase further (Figure 4.6). Using this relationship, changing the soil moisture content from 0.5 to 1.4 g H_2O g dry soil^{-1} increased denitrification by a factor of 2000.

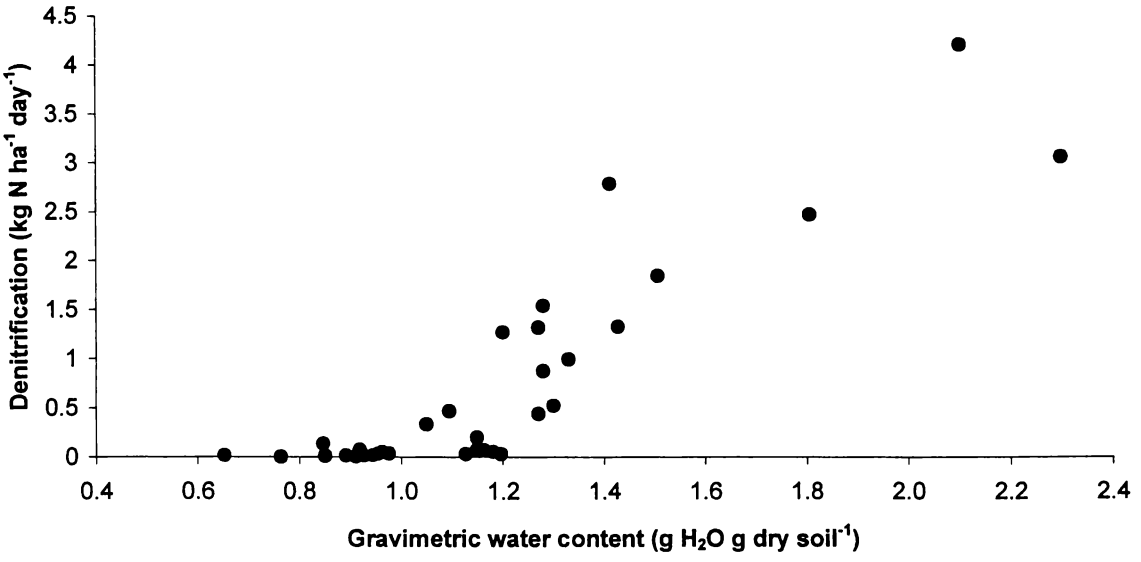

Figure 4.5. Effect of soil water content on denitrification in a laboratory study.

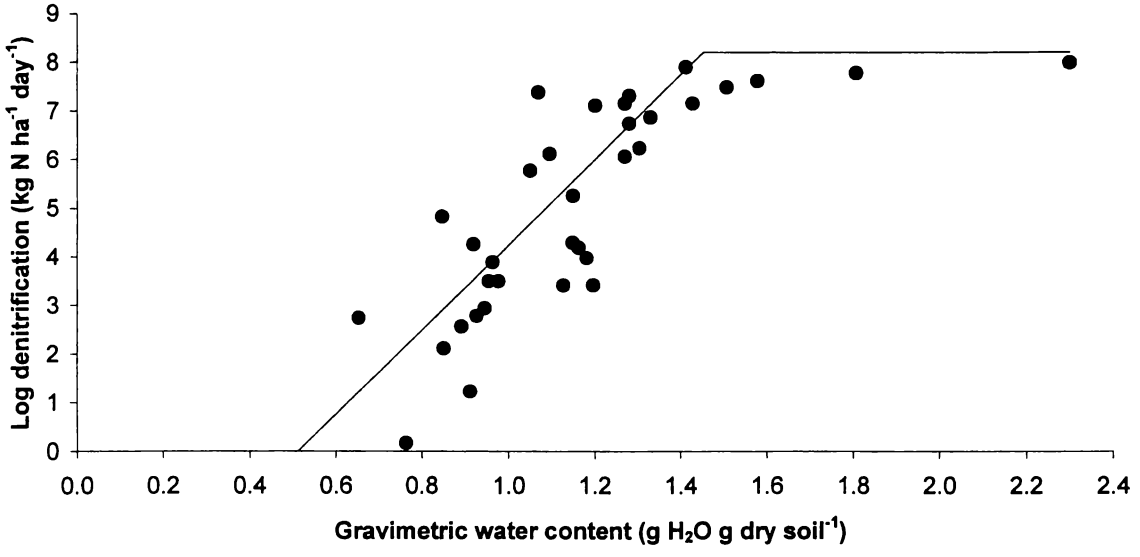

Figure 4.6. Denitrification rates (logarithmic transformed) versus gravimetric water content in a laboratory study.

4.5 DISCUSSION

4.5.1 Effects of wastewater irrigation on denitrification

The addition of treated wastewater does not appear to have greatly stimulated denitrification activity in the upland soils of the RLTS. The denitrification rates were small (2.4 kg N ha^{-1} yr^{-1}) despite regular inputs of water, nitrate and carbon. These rates were comparable to mature, unfertilised conifer forests on upland soil (e.g. Davidson *et al.* 1990; Dutch and Ineson, 1990), but less than rates often recorded in upland agricultural soils receiving nitrogen fertiliser (Colbourn and Dowell, 1984). Annual denitrification rates reported for upland forest soils range from less than 1 (e.g. Myrold *et al.* 1989) to 40 kg N ha^{-1} yr^{-1} (e.g. Dutch and Ineson 1990), whereas rates reported for upland agricultural soils range from less than 1 (e.g. Schwarz *et al.* 1994) to 204 kg N ha^{-1} yr^{-1} (Ryden and Lund 1980). In forest soils, denitrification rates greater than 5 kg N ha^{-1} yr^{-1} have generally only been recorded for fine textured, deciduous forest soils (e.g. Groffman and Tiedje 1989a), or when soil disturbance has increased soil moisture and nitrate availability (e.g. Dutch and Ineson 1990).

Recently, laboratory studies have suggested nitric oxide, a precursor to nitrous oxide during denitrification, reacts with acetylene and oxygen (Bollman and Conrad 1996,; McKenny *et al.* 1996, 1997). If such a reaction was to occur while measuring denitrification, losses could be underestimated. Both *in situ* denitrification rates and potential denitrification losses, as measured by DEA, were low in the RLTS. Denitrifying enzyme activity measures nitrous oxide production in the presence of acetylene, but in the absence of oxygen, therefore interference by acetylene should not have occurred (McKenny *et al.* 1997). Denitrification rates were not measured during the actual irrigation event for practical reasons, and may also have caused denitrification losses to be underestimated. However, surface denitrification rates have previously been shown to be negligible during irrigation (Monnet *et al.* 1995) and the low DEA in this study measured after irrigation, indicates the potential for denitrification in the surface 15 cm of the soil profile is also small.

4.5.2 Spatial and temporal variability of denitrification rates

Daily denitrification rates were found to be highly variable (i.e. CV >100%) in a wastewater-irrigated forest soil. Denitrification rates in agricultural and forestry soils are also highly variable with coefficients of variation greater than 100% (Burton and Beauchamp 1985; Parkin 1987; Groffman and Tiedje 1989a). The variability of denitrification rates in the RLTS was attributed to spatial and temporal effects.

In the RLTS, denitrification rates were spatially variable throughout the year, with denitrification rates mainly varying between, rather than within irrigation blocks. The contribution of irrigation block to spatial denitrification remained important in each of the seasons, indicating that the cause of the variation occurred year-round. Differences in denitrification rates between topographic positions did not contribute greatly to the spatial variability in the RLTS. This result is unexpected as preliminary studies at the RLTS showed that DEA was greater in toeslopes than ridge and midslopes (Chapter 3). In other studies, topography contributed greatly to spatial variation in denitrification rates by altering the availability of soil moisture, nitrate and carbon (Groffman and Tiedje 1989b; Corre *et al.* 1996). For example, greater denitrification rates have generally been observed in toeslope soils, and attributed to higher soil moisture and nitrate contents in toeslope soils than other topographic positions (Groffman and Tiedje 1989a; Corre *et al.* 1996). In the RLTS, topographic position did not affect denitrification rates as strongly as in other studies, and is consistent with the observation that neither nitrate nor carbon availability varied with topographic position in the RLTS. The small contribution of topography to the spatial variation of denitrification probably reflects the youthful nature of the volcanic soils in the RLTS, which have probably undergone less pedogenic development than other soils where topography has had a significant effect on denitrification.

Temporal effects contributed more than spatial effects to the variation in denitrification rates. Denitrification rates varied day-to-day during the weeks studied, but not in the same way each time. Denitrification rates were expected to increase after irrigation as other studies showed surface soil nitrous oxide production greatest immediately after irrigation, and then decreased rapidly as the soil water redistributed and the soil became less anaerobic (Ryden and Lund 1980; Rolsten *et al.* 1982; Sexstone *et al.* 1988). In the RLTS, denitrification rates generally showed no response to irrigation, suggesting that

conditions for denitrification were not greatly improved by wastewater irrigation. A possible reason for this lack of response is discussed later.

Denitrification rates varied seasonally in wastewater-irrigated soils, and in a similar way to unirrigated soils. Denitrification rates generally change with season, and greatest rates occur at those times of the year when soils are moist, and soil nitrate and carbon are available (Groffman and Tiedje 1989a; Ruz-Jerez *et al.* 1994; Corre *et al.* 1996). The season in which the denitrification rates are greatest, however, can vary between ecosystem. For example, in the RLTS, denitrification was greatest in late summer and in autumn, whereas denitrification rates peaked in spring and autumn in a northern temperate forest (Groffman and Tiedje 1989a); summer in a fertilised soil in a semi-arid region in Canada (Corre *et al.* 1996); and in winter in unfertilised pasture soils in New Zealand (Luo *et al.* 1994; Ruz-Jerez 1994).

Accounting for spatial and temporal variability is important if an accurate estimate of annual denitrification rate is to be obtained, as annual losses are often calculated by integrating fluxes of gaseous denitrification products with time. The results from the RLTS indicate that a greater emphasis should be placed on sampling frequently during the year and in as many of the irrigation blocks as possible, with less attention paid to replication and partitioning the landscape into topographic positions. Similarly, Scholefield *et al.* (1990) suggested that an increased frequency of sampling, rather than increased replication at each sample date, should be used to better estimate denitrification losses from long-term grazed grasslands. In the RLTS, decreasing the number of irrigation blocks or sample periods could have significantly under- or over-estimated the annual denitrification rate. For example, had rates only been measured in block 14, the annual rate of denitrification calculated would have been 46% less than the rate reported; while measuring rates only in the winter sample period would have underestimated the rate by 21%.

4.5.3 Factors limiting denitrification rates

Soil denitrification rates in the RLTS were probably restricted by soil aeration, rather than available carbon and nitrate. Soil oxygen is an important regulator of denitrification, inhibiting the synthesis and activity of denitrifying enzymes (Payne 1973). Increasing soil moisture content generally increases denitrification rates by limiting the diffusion of oxygen in the soil. Soil water-filled porosity in the RLTS was low, even immediately after irrigation, and mostly less than the critical threshold value (1.2 g H_2O g^{-1} dry soil or 80% WFP). During the 12 month field trial in the RLTS, WFP averaged 60% (0.9 g H_2O g^{-1} soil), with only 16% (n= 4527) of the samples exceeding 80% WFP. The activity of denitrifiers in the upland soils of the RLTS, therefore, appears to be limited by low soil moisture contents.

The critical WFP required to increase denitrification rates in the irrigated soil of the RLTS was similar to those reported for other soils (Chapter 2). In the RLTS the critical WFP for a sandy loam was 80%, which was only slightly less than 82% reported for a loam (Estavillo *et al.* 1994) and 83% reported for a sand (de Klein and van Logtestijn 1996). The critical WFP for the RLTS was markedly greater than values reported for clay loam (e.g. >62 % WFP), and clay soils (e.g. >60% WFP) by Nommik and Larsson (1991). Groffman and Tiedje (1991) attributed greater threshold WFP with soil coarseness to the effect of soil texture on oxygen availability. Finer-textured soils have smaller pores which leads to greater water retention and a greater opportunity for creating anaerobic microsites in comparison to coarser textured soils, and anaerobic microsites were therefore more likely to be present at lower WFP than in coarser soils. The higher WFP required to further enhance denitrification rates in the RLTS, in comparison to the finer-textured soils reported elsewhere, was probably a function of both the coarse texture of the soil and also the vesicular nature of the pumice soil increasing soil aeration.

Unlike WFP, soil nitrate concentrations in the RLTS were above the value considered critical for denitrification (>5-10 mg NO_3-N kg dry $soil^{-1}$; Jordan 1989; Ryden and Lund 1980; Ryden 1983; Estavillo *et al.* 1994). It is difficult to assess whether carbon availability was adequate for denitrification in the RLTS, as carbon availability has been measured using various techniques in other studies and it is difficult to cite critical values. Generally, soil carbon is not considered limiting to denitrification in forest soils

(Chapter 3; Davidson *et al.* 1990). It is, therefore, proposed that denitrification in the RLTS was not limited by nitrate and carbon availability in the RLTS.

It is hypothesised that denitrification rates did not increase after wastewater irrigation because soil moisture contents did not exceed the threshold value required for denitrification. Poor responses to irrigation or precipitation events have only been recorded when nitrate availability has been low (Rolsten *et al.* 1982; Ryden and Lund 1980), soil temperatures have been limiting (Nommik and Larsson 1989) or, as was the case in the RLTS, when studies have been conducted on free draining sands where high moisture contents have been difficult to maintain for an extended period after irrigation (Bijay-Singh *et al.* 1989). Soil DEA was also probably low in the RLTS because the soils were too aerobic for denitrification. Without sufficient periods of active denitrification it is unlikely that an increase in denitrifying bacteria would eventuate.

4.5.4 Relationship between denitrification rates and soil factors

Denitrification in the upland soils of the RLTS could not be related to any one soil or environmental factor. The lack of significant relationships between denitrification and any of the soil or environmental factors was probably due to low and variable denitrification rates. Correlation analysis between individual soil cores and soil factors accounted for less than 5% of the variation in denitrification rates. Multiple regression analysis improved this result only marginally. This result was not surprising, as denitrification rates are often not related (Hixson *et al.* 1990) or poorly related to bulk soil properties (e.g. Myrold 1988; Parsons *et al.* 1991; Jarvis *et al.* 1994). One of the main problems with relating denitrification rates to soil factors is characterising soil conditions at the microsite scale. Measurements from bulk soil samples, such as soil cores, are not thought to correspond with microsite conditions (Davidson and Hackler 1994). Until techniques are developed to enable field conditions in microsites to be measured, increasing studies across time and space (e.g Ambus and Christensen 1993; Groffman and Tiedje 1989b; Schipper *et al.* 1993) appear to be the only options available to predicting denitrification rates from soil factors. Incorporating stochastic models into the regression analysis has been suggested as a means of improving the relationship between denitrification rates and soil factors (Parkin and Robinson 1989), but appears to have had only limited success to date.

Averaging denitrification rates for each of the sample points across time in each of the irrigation blocks, and relating these to bulk soil properties using multiple regression techniques, slightly improved the prediction of denitrification rates by soil properties. However, soil nitrate or soil moisture content could only account for between 19 and 29 % of the variation in denitrification rates when temporal effects were removed. Bulk soil properties, explained more of the seasonal variations than daily variations in denitrification rates; which suggested that short-term changes (i.e. hourly or daily) in microsite conditions were not reflected in the bulk soil sample. Instead, seasonal changes in substrate availability in the microsite may be proportional to substrate availability in the bulk soil sample.

4.5.5 Predicting annual denitrification rates using DEA

Measuring denitrification rates and developing models based on soil properties requires intensive sampling strategies over an extended period of time. It has been suggested that DEA reflects long term denitrification rates (i.e. annual rates), rather than daily or hourly denitrification rates (Groffman and Tiedje 1989b; Smith and Parsons 1985) and it has been suggested soil DEA may be a simple soil measure to estimate annual denitrification rates (Groffman and Tiedje 1989b). Indeed, DEA has successfully been used to predict denitrification rates at the landscape scale (Groffman and Tiedje 1989b) and when values have been averaged over time (Schipper *et al.* 1993). However, DEA was not effective in explaining the variation in denitrification rates in small scale studies, including this one, or when compared with momentary denitrification rates (e.g. Parsons *et al.*, 1991; Bergstrom and Beauchamp 1993). In the RLTS, DEA did not correspond well with daily denitrification rates or when denitrification rates were averaged over time. This is probably because the range in denitrification rates and DEA values was not large. Soil DEA is therefore probably not a valuable tool for predicting *in situ* denitrification losses in a system where land-use and landscape has very little variation.

4.6 CONCLUSIONS

Denitrification losses in the upland soil of a land treatment system in New Zealand were 2.4 and 1.7 kg N ha^{-1} yr^{-1} in irrigated and unirrigated soils, respectively. The rates in wastewater-irrigated soils were lower than expected and accounted for less than 1% of the annual wastewater-applied nitrogen. Upland denitrification rates appeared to be limited by soil moisture contents which were too low to restrict oxygen availability, and thus increase denitrification rates, despite weekly irrigation for 12 h at 5 mm h^{-1}.

Denitrification rates have not been widely reported for other land treatment systems, although, it might be assumed that denitrification rates will be high given the continuous inputs of nitrogen, carbon and water. However, upland denitrification rates would not be expected to contribute greatly to nitrogen removal in land treatment systems if they are located on free-draining, coarsely textured soils. Instead, if the design of a land treatment system requires large denitrification rates from upland soils, it is suggested that the location needs to contain soil which is not as excessively drained and with finer texture than the soil in this study.

The contribution of upland denitrification to nitrogen removal in other land treatment systems remains largely unexplored. Future work needs to examine aspects of land treatment system design that indirectly affect the regulators of denitrification. This could include the effects of soil texture and drainage, irrigation rates, irrigation frequency, wastewater composition and plant species on annual denitrification rates.

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CHAPTER 5

DENITRIFICATION ENZYME ACTIVITY WAS LIMITED BY SOIL AERATION IN A WASTEWATER-IRRIGATED FOREST SOIL

The results from the field study conducted in Chapter 4 showed that the denitrifying population was small in the Rotorua Land Treatment System. To determine which factors were limiting the denitrifying population, a laboratory experiment compared the individual and combined effects of soil aeration, nitrate and carbon on the denitrifiers in wastewater-irrigated and unirrigated soils.

CHAPTER 5

DENITRIFICATION ENZYME ACTIVITY WAS LIMITED BY SOIL AERATION IN A WASTEWATER-IRRIGATED FOREST SOIL

5.1 ABSTRACT

In a forested land treatment system, previous research has shown the size of the denitrifying population in the upland soils is small. Therefore, this part of the study investigated which factors were limiting the denitrifying population in the Rotorua Land Treatment System (RLTS), by studying the individual and combined effects of soil aeration, nitrate and carbon on denitrification enzyme activity (DEA) in wastewater-irrigated and unirrigated soils. In the study it was found that the size of the soil denitrifying population in the RLTS appeared to be limited by soil aeration. Limiting oxygen availability increased the denitrifying population, and to a greater extent than previously observed in the field. Additions of carbon and nitrate to anaerobic soils, did not further increase the denitrifying population above controls in the irrigated soils.

Although wastewater-irrigation has not increased the size of the denitrifying population after six years, it has changed the factors limiting denitrifiers in the RLTS. For example, in the irrigated soils denitrification is limited by soil aeration, while in the unirrigated soils, denitrification is limited by both soil aeration and nitrate. Furthermore, wastewater-irrigation has altered the short-term response of denitrifiers to anaerobiosis. Under low oxygen conditions, denitrifiers in the wastewater-irrigated soils produced enzymes sooner, and at a greater rate, than soils without a history of wastewater irrigation. It is proposed that the size of the denitrifying population cannot be expected to be large in free-draining, coarsely textured soils, even when provided with additional nitrogen, carbon and water inputs.

5.2 INTRODUCTION

The contribution of denitrification to N cycling varies amongst and within ecosystems. Such differences have been attributed to the size of the denitrifying population, which determines the maximum denitrification rate (Tiedje *et al.* 1982). The size of the denitrifying population is influenced by soil and environmental factors, and is often indirectly measured by doing an assay of denitrification enzyme (Martin *et al.* 1988; Davidson *et al.* 1990), such as the 'Phase I' denitrification enzyme assay (DEA; Smith and Tiedje 1979). In the laboratory, increases in denitrifying populations have occurred after amending soil with nitrate under anaerobic conditions (Jacobson and Alexander 1980), and after adding carbon (Myrold and Tiedje 1985). In the field, differences in the size of denitrifying population between sites have been related to soil texture and drainage (Groffman and Tiedje 1989), and soil respiration and moisture content (Parsons *et al.* 1991).

In a forested land treatment system in New Zealand, *in situ* denitrification rates were found to be small, despite weekly irrigation with tertiary treated wastewater containing nitrate and carbon (Chapter 4). During a 12 month field study, annual denitrification losses in wastewater-irrigated soils (2.4 kg N ha^{-1} yr^{-1}) were not much greater than rates in unirrigated soils (1.7 kg N ha^{-1} yr^{-1}), and rates were considerably less than the amount expected to occur (i.e. 65 kg N ha^{-1} yr^{-1}) when the land treatment system was designed (Cooper pers. com.). Furthermore, the size of the denitrifying population (as determined by DEA) was also small and not significantly different between unirrigated and irrigated sites. The purpose of the following study was to: i) examine which factors may be limiting the size of the denitrifying population by establishing the influence of soil moisture content, nitrate and organic carbon on DEA; and ii) determine if wastewater-irrigation has altered the rate at which denitrifying enzymes are synthesised under anaerobic conditions in the land treatment system.

5.3 MATERIALS AND METHODS

5.3.1 Soil and site details

The Rotorua Land Treatment System (RLTS) is located in Whakarewarewa Forest, New Zealand (38°10'S, 176°16'E) and was established in October 1991 (Tomer *et al.* 1997). The upland soils (soils not influenced by groundwater) form part of a commercial *Pinus radiata* forest (242 ha) which is irrigated, weekly, with tertiary treated wastewater (11 mg N L^{-1}). Soils are pumiceous sandy loams, which are classified as Vitric Orthic Allophanic Soils (New Zealand Soil Classification System; Hewitt 1993), and selected soil properties from irrigated and unirrigated sites are recorded in Table 5.1. In the study area, the topography is mainly moderately steep slopes (12-23°), and supports a *Pinus radiata* forest which was planted in 1975. During the study, soils were collected at least four days after wastewater irrigation.

5.3.2 Effects of adding carbon, nitrate and soil moisture on DEA in wastewater-irrigated soils

To determine the relative importance of carbon, nitrate and moisture additions on the size of the denitrifying population in wastewater irrigated soils, a factorial experiment was conducted with two soils (unirrigated and irrigated), three methods for creating anaerobic soil conditions (none, additions of de-ionised water at 470 g H_2O kg dry $soil^{-1}$, incubation under N_2 head-space), two nitrate additions (0, 100 mg NO_3-N kg dry $soil^{-1}$), and two carbon additions (0, 2 g C-straw kg dry $soil^{-1}$). Four replicates of each treatment were used. At the commencement of the experiment, amendments were added to 150 g samples of sieved (<4 mm), field moist soil in polyethylene bags. Lucerne straw (<2 mm) was added and thoroughly mixed with the soil, followed by either de-ionised water or potassium nitrate solution. Soils were further mixed, before being packed into 250 mL Erlenmeyer flasks to a similar bulk density (0.6 g cm^{-3}). Each flask was capped with a rubber stopper and the N_2 treatments flushed with oxygen-free nitrogen gas for 15 minutes. All treatments were incubated at 25 °C for seven days. Except for the N_2 treatments, flasks were aerated daily by removing caps for 15 minutes. After one week, the flasks were removed from the incubation cabinet and stored overnight at 4 °C until DEA analysis the following day.

To measure soil DEA (Smith and Tiedje 1979), 10 g of fresh soil was weighed into glass screw-top jars (120 mL) with lids fitted with a rubber septum. The jars were flushed with oxygen-free nitrogen gas and to each jar 20 mL of solution containing 1 mM glucose and 1 mM potassium nitrate was added, followed by 10 mL of acetylene gas to inhibit the reduction of N_2O to N_2 (Yoshinari *et al.* 1977). Each jar was shaken at 25 °C for 15 minutes before 5 mL of head-space was removed and stored in a 3 mL evacuated Vacutainer. One hour later, another 5 mL of head-space was removed and stored as before, until N_2O analysis. After DEA analysis, the soil moisture content was determined gravimetrically after drying sub-samples at 104°C for 24 h.

The N_2O concentration of the gas samples was measured using a Hewlett Packard gas chromatograph, fitted with an electron capture detector (350 °C). Gases were separated using a porous packed column (HaySep Q) at 70 °C and at an injector port temperature of 140 °C. The carrier gas (argon with 10 % methane (v/v)) had a flow rate of 30 mL min^{-1} and was passed through a molecular sieve (5A 45/60 mesh).

5.3.3 Effect of wastewater-irrigation on the rate of denitrifying enzyme synthesis

In a second experiment, the rate of nitrous oxide production by denitrifiers under anaerobic conditions was compared for two sources of soil (unirrigated, irrigated) and two rates of chloramphenicol (0, 2.5 mg g fresh $soil^{-1}$). Six replicates of each treatment were used and the soil was incubated for three days at 25 °C. Ten grams of fresh soil (<4 mm) was weighed into glass screw-lid jars (120 mL) fitted with rubber septums. To each jar, 20 mL of solution containing 1 mM glucose and 1 mM potassium nitrate was added, and to half the jars, chloramphenicol was added. A comparison between soils plus chloramphenicol, with soils without chloramphenicol enabled changes in the denitrification rate to be attributed to the synthesis of new enzymes (Smith and Tiedje, 1979). Flasks were flushed with oxygen-free nitrogen gas and amended with 10 mL of acetylene before being placed on a shaker to be continually mixed throughout the incubation. Gas samples was collected every 15 minutes for the first 2.5 h; every 30 minutes between 2.5 and 5 h; hourly between 5 and 12 h, 12 hourly between 12 and 72 h. The N_2O concentration of the gas samples was measured using gas chromatography, as previously described.

5.3.4 Data analysis

Denitrification rates and DEA were corrected for the N_2O dissolved in the soil solution using the Bunsen coefficient (Tiedje 1982). The distribution of the denitrification rates and DEA was negatively skewed and consequently transformed using natural log to normalise data before commencing statistical analyses. Comparisons of treatment means was conducted using the general procedures model using SAS (SAS Institute Inc. 1989). Reported differences were significant at the 5% level.

5.4 RESULTS

5.4.1 Effects of carbon, nitrate and soil moisture on DEA

Initially, soil DEA was small and not different between irrigated and unirrigated sites (Table 5.1). Soil nitrate was greater in the irrigated soil than the unirrigated soil; whereas soil ammonium and total carbon were not different.

Table 5.1. Selected soil properties for wastewater-irrigated and unirrigated soils in the RLTS

Soil	Bulk density ($g\ cm^{-3}$)	DEA ($ng\ N_2O\text{-}N\ g^{-1}\ h^{-1}$)	NO_3-N ($\mu g\ g^{-1}$)	NH_4-N ($\mu g\ g^{-1}$)	Carbon (%)	pH[A]
Irrigated	0.51	0.20	7.0	1.8	6.3	6.5
Unirrigated	0.53	0.30	0.8	2.6	6.1	5.5

[A] 1:2.5 soil:water ratio

All irrigated soil treatments had a larger DEA after seven days incubation than at the beginning of the experiment, although decreasing soil aeration (by either increasing soil moisture content or incubating under N_2) had the greatest effect on DEA (Figure 5.1) in the irrigated soil. After seven days, in the irrigated soil, either decreasing soil aeration alone, or decreasing aeration with additions of carbon and nitrate, increased soil DEA by at least 1000-fold. In contrast, adding nitrate and carbon, without decreasing soil aeration, only increased DEA by 40-fold in the irrigated soil. In the unirrigated soil, only decreasing soil aeration in combination with adding nitrate, increased DEA after seven days (Figure 5.1). Increases in DEA after seven days were greater in irrigated than unirrigated soil treatments. For example, adding nitrate, carbon and water resulted

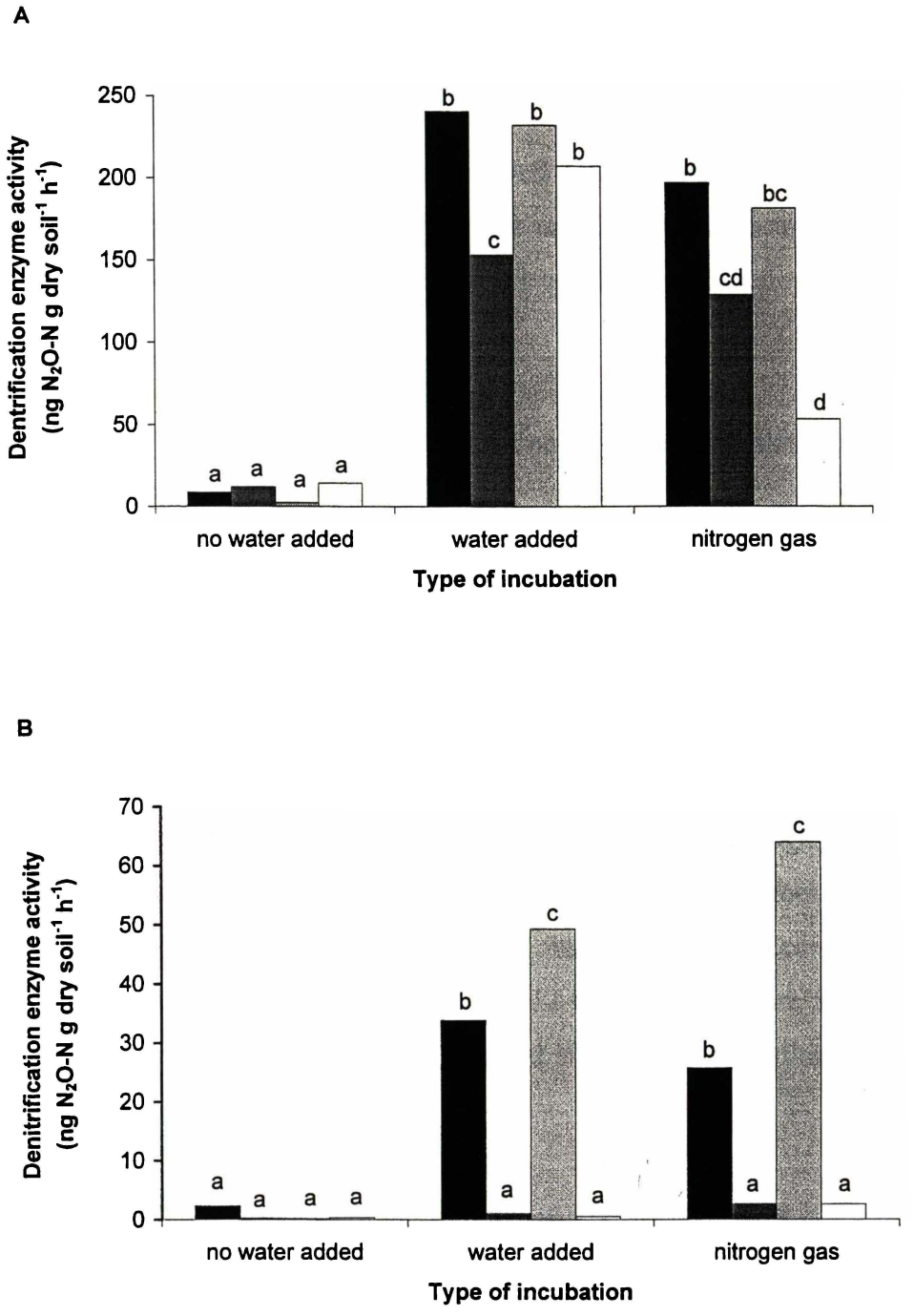

Figure 5.1. Changes in DEA in wastewater-irrigated (A) and unirrigated (B) soils after seven days incubation. Treatments were plus N, plus C(■); plus C (■); plus N (■); control (□). Treatments in the same soil with the same letter are not significantly different ($p < 0.05$). Note differences in y-axis scale.

in a DEA of 240 and 34 ng N_2O-N g dry $soil^{-1}$ h^{-1} in irrigated and unirrigated soils, respectively.

Generally, incubating soils in a N_2 atmosphere increased soil DEA to the same extent as when DI water was added (Figure 5.1). Consequently, DEA was greater in most irrigated treatments, and in the unirrigated plus nitrate treatment, after seven days incubation under N_2. The exception in the unirrigated soil occurred when no nitrate or carbon was added. In this case, DEA was less after N_2 incubation than when water was added.

5.4.2 The effect of wastewater-irrigation on the rate of denitrifying enzyme synthesis

Two or three phases of denitrification were observed in soils without chloramphenicol added. The duration of phases differed between irrigated and unirrigated soils. In the irrigated soil, an initial phase lasted from 15 min to approximately 5 h (Figure 5.2a). The initial phase of denitrification, referred to as Phase I (Smith and Tiedje 1979), was followed by a second increase in rate (transition phase; Smith and Tiedje 1979) until 12 h after which a third linear phase was recorded (Phase II)(Figure 5.2a insert). The denitrification rates of each phase were significantly different from the other phases. In the unirrigated soil, no transition phase was observed. Phase I occurred between 15 min and 12 h, while Phase II occurred between 12 h and 72 h (Figure 5.2b insert). Phase II was greater in soils collected from irrigated sites than soils collected from unirrigated sites (Table 5.2).

The effect of chloramphenicol on irrigated and unirrigated soils is illustrated in Figure 5.2. Chloramphenicol significantly decreased Phase II rate in both soil types, but had no significant effect on Phase I.

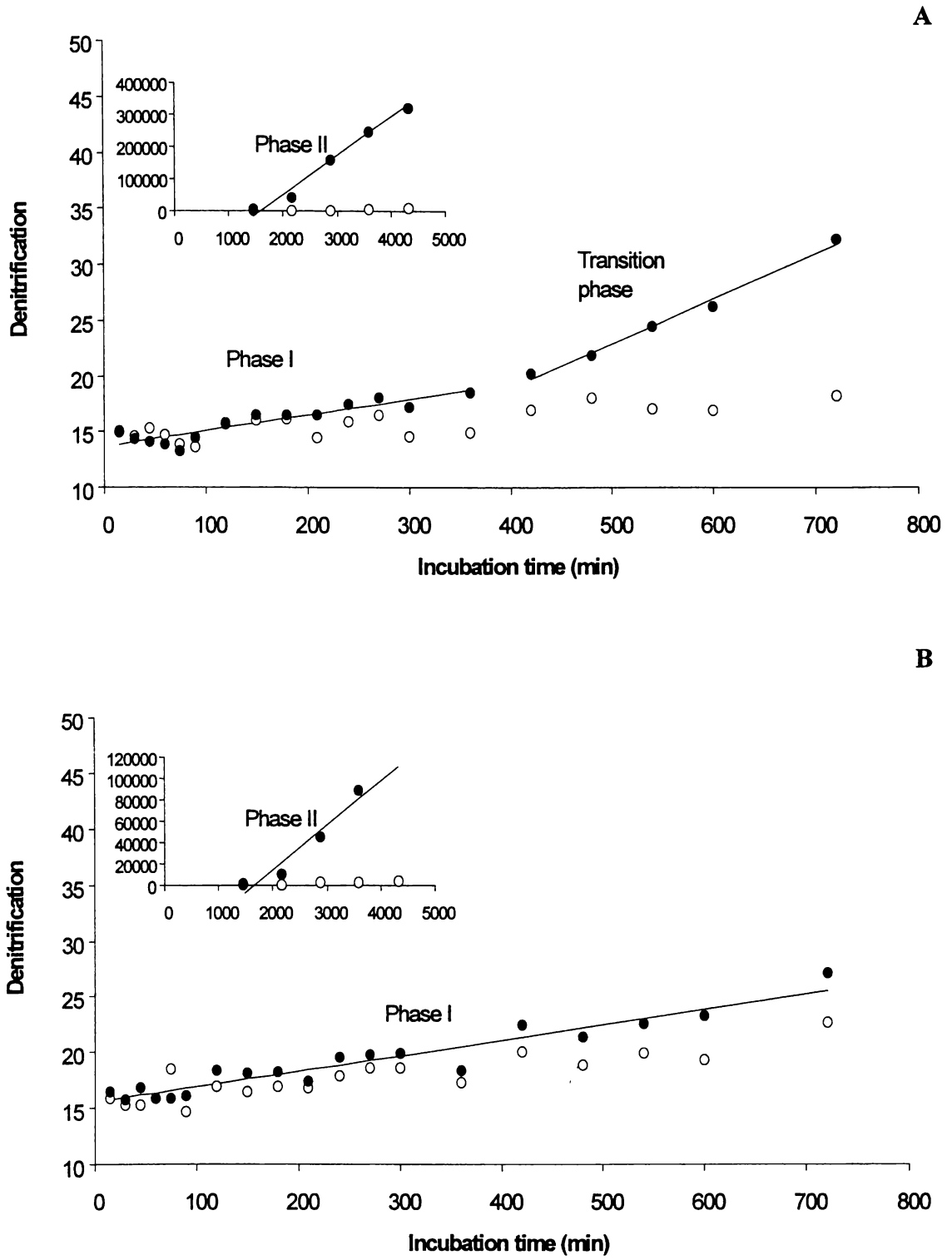

Figure 5.2. Effect of chloramphenicol on denitrification (ng N_2O-N g dry $soil^{-1}$) in wastewater-irrigated (A) and unirrigated soils (B), where soils are treated with (○) and (●) without chloramphenicol. Each value is a mean of six replicates.

Table 5.2. Changes in denitrification rates with time for irrigated and unirrigated soils which did not receive chloramphenicol.

Denitrification rates with the same letter are not significantly different at $p < 0.05$.

Phase	Time of anaerobic incubation (h)	Denitrification rate (ng N_2O-N g dry soil h^{-1})
Irrigated soil		
Phase I	0 - 5	0.01[a]
Transition	5 - 12	0.04[b]
Phase II	12 - 72	122[c]
Unirrigated soil		
Phase I	0 - 12	0.01[a]
Transition	not detected	not detected
Phase II	12 - 72	41.4[d]

5.5 DISCUSSION

5.5.1 Factors restricting denitrification

Denitrification is a biological process, which only occurs when oxygen is absent and carbon and nitrate are present (Tiedje 1988). Oxygen is known to decrease denitrification by two mechanisms: it inhibits the activity of denitrifying enzymes present and represses the synthesis of new enzymes i.e. the size of the denitrifying population (Smith and Tiedje 1979). In the laboratory, limiting oxygen availability in wastewater-irrigated soils increased the denitrifying population, and to a greater extent than previously observed in the field (Chapter Four). Additions of carbon and nitrate to anaerobic soils, however, did not further increase the denitrifying population above controls in the irrigated soils. The size of the denitrifying population in the top 15 cm of the wastewater-irrigated soils therefore appears to be limited by soil aeration, despite weekly wastewater-irrigation, rather than nitrate and carbon. It is likely that soils are rarely anaerobic enough to promote denitrification activity because of the very free-draining nature of the soil (Cook *et al.* 1994).

Wastewater irrigation appears to have altered the factors limiting denitrification in the RLTS. In the unirrigated soils, the denitrifying population was limited by both aeration and nitrate availability. In the irrigated soils, application of nitrate-containing wastewater has increased soil nitrate so that it is no longer a limiting factor. In the

RLTS, soils collected from unirrigated sites had nitrate concentrations less than has been suggested to be necessary to support denitrifying biomass. For example, Jacobson and Alexander (1980) suggested at <1 mg N kg^{-1} the size of the denitrifying population may be restricted. Denitrification activity in the unirrigated soils in the RLTS is limited by the same factors normally reported for unfertilised forest soils (Roberston and Tiedje 1984; Robertson *et al.* 1987).

In irrigated and unirrigated soils, soil moisture content mostly increased enzyme synthesis by limiting soil aeration. However, in addition to restricting oxygen diffusion, increasing soil moisture appears to have increased the denitrifying population by redistributing carbon and nitrate to denitrifying microsites in irrigated soils, unamended with carbon and nitrate. Consequently, while incubating irrigated soils with nitrogen gas generally increased the denitrifying population to a similar amount as when soils were incubated with water, the increase was not as great when nitrate and carbon were not added.

5.5.2 The effect of wastewater-irrigation on the rate of denitrifying enzyme synthesis

The denitrification response to anaerobic conditions in soils collected from the RLTS was consistent with the findings of Smith and Tiedje (1979). An initial constant rate, Phase I, was attributed to the activity of enzymes already present in the soil. Phase I was not different between irrigated and unirrigated soils, suggesting that there was no difference in the initial size of the denitrifying population between the two soils (c.f. Chapter 4). After 5 h in the irrigated soil, and 12 h in the unirrigated soil, nitrous oxide production occurred at a greater rate than Phase I, and was attributed to enzyme synthesis (Phase II) as chloramphenicol inhibited its increase.

Although wastewater irrigation has not increased the size of the denitrifying population in the RLTS, it appears to have altered the response of the denitrifying population to anaerobiosis. In the laboratory, the onset of enzyme synthesis and the rate of enzyme synthesis differed between irrigated and unirrigated soils. Firstly, enzyme synthesis occurred earlier in the irrigated soil than the unirrigated soil. Secondly, enzyme synthesis was three times greater in the wastewater-irrigated soils than the unirrigated

soils. Sotomayor and Rice (1996) also showed the rate of denitrifying enzyme synthesis varied between land use, with greater enzyme synthesis occurring in grassland than cultivated soils. The results from the RLTS indicate that although both irrigated and unirrigated soils might have had the same initial DEA, denitrifiers in the irrigated soils were more responsive to changes in soil oxygen concentrations. The reason for the denitrifying population growing at a faster rate in the irrigated than unirrigated soil was not determined in this study. However, as the onset and rate of enzyme synthesis has been shown to vary between denitrifier species (Smith and Tiedje 1979), it is possible that the wastewater-irrigated soils contains different denitrifier species to the unirrigated soil.

In the RLTS, field soil temperatures are less than 25 °C, and there may be less potential, therefore, for denitrifier growth in the field in comparison to laboratory results (Focht 1974). Currently in the RLTS, irrigation occurs for 12 h, after which the soil remains near-saturated for approximately 1-3 h (Tomer 1998). Under current irrigation scheduling, soils in the RLTS are therefore unlikely to remain anaerobic for a sufficiently long period of time (i.e. at least 5 to 12 h) to induce denitrifier growth.

In conclusion, the size of the soil denitrifying population in the irrigated soils of the RLTS was limited by low soil moisture contents. Soil moisture contents were too low to restrict oxygen availability, despite weekly irrigation for 12 h at 5 mm h^{-1}, and thus promote denitrification enzyme synthesis. However, wastewater-irrigation has increased soil nitrate such that nitrate is no longer limiting denitrification in the forest soils of the RLTS. The results suggest that the size of the denitrifying population cannot be expected to be large in free-draining, coarsely textured soils, even when provided with additional nitrogen, carbon and water inputs.

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CHAPTER 6

SYNTHESIS AND CONCLUSIONS

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6.1 OVERVIEW AND SYNTHESIS OF RESULTS

6.1.1 Introduction

In land-based treatment systems, applied nitrogen is often assumed to be partially removed by upland soil denitrification. Applying wastewater to soil is thought to enhance conditions required for upland denitrification by i) increasing soil moisture contents, and thus decreasing soil oxygen availability and increasing substrate transport, ii) increasing soil nitrate contents, and iii) increasing available organic carbon. Although many field experiments have quantified the affects of nitrogen fertilisers on denitrification, there is a distinct lack of information regarding the affects of wastewater application on denitrification losses and denitrifying populations in land treatment systems. Furthermore, the effects of irrigation and N fertilisation on annual denitrification rates in upland soils have not been widely reported. Instead, *in situ* denitrification rates and the size of denitrifying populations have mainly been studied in unfertilised forest soils, and fertilised grassland soils, as discussed in Chapter 2.

6.1.2 The effects of wastewater-irrigation on *in situ* denitrification rates in a forested land treatment system

In the Rotorua Land Treatment System (RLTS), wastewater-irrigation does not appear to have greatly enhanced upland denitrification rates (Chapter 4). Although wastewater irrigation increased upland denitrification rates by 40%, the annual rate was only 2.4 kg N ha^{-1} yr^{-1}. Consequently, in the RLTS, upland denitrification removed less than 1% of the wastewater-applied nitrogen. Although upland denitrification rates were small in the RLTS, the annual rate was comparable to those reported in the literature for unfertilised coniferous forest soils, and fertilised sandy and sandy loam grassland soils (Table 6.1). In unfertilised conifer forests, annual denitrification rates have ranged from less than 0.1 to 2.4 kg N ha^{-1} yr^{-1}, while in fertilised sandy, and coarse sandy loam grassland soils, reported annual denitrification rates have not exceeded 6.9 kg N ha^{-1} yr^{-1} (despite nitrogen applications of up to 450 kg N ha^{-1} yr^{-1}).

Table 6.1 Summary of annual denitrification rates in selected forest and agricultural systems.

Summarised from Tables 2.1 and 2.1.

System	Number of studies	Geometric mean (kg N ha^{-1} yr^{-1})	Range (kg N ha^{-1} yr^{-1})
Forest			
Coniferous	9	0.22	<0.1-2.4
Coniferous, plus N	1	0.1	-
Deciduous	9	3.2	0.4-28
Deciduous, plus N	1	11.9	-
Agricultural			
Sand to coarse, sandy loam	1	0.3	-
Sand to coarse, sandy loam, plus N	4	3.0	0.65-6.9
Loam	4	9.8	1.6-17.4
Loam, plus N	20	26	4.5-110

In situ denitrification rates varied considerably in the wastewater-irrigated soils of the RLTS. Spatially, *in situ* denitrification rates varied between irrigation blocks, between topographic positions and within topographic positions. Temporally, denitrification rates varied both between seasons and throughout the week following irrigation. Seasonally, the largest denitrification rates occurred in autumn relative to other times of the year. The pattern of daily denitrification rates after a wastewater-irrigation event changed throughout the year, and as a consequence, there was no consistent denitrification response to irrigation. In accordance with previous studies, the large variation required log-transformation of data prior to statistical analysis and justified the need for large numbers of replicate samples, as was estimated from the initial survey (Chapter 3).

Temporal effects generally contributed more than spatial effects to the total variation in upland denitrification rates in the RLTS (Chapter 4). In the wastewater-irrigated soils, seasonal and day-to-day variation in daily denitrification rates accounted for approximately 76 % of the total variation in upland denitrification rates. Spatially, the difference in denitrification rates between irrigation blocks was the greatest source of spatial variation, contributing 23 % to the total variation in daily denitrification rates, while differences between topographic position and within topographic position only contributed approximately 1% to total variation. It is suggested, therefore, that in the

RLTS, a greater emphasis should be placed on sampling as frequently as possible during the year and in as many of the irrigation blocks as possible, with less attention paid to replicating denitrification measurements at one location and partitioning the landscape into topographic positions.

Most studies do not consider the contributions of different sources of spatial and temporal variability when designing sampling procedures for denitrification measurements. This investigation showed that to determine annual rates of denitrification in the RLTS, it is more important to measure denitrification rates frequently during the year rather than intensively measure denitrification rates at one point in time (Chapter 4). Although other studies have suggested how to account for either spatial (e.g. Parkin *et al.* 1987; Starr *et al.* 1995) or temporal variability (e.g. Groffman and Tiedje 1989; Tiedje *et al.* 1989) individually, the relative importance of both temporal and spatial sources of denitrification to variability does not appear to have been assessed simultaneously.

In the RLTS, *in situ* denitrification rates were found to be low because soil moisture contents were rarely sufficient to create the anaerobic soil conditions required for denitrification. In Chapter Four, a laboratory study (using intact soil cores) showed that denitrification rates only increased when soil moisture contents caused water-filled porosity (WFP) to exceed 80%. During the field study, however, WFP averaged only 60%, with only 16% of the samples exceeding the critical 80% WFP. Furthermore, WFP averaged 70% immediately after irrigation, despite a hydraulic loading of 55 mm over 12 h, and decreased to an average of 63% WFP by day four. The critical moisture content required for upland denitrification to be markedly enhanced in the RLTS is probably rarely achieved because of the very free-drained nature of the pumiceous, sandy-loam soils (Cook *et al.* 1994).

6.1.3 Relating *in situ* denitrification rates to other soil properties

In situ denitrification rates in the upland soil of the RLTS could not simply be related to any one soil or environmental factor. It is suggested that the lack of significant relationships between denitrification and any of the soil or environmental factors was probably due to the lack of variation in denitrification rates generally (i.e. the denitrification rates were low and fairly similar). Correlation analysis between

denitrification rates of individual soil cores and soil factors accounted for less than 5% of the variation in denitrification rates. Multiple regression analysis improved this result only marginally, with soil moisture content, soil respiration, soil temperature and rainfall all significant variables, but only explaining up to 11% of the variability. Furthermore, excluding soil cores which did not exceed the critical moisture content (i.e. 80% WFP) from the analysis did not improve the relationship between denitrification rates and soil properties. Averaging denitrification rates for each of the sample points across time and in each of the irrigation blocks, and relating these to bulk soil properties using multiple regression techniques, slightly improved the prediction of denitrification rates by soil properties, though still only accounted for between 19 and 29 % of the variation in denitrification rates.

In other field studies, multiple regression analysis has only successfully related denitrification rates to soil properties when denitrification rates have differed significantly between sample sites (Table 2.6). For example, denitrification rates have been related to factors which regulate denitrification in landscape studies where topography has significantly affected rates (Groffman and Tiedje 1989) and in field-scale studies investigating the effects of different rates of nitrogen fertiliser on denitrification (Bailey 1997). In the RLTS, and other field-scale studies (e.g. Myrold 1988; Jarvis *et al.* 1994; Roberston and Klemedtsson 1996), where denitrification rates have not varied greatly between sites, denitrification rates may not have been related to soil properties because bulk soil samples do not adequately characterise conditions at the microsite scale (Davidson and Hackler 1994). From this study in the RLTS, and other field-scale studies, it would appear inadvisable to attempt to relate denitrification rates to soil properties using bulk soil properties and multiple regression, unless denitrification rates are expected to vary significantly between sample sites.

6.1.4 Effects of wastewater irrigation on the denitrifying population

In the RLTS, the size of the denitrifying population was small and not greatly enhanced by wastewater-irrigation. In addition, four years of wastewater irrigation had not markedly affected the distribution of the denitrifying population through the soil profile, with most denitrification activity remaining near the surface 10 cm. Consequently, given the size of the denitrifying population, *in situ* denitrification rates would not be expected to exceed 13 kg N ha^{-1} yr^{-1} in the RLTS (Section 4.4.1).

In Chapter Five, laboratory studies indicated that the size and the activity of the denitrifying population in the RLTS was limited by soil aeration. Oxygen decreases denitrification by inhibiting the activity of the denitrifying enzymes present (i.e. denitrification rates) and repressing the synthesis of new enzymes (i.e. the size of the denitrifying population) (Smith and Tiedje 1979). In wastewater-irrigated soils, limiting the oxygen availability in the soils resulted in an increase in the size of the denitrifying population above that previously observed in the field. Additions of nitrate and organic-carbon to anaerobic soils, however, did not further increase the denitrifying population in comparison to controls. Therefore, it was concluded that the denitrifying population in the top 15 cm of the wastewater-irrigated soils in the RLTS, appeared to be limited by soil aeration rather than soil nitrate and carbon availability.

Wastewater-irrigation appears to have increased soil nitrate concentrations to the extent that soil nitrate does not limit the denitrifying population in the RLTS. In unfertilised conifer forests, such as the unirrigated soils in this study, denitrification has often been limited by low nitrate availability (e.g. Vermes and Myrold 1992; Henrich and Haselwander 1997). Furthermore, although wastewater irrigation decreased carbon availability in the field in the RLTS, organic-carbon availability was sufficient for denitrification enzyme synthesis in the wastewater-irrigated soils.

Although wastewater-irrigation has not increased the size of the denitrifying population in the RLTS, it is possible that it may have altered the composition of the denitrifying species in the irrigated soils in comparison to the unirrigated soils. In a laboratory study, denitrifiers in wastewater-irrigated soils were more responsive than denitrifiers in unirrigated soil to changes in soil oxygen concentrations (Chapter 5). Enzyme synthesis occurred earlier, and at a greater rate, in wastewater-irrigated soils than unirrigated soils. In other studies, both the onset and rate of enzyme synthesis has been shown to vary between denitrifier species (Smith and Tiedje 1979), and it is therefore possible that wastewater-irrigated soils contained different denitrifier species to the unirrigated soil in the RLTS. These organisms would appear to be responsive to periodic decreases in oxygen as might occur after an irrigation or rainfall event.

6.2 IMPLICATIONS OF THE STUDY

The results from this study have implications for the performance of the RLTS, land treatment design and approaches to measuring *in situ* denitrification rates in pumiceous volcanic soils, and possibly other free-draining soils.

6.2.1 The contribution of upland denitrification to nitrogen removal in the RLTS

Designers anticipated that upland denitrification, tree uptake and wetland denitrification would contribute markedly to wastewater N renovation in the RLTS (Table 6.2). Upland denitrification was expected to remove 21% of annually-applied nitrogen, based on an expected annual input of 312 kg N ha^{-1} yr^{-1}. Since the commencement of wastewater irrigation, studies have investigated upland denitrification (Chapter 4), tree uptake (Thorn *et al.* 1997), nitrogen storage in the upland soils (Hopkins 1997), soil leachate chemistry and nutrient fluxes (Gielen *et al.* 1997), and wetland nutrient budgets (Tomer *et al.* 1997a; Peacock 1998) (Table 6.2). This thesis has shown that upland denitrification removes less nitrogen than was originally anticipated by RLTS designers (i.e < 1% based on an actual annual input of 300 kg N ha^{-1} yr^{-1}). In addition, upland denitrification renovates substantially less wastewater-applied nitrogen than other processes in the RLTS. For example, tree uptake and wetland denitrification appear to remove 14 and 17 times more nitrogen than upland denitrification, respectively.

Table 6.2 Predicted and measured contributions of different components to nitrogen renovation (kg N ha^{-1} yr^{-1}) in the RLTS.

Numbers in brackets show proportion of wastewater-applied nitrogen removed (%).

Component	Predicted	Measured	Reference
Tree uptake	35 (11)	34 (11)	Thorn *et al.* 1997
Upland soil storage	nd[A]	750 kg N ha^{-1} in first 4 years (60)	Hopkins 1997
Upland denitrification	65 (21)	2.4 (0.8)	Chapter 4
Wetland denitrification	212 (68)	40 (13)	Peacock 1998

[A] nd, not determined

Although upland denitrification in the RLTS is less than anticipated by designers, a review of literature indicates that annual denitrification rates equal to 65 kg N ha^{-1} yr^{-1} have only occurred in N fertilised, loam soils (Figure 2.3). In soils of similar texture to

the upland soil of the RLTS, annual denitrification rates have rarely exceeded 7 kg N ha^{-1} yr^{-1} (despite applications of up to 450 kg N ha^{-1} yr^{-1}).

Although the soil conditions in the RLTS may not be conducive to upland denitrification, volcanic ash soils do have several other physical and chemical properties which make them suitable for effluent irrigation and treatment, including good drainage characteristics (Cook *et al.* 1994) and a large capacity for phosphorus adsorption (Wada 1985). The free-draining nature of the soils allows the profile to withstand large hydraulic loadings without becoming waterlogged, an important consideration if the cover crop is not tolerant to waterlogging. To date, the volcanic soils have successfully removed the phosphorous, with much of the applied phosphorous accounted for in the top 10 cm of the of the soil profile in the RLTS (Hopkins 1997). Feasibility studies have suggested that the soil profile could adsorb the total loading of P from the wastewater for 70 to 80 years (Tomer *et al.* 1997b).

6.2.2 Conceptual model of upland denitrification in forested land treatment systems

From the results of this study and previous literature, a conceptual model is proposed that outlines the combination of soil processes and conditions that must occur to increase upland denitrification rates in a forested land treatment system (Figure 6.1). Alternatively, the model also illustrates how the absence of particular soil processes and conditions will not result in upland denitrification rates being increased. The model has been developed assuming carbon does not limit denitrification in forest soils (section 2.5.3; Chapter 6), consequently this model may not be applicable to soil if carbon availability naturally limits denitrification and carbon is not applied in wastewater.

Applying wastewater to forest soils will only increase upland denitrification rates if the critical moisture content required for denitrification is regularly obtained, and if the soil contains sufficient nitrate and carbon (Figure 6.1). The critical moisture content required for denitrification will vary with soil texture and drainage (Figure 6.1). In a volcanic, sandy loam soil, the results from this study suggests a WFP greater than 80% is required for significant denitrification activity. The frequency with which the critical moisture content is obtained will depend upon the soil drainage characteristics, the wastewater irrigation schedule and rainfall. The availability of soil nitrate and carbon in

a land treatment system will depend upon soil type and wastewater composition. In forest soils, this study (Chapter 5) and previous studies in unirrigated soils (Chapter 2), have indicated that carbon availability will be sufficient in the upper 15 cm of the soil profile for upland denitrification. In contrast, nitrate availability is likely to be too low for upland denitrification in forest soils (Chapter 2), unless wastewater irrigation increases the soil nitrate contents sufficiently, as occurred in the RLTS (Chapter 5). The critical soil nitrate concentration for denitrification in soil ranges from greater than 2 to 15 mg NO_3-N kg $soil^{-1}$ (Figure 6.1).

The likelihood of upland denitrification contributing to nitrogen removal in proposed land treatment sites could be assessed by conducting a few, short-term, laboratory experiments. Firstly, the critical moisture content required for denitrification could be determined (using a method similar to that described in Chapter 4) and compared with expected moisture contents. If it was shown that critical moisture content was likely to be met in a proposed land treatment site, a second experiment could assess if soil nitrate and carbon availability is likely to limit denitrification. However, even if soil conditions are expected to be suitable for denitrification the means for predicting the actual denitrification rate has yet to be developed.

Upland denitrification should not be assumed to contribute substantially to nitrogen removal in forested land treatment systems which are located on free-draining soils. In this study, the excessive drainage of the soils prevented soil moisture contents from becoming large enough to sufficiently limit oxygen availability for denitrification (Figure 6.1). Consequently, in forested land treatment systems which are located on free-draining soils, excess soil nitrate will need to be removed by other mechanisms, such as on-site removal by plant uptake, storage in soil organic and inorganic fractions, and off-site removal by denitrification and plant uptake in wetland soils. Alternatively, the amount of nitrate applied could be decreased by improving wastewater treatment prior to land application, which is an option the Rotorua District Council is currently considering.

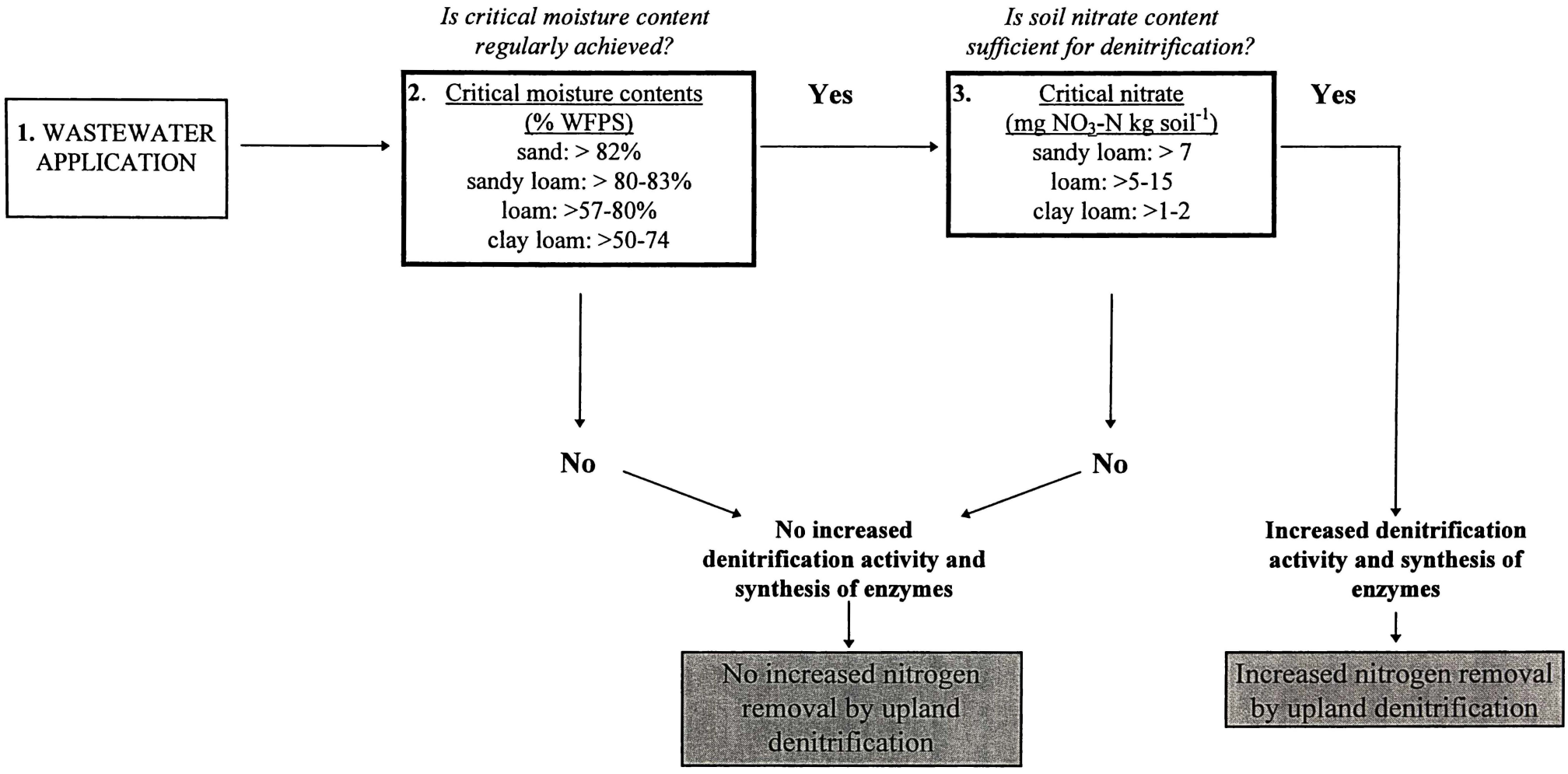

Figure 6.1. Conceptual decision model for soil processes and conditions required to increase nitrogen removal in forest land treatment systems. (Explanatory notes are given in text. Note that carbon is generally not limiting to upland denitrification in forest)

[a] Results from this thesis

[b] Table 2.4

[c] Table 2.5

6.2.3 Measuring *in situ* denitrification rates in land treatment systems

When designing field studies to estimate annual denitrification rates, questions regarding the method for measuring *in situ* denitrification rates, where to sample within the study area, and how frequently samples should be taken during the study, are often asked. The following recommendations for measuring annual denitrification rates in a forested land treatment system, located on young pumiceous soils, are made on the basis of this study in the RLTS:

1. Choosing a method for measuring *in situ* denitrification rates

In the RLTS, and in most upland soils, *in situ* denitrification rates are highly variable. Consequently high sample frequency is required to obtain a precise estimate of denitrification. Soil core techniques using acetylene inhibition are recommended for measuring *in situ* denitrification rates a forested land treatment system because i) the method allows more samples to be collected than other techniques (Tiedje *et al.* 1989); ii) rates measured using soils cores have compared favourably with other techniques (Tiedje *et al.* 1989); and iii) the equipment is easily transported into forests.

2. Number of sample dates during a year

In the RLTS, denitrification rates varied with season, and from day-to-day between irrigation events. To determine annual denitrification rates in land treatment systems located on different soil types, or with different irrigation schedules than the RLTS, it is recommended that: i) a sample procedure for estimating denitrification losses between irrigation events is established using a similar approach to that described in Chapter 3; and ii) denitrification losses between irrigation events are measured as frequently as possible during a 12 month period to account for the seasonal variability of denitrification rates. For example, in the RLTS, denitrification losses between irrigation events were calculated by measuring daily denitrification rates between irrigation events, which was then repeated on 21 separate occasions during a year to determine the annual denitrification rate (Chapter 4).

3. Sampling locations

In young pumiceous landscapes, spatial analysis of the variability of denitrification suggested it is more important to measure denitrification rates in many locations (e.g. there were 18 locations used in this study), rather than stratify the landscape into topographic positions (Table 6.3). At each location in this study (i.e. 'field site'), *in situ* denitrification

rates were not highly variable, and four replicate samples precisely determined denitrification rates within a 200 m^2 area.

In unirrigated, young, volcanic forest soils, the recommendation for measuring *in situ* denitrification rates are similar for irrigated volcanic soils with the exception that denitrification losses are characterised following rainfall events, rather than irrigation.

Table 6.3. The contribution of different sources to the spatial variation of denitrification rates in the RLTS.

Source of variation	% of total variance
Irrigation block	86
Topographic position	5
Field site	0
Sample point	9

6.3 CONCLUSIONS

The main conclusions from this study were:

1. Upland denitrification rates were small (2.4 kg N ha^{-1} yr^{-1}), and contributed less than 1% to removal of wastewater-applied nitrogen in a forested land treatment system located on a pumiceous soil.

2. *In situ* denitrification rates were considerably spatially and temporally variable in the upland soils of a forested land treatment system. Temporal sources (i.e. seasonal and day-to-day variation) contributed more than spatial sources (i.e. irrigation block, topographic position and within topographic position variation) to the variation in daily *in situ* denitrification rates.

3. The size of the denitrifying population in the forested land treatment system was small, and even under optimum conditions for denitrification, would not be expected to remove more than 13 kg N ha^{-1} yr^{-1} from the land treatment system.

4. The main limitation to denitrification in the RLTS appeared to be excessive aeration.

5. Wastewater irrigation altered the response of denitrifiers to anaerobic soil conditions. Denitrifiers in the wastewater-irrigated soils produced enzymes sooner and at a greater rate than denitrifiers in unirrigated soils.

6. To measure *in situ* denitrification rates in a forested land treatment system, measurements need to be taken from at least the upper 10 cm of the soil, including the litter layer. To estimate annual denitrification rates in a forested land treatment system, denitrification rates need to be measured on a daily basis between irrigation events and repeatedly throughout the year. At each sample date, samples need to be taken from locations widely spaced apart, with less attention paid to sampling between or within topographic positions.

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