THE UNIVERSITY of EDINBURGH

Title	Nitric oxide emissions from agricultural soils
Author	Thorman, Rachel E.
Qualification	PhD
Year	2003

Digitisation Notes:

- Pages 107, 109, 123 appear twice in original
- Pages 248 - 250 missing from original

Nitric Oxide Emissions from Agricultural Soils

Rachel E. Thorman

Doctor of Philosophy – The University of Edinburgh – 2003

Declaration

I am responsible for composing this thesis. It represents my own work and where the work of others has been used it is duly acknowledged.

Signed.....
Date.....

Abstract

Nitric oxide (NO) plays a crucial role in photochemistry, particularly in the formation of tropospheric ozone. In soil the biogenic production of NO is primarily conducted by the microbial processes of nitrification and denitrification. The management of soils may, therefore, significantly impact on local atmospheric NO concentrations. The aim of this study was to investigate the influence of various agricultural practices on the magnitude of NO flux, specifically the role of tillage technique in an arable system and the comparative effect of organic wastes and inorganic fertilisers applied to a grassland system.

Fluxes of NO from a sandy loam/silty clay loam soil cropped with spring barley, with and without the addition of NH_4NO_3 fertiliser (80 kg N ha^{-1}), were measured using a static chamber method. The site was managed to compare the influence of 3 tillage regimes; conventional mouldboard ploughing, deep ploughing and direct drilling. There was a marked effect on the magnitude of NO fluxes from both the nitrogen and tillage treatment. Nitric oxide fluxes ranged between deposition and emission from -2.6-49.5 μg NO-N m^{-2} h^{-1} (fertilised & ploughed) and -2.0-2.0 μg NO-N m^{-2} h^{-1} (unfertilised & direct drilled). Emissions of NO were significantly larger from the ploughed soils than from the direct drilled soils, primarily due to the increased water filled pore space stimulating denitrification and reducing NO emission. Of the fertiliser N added 0.002-0.011% was lost as NO.

The flux of NO between ungrazed grassland (clay loam) and the atmosphere was measured following the application, at a target rate of 120 kg available N ha^{-1}, of either cattle slurry, anaerobically digested sewage sludge, thermally dried sewage sludge pellets, mineral NPK fertiliser & Ficote 70® slow release fertiliser or no fertiliser addition. Nitric oxide emissions were stimulated by both organic wastes and NPK inorganic fertiliser, with cumulative fluxes markedly higher from the organic wastes, particularly from the sewage sludge pellets, which were 1.3-42.3 times larger than the other treatments. It was estimated that 0.0004-0.03% of the applied total N was released as NO.

Complementary laboratory studies designed to investigate the influence of dominant environmental factors on NO emission from repacked soil cores under controlled conditions showed that NO emission was 2.2-23.5 times larger from soil amended with sewage sludge pellets. The magnitude of the flux was associated with a soil saprophytic fungus and incorporation of the pellets appeared to reduce the cumulative NO loss. In field and laboratory studies NO flux rate was strongly dependent on soil NH_4^+-N, soil NO_3^--N, soil water filled pore space and the pattern of precipitation, particularly around fertiliser application. The data suggest that NO was primarily produced by nitrification in the grassland soil and a combination of both denitrification and nitrification in the arable soil.

The total flux from UK agricultural land was estimated as 0.007 Tg of NO-N. This is approximately 1.5% of the annual UK total NO-N production. Based on the evidence collected from the 2 field studies, therefore, the emission of NO from agricultural soils in the UK is not significant in terms of its contribution to the NO-N total. However, agricultural soils may emit NO to the atmosphere and produce localised concentrations high enough (e.g. after fertiliser application) to generate harmful levels of tropospheric O_3.

Acknowledgements

My thanks to MAFF who funded the studentship and allowed me to carry out this research.

I would like to express my thanks to my supervisors, Dr. Ute Skiba at the Centre for Ecology and Hydrology, Edinburgh, and Dr. Bob Rees in the Land Management Department of the Scottish Agricultural College, Edinburgh, for their invaluable advice, encouragement and support throughout my PhD studies and particularly in the last year and a half.

Special thanks to Dr. Iain McTaggart, who kindly allowed me to use the plots in his field trial, to have access to his preliminary results and to seemingly never tire of me pestering him for information!

My sincere thanks to all the staff in the Land Management Department, SAC. Many people provided invaluable advice and assistance. In particular, Lesley Swan and Julia Asrih who provided fantastic assistance and humour during soil analysis work. Thanks also to Robert Ritchie and Colin Crawford who provided invaluable help and support in the field and regularly allowed me to pinch their soil augers! I am very grateful for the help, guidance and access to data given by, Albert Scott, Bruce Ball and Ed Robertson. At the Centre of Ecology and Hydrology (CEH), Edinburgh, many people contributed to the successful completion of this thesis. Particular thanks to Robert Storeton-West for his help in all matters concerning trace gas analysers, and to Gordon McFadyen for trying to teach me about physics!. Many thanks to Professor David Fowler for somewhere finding the money to enable me to attend the 10th Nitrogen Workshop in Copenhagen. It was of immense benefit to my academic development. I am also grateful to George, Dave, Alex, Nancy, Davis, Mark, Eiko, Kevin and the many others who helped me.

I am indebted to the staff at ADAS (particularly the soils team, Denise Ginsburg, Jack Harmer, Chris Dyer and Geoff Denton) who have been exceptionally tolerant of me writing up and have provided constant help and support.

Particular thanks to my various office mates during the course of this research, for putting up with me and for all their help and constant laughter; Chris, Trev, Uli, Debbie, Karen, Petra and Franz. Special thanks to Dougal Bruce for his constant support (and for his maths skills!), only hoping I can be half as wonderful this summer.

Finally, thank you to my parents, Michael and Kim Thorman, and to my sister Sarah and her family, without whose unconditional love, support and provision of a great place to work with a constant supply of food, this thesis would never have happened.

Table of Contents

List of figures

List of tables

List of photographs

List of acronyms

CON	Control
CP	Conventionally ploughed
CS	Cattle slurry
DD	Direct drilled
DP	Deep ploughed
GS	Growth stage
MIX	Pellets mixed into the top 7 cm of soil
NPK	Mineral nitrogen NPK fertiliser
N-	Unfertilised
N+	Fertilised
SR	Slow release fertiliser
SSL	Sewage sludge liquid
SSP	Sewage sludge pellets
SUF	Surface applied pellets
TAN	Total ammoniacal nitrogen
WFPS	Water filled pore space

Chapter 1. Literature review

1.1. Introduction

This review aims to evaluate the current knowledge of the processes and the environmental factors affecting them, contributing to NO and N_2O emission. The initial sections are intended to demonstrate the importance of NO and N_2O to atmospheric chemistry and to specifically relate their production to the impact they may have on the local and global environment.

Nitrogen gas (N_2) is the predominant (78%) constituent of the earth's atmosphere, (Prather *et al.* 1995). Less than 1% of the atmosphere, however, is comprised of trace gases including; nitric oxide (NO), nitrogen dioxide (NO_2) and nitrous oxide (N_2O) (Prather *et al.* 1995). Nitrogen in its various forms has historically been greatly influenced by humans (Meixner, 1994). It has been estimated that *circa.* 70% of gaseous emissions of both oxidised and reduced nitrogen species are human induced (NEGTAP, 2001). Accordingly, gaseous nitrogen species play important roles in current environmental issues (Jaffe, 1992). These high profile, nitrogenous trace gases are disproportionately important in atmospheric chemistry. The two gases, NO and NO_2 contribute to acid deposition and are of direct importance in tropospheric ozone (O_3) pollution, due to their role in atmospheric photochemistry. The radiatively significant gases of N_2O and O_3 contribute to the phenomenon of enhanced global warming. Furthermore, N_2O indirectly contributes to regulating the stratospheric ozone concentration, which is currently diminishing, raising fears over growing cancer rates (Anon, 1997).

1.1.1. Nitrogenous gases

The nitrogen compounds of particular interest in relation to this work are the gaseous species, nitrous oxide (N_2O), nitrogen dioxide (NO_2) and of primary concern nitric oxide (NO). The nitrogen oxides of NO and NO_2 are so closely related that when in photochemical equilibrium they are collectively known as NO_x (Conrad,

1990). It is generally assumed that NO contributes to the greater fraction of NO_x emission (Conrad, 1990; Prather *et al.* 1995). This proportion has been estimated by numerous researchers to be ≥ 90% (Slemr & Seiler, 1984; Finlayson-Pitts & Pitts, 1986; Galbally, 1989; Conrad, 1990; Hutchinson & Brams, 1992; Hutchinson & Davidson, 1993; Hutchinson *et al.* 1993a; Hutchinson *et al.* 1993b). The two NO_x gases are highly reactive and hence are short lived in the atmosphere (Slemr & Seiler, 1991; Meixner, 1994). The lifetime of NO_x is estimated to be 1-1.5 days (Crutzen, 1988; Conrad, 1990). Conversely, N_2O has a low reactivity and therefore a longer atmospheric residence time of 100-200 years (Jaffe, 1992; Meixner, 1994). The short lifetime of NO_x means that emissions are not transported far, therefore, they have a dominant effect on the local NO_x mixing ratios. N_2O however, can be transported over a much larger range, and consequently has an impact on atmospheric chemistry some distance from its source.

1.2. Environmental effects of the nitrogenous trace gas species

1.2.1. Ozone

1.2.1.1. Tropospheric O_3

Over the last thirty years rising concentrations of tropospheric O_3 have been reported especially in the Northern Hemisphere and Europe. European protocols, implemented in the early nineties aimed at reducing the concentrations of tropospheric O_3 precursors have been successful, subsequently it is likely that peak O_3 concentrations will continue to fall.

The production, loss and transport of O_3 is regulated by a suite of atmospheric chemical reactions. The principal control on the concentration of O_3 in the troposphere is the level of NO_x, primarily NO. There is a threshold level of NO above which there is formation of O_3 and below which O_3 is lost (Lammel & Graßl, 1995; Prather *et al.* 1995). The critical level of NO has been estimated at 10-30 pptv (Lammel & Graßl, 1995), 5-10 ppt (Crutzen, 1988), < 5 pptv (Conrad, 1990) and 5-10 pptv (Meixner, 1994).

The vast majority of O_3 in the troposphere is produced from photochemical oxidation of primarily methane (CH_4) and carbon monoxide (CO), as well as other non-methane hydrocarbons (NMHC). It is NO which regulates the concentration of hydroxyl (OH), which is the most crucial tropospheric oxidising agent and hence the most important chemical removal agent in the troposphere (Prather *et al.* 1995).

Elevated O_3 concentrations at the earth's surface are a cause of great concern. Ozone is a highly reactive gas and as such has the potential to damage various materials such as paint and rubber. Ozone is perhaps more importantly phytotoxic affecting photosynthesis and causing early senescence of leaves and needles resulting in vegetation die-back (Prinz, 1988). Ground level O_3 pollution has been attributed as the cause of crop damage in the USA (Logan, 1988) and to forest decline in Southern Germany and Southern California (Prinz, 1988). Not only is O_3 phytotoxic and damages everyday materials, but it is also harmful to human health. Headaches and disorientation as well as eye and respiratory irritation are stimulated as the result of inflammation of both the lungs and respiratory tract following oxidation of O_3 (Tortoso & Hutchinson, 1990; Ormeci *et al.* 1999; NEGTAP, 2001).

1.2.1.2. Stratospheric O_3

There is currently much concern over the reported losses of stratospheric O_3. The most dramatic decreases in stratospheric O_3 have been studied over the South Pole. The Antarctic O_3 hole commonly appears from September to October with October averages of 50-70% lower than in the 1960's (Prather *et al.* 1995).

Anxiety over the diminishing stratospheric O_3 concentrations are related to human health worries. The O_3 in the stratosphere prevents harmful UV-B radiation from reaching the earth's surface (Smith, 1997). The O_3 depletion has heightened fears of an escalation in cancer occurrence (especially of skin cancer) due to a rise in the influx of UV radiation to the earths' surface. Nitrous oxide significantly contributes to the regulation of O_3 in the stratosphere.

1.2.2 The Greenhouse effect

The greenhouse effect is an entirely natural process, raising the temperature of the surface of the earth by 33 °C (Duxbury *et al.* 1993). Without this essential process the normal balance of the earth's energy budget would create a much colder global climate. It is now widely believed that the natural greenhouse effect is undergoing enhancement due to the actions of humans. Anthropogenically produced greenhouse gases e.g. CO_2, N_2O, CH_4, chloroflorocarbons-CFC's are being added to the atmosphere. The gases absorb a larger quantity of infrared radiation resulting in enhanced temperatures. It has been predicted that over the next century the average global temperature may rise by 1.5-4.5 °C (Smith *et al.* 1995b). A rise in the earth's surface temperature has wide reaching environmental consequences. Coastal development, agriculture (in particular food production), water supplies and other aspects of society are at risk (Smith *et al.* 1995b).

Nitrous oxide is a naturally produced gas contributing to global warming and atmospheric concentrations are rising primarily as a direct result of the actions of man. Although on a global scale it has been estimated to contribute only 4% to greenhouse warming, N_2O possesses a radiative absorption potential 150 times greater than carbon dioxide, the largest contributor at approximately 50% (Bouwman, 1990; Conrad, 1995). As a consequence N_2O plays a crucial role in atmospheric chemistry (Bouwman, 1990).

1.2.3 Acid deposition and Eutrophication

The major loss of NO from the troposphere is via wet or dry acid deposition (Meixner, 1994; Prather *et al.* 1995; PORG, 1997). Wet deposition is the process which has given rise to the term, 'acid rain'. Acid deposition has the potential to acidify both terrestrial and aquatic ecosystems and nitrogen deposited from the atmosphere may cause adverse effects to these ecosystems. In its capacity as a major plant nutrient, excess N can result in damage to semi-natural plant communities adapted to nutrient-poor habitats. In water bodies, the nitrogen-enriched system can initially support a large population of aquatic flora and fauna, particularly of algae in what is known as an algal bloom. Ultimately, though, this can lead to a depletion in

the water's O_2 supply, due to heterotrophic microbial breakdown of the algae, resulting in subsequent death of fish and other aquatic organisms. Additionally, the algae are often toxic to both animals and humans.

1.3. Global distribution of NO_x and N_2O

1.3.1. NO_x

Although the removal processes of NO_x from the troposphere are well understood, the rate of loss of NO_x from the troposphere is extremely unpredictable. Consequently this variable loss coupled with its short lifetime, high reactivity and its complicated geographical distribution of sources and sinks dictates that both the spatial and temporal global distribution of tropospheric NO_x is not well defined (Conrad, 1990; Prather *et al.* 1995). Consequently, tropospheric mixing ratios of NO_x extend over four orders of magnitude (Conrad, 1990).

1.3.2. N_2O

In contrast to NO_x and as a consequence of its long atmospheric residence time, N_2O displays a relatively stable atmospheric distribution with only small temporal and spatial deviations (Prather *et al.* 1995). There is however a source imbalance between hemispheres resulting in an excess source of 5 Tg N y^{-1} in the Northern hemisphere (Prather *et al.* 1995).

1.4. Sources of Tropospheric gases

As a signatory state to the United Nations Framework Convention on Climate Change (UNFCCC) (1992) the UK is obliged to establish a national emissions inventory. The inventory aims to accurately assess all anthropogenic sources of greenhouse gases, including nitrous oxide (N_2O). Currently, the UK emission inventory of N_2O loss from agricultural soils is derived using emission factors based on the Intergovernmental Panel on Climate Change (IPCC) methodology.

Additionally, NO_x emissions are reported by member states of the United Nations Economic Commission for Europe (UNECE) under the Convention on Long-Range Transboundary Air Pollution (CLRTAP) (1988). The inventories for NO_x and N_2O are revised annually in order to take into consideration new information regarding improved methodologies and activity data (NEGTAP, 2001).

1.4.1. NO_x

NO_x may be emitted into the troposphere from a wide range of sources, either biogenic or non-biogenic (Table 1.1). The contribution of each source varies depending on the geographical location and type. Non-biogenic sources are generally restricted to small geographical areas where emissions are larger and fairly easy to gauge (Williams *et al.* 1992). Biogenic sources however, tend to be smaller and more diffuse so quantification of emissions is harder (Williams *et al.* 1992).

1.4.1.1. Non-biogenic

1.4.1.1.1. Fossil fuel combustion

In the industrialised countries of northern America and Europe by far the most important source of tropospheric NO_x is the burning of fossil fuels. The largest regional sources globally are from fossil fuel combustion in North America and Europe where 6 Tg (N) yr^{-1} and 7.3 Tg (N) yr^{-1} respectively are emitted (Prather *et al.* 1995). Approximately, 94% of NO_x emissions in the UK, Germany, France, Belgium and the Netherlands have been reported to have been released from fossil fuel combustion (Simpson, 1993).

In an effort to reduce NO_x emissions over the last 17 years, UNECE has introduced several international pollutant emission control protocols e.g. CLRTAP, Gothenburg protocol. Annual figures for the UK indicate that NO_x emissions have actually decreased from a peak of 850 kt NO_x-N in 1980 to 500 kt NO_x-N in 1997 primarily from a reduction in anthropogenic emissions (NEGTAP, 2001). Model predictions estimate that this decline will continue so that in 2010 the UK NO_x emissions are estimated to be only 359 kt-N (NEGTAP, 2001).

Table 1.1. The estimated annual global emissions of NO_x taken from various sources.

	Magnitude (Tg (N) yr^{-1})			
	Crutzen, 1988	Bouwman, 1990	Prather *et al.* 1995	Skiba *et al.* 1997
Sources				
Fossil fuel combustion	20	14-28	24	21
Soil	5-15	4-16	12	6-20
Biomass burning	5-10	3.6-6.7	8	
Lightning	3-8	2-20	5	
NH_3 oxidation		1-10	3	
Aircraft			0.4	
Transport from stratosphere		1-10	0.1	

1.4.1.1.2. Aircraft

Combustion of aviation fuel accounts for only *circa.* 3% of the total fossil fuel combustion, consequently emissions from aircraft engines contribute a relatively small amount to the global NO_x source (Table 1.1.).

1.4.1.1.3. Lightning

Globally the contribution of lightning to the total NO emission has been assessed at 5 Tg N y^{-1} (Prather *et al.* 1995) of which Europe has been estimated to contribute 0.02 Tg N y^{-1} (Simpson *et al.* 1999).

1.4.1.1.4. Ammonia Oxidation

An estimated 10-20% of atmospheric NH_3 is oxidised via OH• to yield a variety of nitrogen compounds including both NO_x and N_2O (Bouwman, 1990). Above NO_x concentrations of 60 ppt however, NH_3 oxidation may act as a sink for NO_x (Bouwman, 1990).

1.4.1.2. Biogenic

In industrialised countries biogenic sources of NO_x are much smaller compared to anthropogenic sources (Williams & Fehsenfeld, 1991).

1.4.1.2.1. Soil

In rural areas remote from polluting anthropogenic emissions however, natural sources of NO_x, especially from soil, may well be more important to tropospheric chemistry than emissions released non-biogenically (Conrad, 1990; Williams *et al.* 1992; Prather *et al.* 1995). However, figures for the emissions of NO_x from soil still remain highly uncertain and exceedingly variable both spatially and temporally (Yienger & Levy, 1995; Stohl *et al.* 1996; Simpson *et al.* 1999).

Davidson & Kingerlee (1997) predicted an inventory estimate of the global NO soil source to be 21 Tg N y^{-1}, although in the same publication Skiba *et al.* (1997) stated that globally NO_x release from soils may account for approximately half of that attributed to the combustion of fossil fuels, i.e. in the order of 10 Tg N y^{-1}. This is a similar figure to that obtained by Yienger & Levy (1995) who used a combination of an inventory and modelling to calculate the global emission of NO at 10.2 Tg N y^{-1}.

On a global scale Europe contributes to > 20% of the global NO_x total, but unlike other continents biogenic NO_x emissions are not significant (Davidson & Kingerlee, 1997; Simpson *et al.* 1999). This is primarily due to the climate and size of Europe, which represents only 7% of the global land area (Davidson & Kingerlee, 1997). European biogenic emissions, which are dominated by loss from soils, may be substantial though within individual countries (Simpson *et al.* 1995; Simpson *et al.*

1999). Certainly in less industrial, warmer countries of southern Europe that are identified as low NO_x areas (e.g. Albania, Spain, Portugal and Turkey), it has been predicted that emissions from soils could account for 24 - 62% of the total annual emission (Simpson, 1993; Simpson, 1995). Conversely, in the more developed, cooler countries of Europe that are generally high NO_x areas, (e.g. UK, Germany and France) it is estimated that soils only contribute to < 6% of the total annual emission. (Simpson, 1993; Simpson *et al*, 1995).

Studies carried out, particularly in developed countries, indicate that the largest NO_x emissions from soil are released from areas employed in agriculture. One of the largest uncertainties in estimating soil NO emissions in developed regions of the world (e.g. Europe) is the contribution from intensively fertilised agricultural land (Simpson *et al.* 1999). Nonetheless, Stohl *et al.* (1996) suggested that 72-81% of European soil NO emissions is from arable land. In particular it has been estimated that globally fertiliser stimulated NO emissions amount to 0.5-5 Tg N y^{-1} (Cole *et al.* 1996). It is apparent that cultivated land, tropical savanna, chaparral and potentially tropical forests are all globally significant sources of NO.

1.4.1.2.2. Biomass burning

In addition to soil as a significant natural source of NO_x, biomass burning can substantially contribute to the global total. Indeed, biomass burning has been estimated to contribute 15 (Prather *et al.* 1995) to > 30% (Simpson *et al.* 1999) of global NO emissions. Similarly to the pattern of emissions from soil, generation of NO from biomass burning varies greatly between regions of the world. Predominantly burning takes place in tropical and subtropical regions, whilst temperate areas e.g. in Europe are less important. Many fires in the tropics/subtropics are induced by humans especially during the conversion of forested areas to land suitable for agriculture (slash and burn). In relation to global sources there is, however, little distinction made between truly biogenic fires and those which are generated by humans, primarily as a result of sparse information.

1.4.2. N_2O

Prather *et al.* (1995) estimated that atmospheric N_2O concentrations have increased from *circa.* 275 ppbv, prior to the industrial revolution, to *circa.* 311 ppbv in 1992. This has resulted in an estimated yearly increase of 0.5 ppb (Fowler *et al.* 1997). Although the global budget of N_2O is relatively well known the individual sources and sinks remain poorly understood (Bouwman, 1990) and vary widely between countries, especially between industrial nations and those which are still developing. As with NO_x emissions, sources may be of a biogenic or of non-biogenic origin, but globally are very differently partitioned compared to NO_x (Table 1.2).

The dominant source of atmospheric N_2O is from soil (Bouwman, 1990; Fowler *et al.* 1997; Oenema *et al.* 1997) contributing to approximately 65% of the global total, which is roughly equivalent to 9.5 Tg N y^{-1} (Prather *et al.* 1995; Smith, 1997).

1.4.2.1. Non-biogenic

1.4.2.1.1 Industrial sources

Fossil fuel combustion is a very minor source (0.1-0.6 Tg Ny^{-1}) of global N_2O The contribution of fossil fuel combustion to the global N_2O concentration is, however, set to rise, largely as a consequence of the increased use of catalytic converters. In the UK catalytic converters are fitted to all new petrol-engine cars and are successful at cutting > 80% of NO_x emissions, but generate N_2O instead (Anon, 1997).

Nitrous oxide is also a by product of the production of adipic acid, used in the manufacture of nylon (Prather *et al.* 1995) and although on a global scale production from this source is relatively small, in the UK in 1990 it accounted for 72% of national N_2O emissions, releasing 81 kt of N_2O into the atmosphere (Anon, 1997). In 1998 the only adipic acid plant in the UK achieved emission cuts of > 95% (Anon, 1997).

Table 1.2. The estimated annual global emissions of N_2O taken from various sources

			Magnitude (Tg (N) yr^{-1})	
			Prather *et al.* 1995	
Sources			**Range**	**Likely**
Biogenic	Oceans		1-5	3
	Tropical soils	Wet forests	2.2-3.7	3
		Dry savannas	0.5-2.0	1
	Temperate soils	Forests	0.1-2.0	1
		Grasslands	0.5-2.0	1
	Cultivated soils		1.8-5.3	3.5
	Biomass burning		0.2-1.0	0.5
Non-biogenic	Industrial sources		0.7-1.8	1.3
	Cattle & feed lots		0.2-0.5	0.4

1.4.2.2. Biogenic

1.4.2.2.1. Soil

The most important biogenic sources of N_2O are oceans and tropical forest and savanna soils. In fact tropical soils in themselves are probably the single largest source of global atmospheric N_2O (Prather *et al.* 1995; Smith, 1997), although it should be noted that emissions resulting from tropical agricultural soil as a result of human activity is included under the biogenic umbrella (Prather *et al.* 1995).

Cultivated soils contribute an estimated 3.5 Tg N y^{-1} to the global total. (Prather *et al.* 1995) and as a source have contributed to the global rise in

atmospheric N_2O primarily through the intensive use of synthetic nitrogenous fertilisers (Bouwman, 1990). Consequently, chemical nitrogenous fertilisers alone are estimated to contribute 1.5 Tg N y^{-1} to the global N_2O total attributed to cultivated soils (Cole *et al.* 1996). As a result of the Kyoto Protocol (1998) the UK (in its role as an EU member) has agreed to undertake legally binding reductions in greenhouse gas emissions of 12.5% of 1990 levels by 2008-2012 (http://unfccc.int/resource/convkp.html).

1.4.2.2.2. Biomass burning

The magnitude of emissions of N_2O from the burning of biomass is relatively small (0.5 Tg Ny^{-1}), but problematical to assess and relatively few studies have been carried out, especially in the tropics (Prather *et al.* 1995). The accuracy of emission estimates, therefore, remains uncertain (Prather *et al.* 1995).

1.4.2.2.3. Oceans

Oceans are significant emitters of N_2O (3 Tg Ny^{-1}) as a result of upwelling regions within the Indian and Pacific oceans (Prather *et al.* 1995).

1.5. Variability of NO_x and N_2O emissions

The release of NO_x and N_2O from soils is highly variable both temporally (Goodroad & Keeney, 1984) and spatially (Stohl *et al.* 1996), which renders the quantification of emissions very difficult. Soil is known to be a highly heterogeneous substance containing microsites where the microbial community experiences different conditions compared to the bulk soil. Processes influenced by micro-organisms often display a positively skewed, log normal frequency distribution where the majority of measurements are low, but a few are exceedingly high (Parkin, 1987). The spatial variability of both N_2O (Goodroad & Keeney, 1985) and NO is often greater when the gas concentrations are highest e.g. on cultivated soils after application of fertilisers, when nutrients will concentrate in patches. As a result of

variation it is desirable to increase the number of replicates in an experiment, since the estimation variance may decrease, however, this is usually impracticable due to lack of time and an increase in cost (Velthof & Oenema, 1995a).

1.6. Production of NO and N_2O

1.6.1. Biological

There are two main processes involved in the biological production of NO and N_2O in the soil environment; microbial nitrification and denitrification.

1.6.1.1. Nitrification

1.6.1.1.1. Chemoautotrophic nitrification

Nitrification is the biological oxidation of ammonium, to nitrate and nitrite in the presence of oxygen. The nitrogen oxides, NO and N_2O are produced as a by-product, when soil conditions for the completion of nitrification become sub-optimal (Davidson, 1991; Conrad, 1990). The current hypothesis is that the bulk of nitrification, especially in neutral agricultural soils, is carried out by chemoautotrophic bacteria (Conrad, 1995). Chemoautotrophs are obligate aerobes (i.e. microbes that can only respire aerobically), that is they gain their energy from the oxidation of inorganic substrates and fix their carbon in the form of atmospheric CO_2 (Killham, 1994; Davidson & Schimel, 1995).

The dominant chemoautotrophic nitrifying bacteria, *Nitrosomonas* and *Nitrobacter* exhibit substrate/product interaction (Killham, 1994). Nitrification of ammonium by the bacteria *Nitrosomonas* (also *Nitrosococcus*, *Nitrosolobus* and *Nitrospira*) generate the product nitrite, which *Nitrobacter* subsequently utilises as a substrate during further nitrification to nitrate (Equation 1.1).

$$NH_4^+ \rightarrow NH_2OH \rightarrow (HNO) \rightarrow NO_2^- \rightarrow NO_3^- \qquad (1.1)$$

$$\downarrow \qquad \downarrow$$

$$(NO) \qquad NO$$

$$\downarrow \qquad \downarrow$$

$$(N_2O) \qquad N_2O$$

The production of N_2O arises during the first oxidation stage via (mainly aerobic) nitrifier denitrification. The reduction of NO_2^- is used as a substitute electron acceptor when O_2 is limiting (Anderson & Levine, 1986; Firestone & Davidson, 1989; Conrad, 1990; Klemedtsson *et al.* 1990; Umarov, 1990; Davidson, 1993). It is therefore necessary to have enough O_2 present to permit NH_4^+ oxidation, but for O_2 to be partially limiting in some microsites to allow the generation of N_2O (Davidson, 1993). The pathway for the production of NO during nitrification is less well understood, indeed more than one pathway may be present. It is possible that NO is produced from the same mechanism that generates N_2O, or NO may be generated as a by-product from an intermediate (hydroxylamine/nitroxyl) formed during the oxidation of NH_4^+ to NO_2^- (Anderson & Levine, 1986; Firestone & Davidson, 1989; Davidson, 1993).

1.6.1.1.2. Heterotrophic nitrification

NO and N_2O are not only generated during chemoautotrophic nitrification, but in acidic conditions they may also be produced as a by-product of heterotrophic nitrification. Nitrification by heterotrophs is a common process carried out by both fungi and bacteria, but fungi, e.g. *Aspergillus flavus*, are regarded as the most abundant and efficient microbial group (Killham, 1986; Robertson & Kuenen, 1990).

Studies have demonstrated that heterotrophic nitrification in agricultural or even acid upland soils is minimal (Killham, 1986), however, it is believed that in

acidic heath (Papen *et al.* 1989) and forest soils fungal heterotrophic nitrification may be dominant (Killham, 1986; Umarov, 1990; Killham, 1994; Conrad, 1995). Schimel *et al.* (1984) studied an acid (pH 5.8) coniferous forest soil in Sierra Nevada, California, USA and observed that neither chlorate nor acetylene, which are both known to inhibit autotrophic nitrification, significantly affected the production of NO_3^-. Moreover, the addition of the bacteriocide streptomycin had a negligible effect on production, whereas when the fungicide cycloheximide was added NO_3^- production ceased. The results therefore, strongly suggest that the dominant nitrogen gas generation process in the soil was heterotrophic nitrification.

1.6.1.2. Denitrification

Denitrification is the anaerobic reduction of nitrate to N_2O and dinitrogen (N_2) via the intermediate products of nitrite and NO, and is frequently termed dissimilatory nitrate/nitrite reduction (Bouwman, 1990; Killham, 1994). Primarily, denitrification in soil is carried out by heterotrophic bacteria, of which there are many genera, e.g. *Pseudomonas, Alcaligenes* & *Bacillus*. Heterotrophic bacteria are able to utilise nitrate and nitrite in conjunction with a readily oxidisable organic carbon supply for growth. The majority of denitrifying microorganisms are facultative aerobes undergoing respiration, but switch from aerobic metabolism using oxygen as the terminal electron acceptor to anaerobic metabolism utilising nitrate or nitrogen oxides, in the absence or at low partial pressures of oxygen (Bouwman, 1990; Killham, 1994; Davidson & Schimel, 1995). The decline in the O_2 diffusion rate in the soil is generated from either a high soil water content that precludes O_2, or from a large O_2 consumption rate or both.

For denitrification to proceed however, it is not essential for the bulk soil to be under anaerobic conditions. Indeed, it is highly likely, especially in an agricultural soil, that both nitrification and denitrification proceed simultaneously (Arah & Smith, 1990; Davidson, 1993). Aerobic conditions may be dominant in a generally well aerated soil, but pockets of anaerobic microsites may develop (Arah & Smith, 1990) resulting in the establishment of 'hot-spots', that is restricted zones of high denitrification (Parkin, 1987). Pockets of anaerobicity may arise in aggregates, which

remain virtually water filled (following drainage of pores between aggregates), if they are large enough to restrict O_2 diffusion and hence provide a suitable environment for denitrification (Smith, 1980). Additionally, anaerobicity may develop (not necessarily within aggregates) in microsites largely as a result of the stimulation of heterotrophic activity associated with high levels of readily oxidisable organic matter e.g. available carbon added as manure (Parkin, 1987). During denitrification the sequential reduction of nitrate may proceed in one of two ways depending on the position of NO as an intermediate (Conrad, 1990). Firstly, NO may act as an obligatory intermediate (1.2)

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \qquad (1.2)$$

or NO may reach equilibrium with another intermediate (1.3) (Remde *et al.* 1989).

$$\begin{array}{ccccccccc} NO_3^- & \rightarrow & NO_2^- & \rightarrow & (X) & \rightarrow & N_2O & \rightarrow & N_2 \\ & & & & \uparrow\downarrow & & & & \\ & & & & NO & & & & \end{array} \qquad (1.3)$$

Although most denitrification is performed by heterotrophs, there is evidence that autotrophs can denitrify anaerobically (Killham, 1994), e.g. nitrifiers can convert nitrite to NO under anoxic conditions (Conrad, 1995).

1.6.1.2.1. Aerobic denitrification

Alternatively, experiments have indicated that bacterial denitrification may be widespread in aerobic conditions. A number of species of bacteria have been identified which simultaneously utilise O_2 and denitrify, e.g. *Alcaligenes faecalis* produced both NO and N_2O in conditions of air saturation (Anderson & Levine, 1986). The response to O_2 varies widely between species, e.g. *Thiosphaera pantotropha* is able to denitrify at air saturation, but denitrification by *Hyphomicrobium X* is inhibited at relatively low O_2 concentrations (Robertson & Kuenen, 1990). The environmental conditions and nutrient supply are likely to

govern whether versatile aerobic denitrifiers or specialist denitrifiers occur in any given ecosystem.

1.6.1.3. Dissimilatory reduction of nitrate to ammonium

In addition, there is a group of non-denitrifying, fermentative bacteria e.g. *Serratia marcescens*, which produces NO, N_2O and ultimately N_2 in anaerobic conditions during dissimilatory reduction of nitrate and sequentially nitrite to ammonium (Anderson & Levine, 1986; Sahrawat & Keeney, 1986; Robertson & Tiedje, 1987; Conrad, 1995). Dissimilatory reduction to ammonium requires a C:N ratio of > 10, therefore a high availability of organic matter (Umarov, 1990).

1.6.1.4. Assimilatory nitrate/nitrite reduction

Microorganisms (particularly fungi and yeasts) have been shown to produce N_2O and NO from assimilatory NO_3^- (Sahrawat & Keeney, 1986; Conrad, 1990; Hutchinson & Davidson, 1993) and NO_2^- (Sahrawat & Keeney, 1986; Robertson & Tiedje, 1987; Umarov, 1990) reduction. This process arises when e.g. NO_3^- is immobilised by cells and reduced for synthesis of cell constituents (Sahrawat & Keeney, 1986; Killham, 1994).

1.6.2. Abiological

1.6.2.1. Chemodenitrification

Chemodenitrification is the general term given to a group of nonenzymatic, chemical reactions producing nitrogen oxides and in particular NO (Firestone & Davidson, 1989; Bouwman, 1990; Conrad, 1990; Davidson, 1993; Hutchinson *et al.* 1993b). Chemical production of NO_x may be significant under certain environmental conditions, but abiological generation of N_2O remains negligible (Conrad, 1990; Conrad, 1995) especially in anaerobic conditions (Bouwman, 1990). The most important of the reactions is the chemical decomposition of HNO_2 to form NO and

NO_2 (Meixner, 1984) and to a much lesser extent N_2O (Sahrawat & Keeney, 1986) and N_2 (Equation 1.4) (Blackmer & Cerrato, 1986).

$$3HNO_2 \rightarrow 2NO + HNO_3 + H_2O \qquad (1.4)$$

This reaction requires a low soil pH and is accelerated by high levels of organic matter (Blackmer & Cerrato, 1986; Firestone & Davidson, 1989). Commonly the dominant gaseous end product is NO (Galbally, 1989; Conrad, 1990).

Davidson (1992) comments that soil pH is a controlling factor in chemodenitrification of NO_2^-. Above pH 5 reaction rates are insignificant (Firestone & Davidson, 1989; Davidson & Schimel, 1995; Conrad, 1995), but if soil microsites are sufficiently supplied with NO_2^- and have a low pH, production could be substantial (Davidson, 1992).

Accumulation of nitrite is an important factor in the generation of NO abiotically, although this is in part regulated through biological processes, e.g. accumulation is a function of the proximity to NH_4^+ oxidisers during nitrification (Firestone & Davidson, 1989; Davidson, 1993; Hutchinson *et al.* 1993b). Soil physical processes, such as freeze-thaw and wetting and drying of soil could be responsible for a concentration of H^+ and NO_2^- ions in thin water films in soil microsites, which may result in abiological emissions of NO (Davidson, 1991; Hutchinson *et al.* 1993b).

1.7. Consumption of NO_x and N_2O

Studies in both the field (Johansson, 1984; Johansson & Granat, 1984; Slemr & Seiler, 1984) and laboratory (Johansson & Galbally, 1984) have demonstrated that soil may not only act just as a source of atmospheric NO_x and N_2O, but particularly with respect to NO, it may also function as a sink. NO and N_2O are taken up by soils, primarily via microbial processes.

The emission flux of NO_x and N_2O from the soil is a balance between the simultaneous production and consumption of the gases. In both field (Johansson & Granat, 1984; Slemr & Seiler, 1984; Slemr & Seiler, 1991) and laboratory studies (Johansson & Galbally, 1984; Remde *et al.* 1989) the development of a

compensation mixing ratio at the point when the rate of uptake (consumption) of a particular gas species is equal to the rate of its release (production) has been observed (Conrad, 1994). It is the magnitude of this compensation mixing ratio in relation to the size of the ambient atmospheric mixing ratio, which controls the direction and amount of the overall net flux to and from the soil (Conrad, 1990).

When the soil compensation point is lower than that of the mixing ratio of the atmosphere, e.g. if the soil consumption processes are proceeding at a higher rate than production or during an atmospheric pollution episode (Slemr & Seiler, 1984; Conrad, 1995), the soil will act as a sink for NO_x/N_2O. Conversely, when the compensation point is higher than the atmospheric mixing ratio, gas will be emitted from the soil e.g. Remde & Conrad, (1991b) reported an increase in the NO compensation point in anaerobic soil conditions as a result of greater stimulation of NO production versus NO consumption.

The NO_x compensation concentration has been estimated to lie in the range of < 0.1 to 163 ppb, whilst the ambient NO_x is expected to have a concentration of < 0.1 to 24 ppb (Slemr & Seiler, 1991). Consequently, the NO_x compensation point is frequently larger than the ambient mixing ratio and thus produces a net emission, which primarily consists of NO (Delany *et al.* 1986).

In contrast to NO_x the compensation point of N_2O in the field (*ca.* 500 ppb) is generally greater than the atmospheric mixing ratio (*ca.* 350 ppb) (Schuster & Conrad, 1992; Conrad, 1994; Conrad, 1995). Subsequently, N_2O emission from field soil is the dominant flux, although occasionally the soil may function as a sink (Conrad, 1990; Conrad, 1994, Conrad, 1995), e.g. N_2O uptake (peak flux of -11.6 ng N m^{-2} s^{-1}) from a perennial ryegrass sward in Berkshire (UK) was recorded from both fertilised and unfertilised plots when the soil NO_3^--N was < 1 μg N g^{-1} and the soil water content was $> 20\%$ Ryden (1981). The primary route of NO consumption in soil is thought to be a microbial process, specifically via reduction during denitrification (Remde *et al.* 1989).

It is highly possible that before release into the atmosphere any NO produced in the soil from nitrification may be consumed during the reductive process of respiratory denitrification, (Baumgärtner & Conrad, 1992), resulting in an end product of either N_2O or N_2. During a laboratory study in anaerobic soil, NO uptake

was stimulated by both nitrate and glucose addition (Rudolf *et al.* 1996), which suggests that NO consumption was a result of the action of denitrifying organisms, which is not unexpected since denitrifiers possess an extremely high affinity for NO (Remde & Conrad, 1991a; Baumgärtner & Conrad, 1992). Denitrification is also the only process in which significant amounts of N_2O can be consumed and prevented from entering the atmosphere (Klemedtsson *et al.* 1990).

During denitrification the consumption rate of a specific gas is related to both its concentration in the soil and its concurrent rate of production, although the rate of production is independent of the gas concentration (Baumgärtner & Conrad, 1992; Conrad, 1994; Dunfield & Knowles, 1998). Specifically, the quantity of NO is balanced between the production by nitrite reductase and consumption by nitric oxide reductase (Conrad, 1995), whilst the amount of N_2O is a function of the production by nitric oxide reductase and consumption by nitrous oxide reductase (Conrad, 1995).

Denitrifiers have the ability to potentially undergo NO consumption in aerobic soils as NO reductase functions remarkable well due to its relative insensitivity against inactivation by O_2 (Remde & Conrad, 1991b; Conrad, 1995). Nevertheless, given that in some soils consumption (particularly of NO) has been initiated by aerobic rather than anaerobic experimental conditions, anaerobic denitrifiers are not the only group of microbes which are capable of consuming NO and N_2O (Conrad, 1995).

1.7.1. Effect of soil physical properties

Not only does the magnitude of the gas flux depend upon net production, but it is also a function of diffusion (Conrad, 1994). Consumption of N_2O and particularly NO, can be affected by soil physical factors. Molecular diffusion within soil pores, and advective and convective airflows through the soil as a result of pressure and thermal forcing drives the physical transport of NO and N_2O to the soil surface and into the atmosphere (Galbally, 1989). Soil structure and texture can influence the size and connectivity of the soil pores, which in turn affect gaseous diffusion. A soil with a more tortuous pathway for gaseous diffusion is more likely to lose NO through consumption before it can escape to the atmosphere.

Additionally, gaseous diffusion may be impeded via the presence of water (Firestone & Davidson, 1989; Galbally, 1989), which can close or reduce the necks that connect adjacent soil pores (Marshall, *et al.* 1996). It has been noted consequently, that N_2O generated during nitrification has a greater chance of escaping from the soil, than that produced from denitrification (Byrnes *et al.* 1990). Specifically, the ratio of N_2O:N_2 depends on the balance between the diffusion of N_2O away from the site of production, with the rate of reduction to N_2 (Smith, 1980). The slower the diffusion the greater the chance that N_2O will be consumed (Smith, 1980).

1.8. Controls on NO_x and N_2O emissions

The microbial processes, autotrophic nitrification and anaerobic respiratory denitrification, are generally thought of as the largest contributors to the emission of NO_x and N_2O from soil. The production of these gases by nitrification and denitrification is not only controlled by consumption, but also at two other levels; the rate at which nitrification & denitrification proceed and the relative proportions of the final end products (Firestone & Davidson, 1989; Conrad, 1990). This process regulation has been described by the 'hole-in-the-pipe' conceptual model (Figure 1.1.), which proposes that NO and N_2O loss are analogous to liquid flowing through holes in a leaky pipe (Firestone & Davidson, 1989).

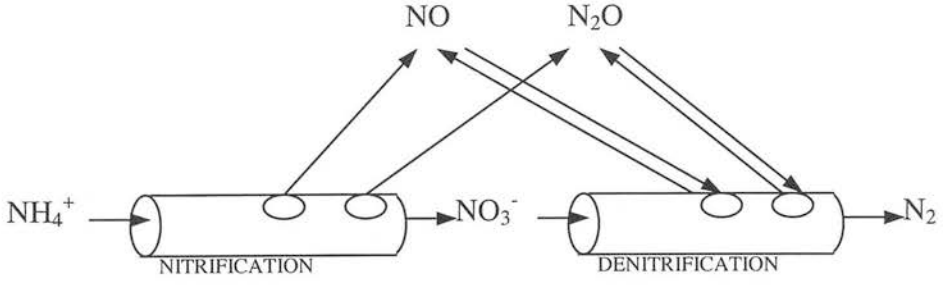

Figure 1.1. The 'hole-in-the-pipe' conceptual model, after Firestone & Davidson, (1989) and Davidson (1993).

In this model the transfer rate of nitrogen along the 'process pipe' is comparable to the rate of nitrogen cycling within the soil and the size of the holes in the pipe through which the gases 'leak' are analogous to the factors which control the partitioning of the products (NO, N_2O or a more oxidised/reduced product) (Firestone & Davidson, 1989). The significance of the 'leaks' is governed by the rate at which nitrogen progresses through the process pipe, e.g. factors which regulate the magnitude of the 'leaks' are crucial in the control of nitrogen gas generation during high levels of denitrification (Firestone & Davidson, 1989). Indeed, the accumulation of N_2O as the main end product will occur due to any factor which reduces the denitrification rate (Firestone & Davidson, 1989), e.g. an increase in soil acidity.

There are numerous biological and non-biological factors which regulate the gaseous emissions of NO_x and N_2O, which Firestone and Davidson (1989) have described as either cellular (proximal) or environmental (distal). The cellular controllers are arguably the most critical parameters controlling nitrification and denitrification and themselves are greatly influenced by environmental factors. The cellular factors and their hierarchy of importance differ between nitrification and denitrification. In most soils the process of nitrification is principally controlled by the soil NH_4^+ content and secondly by the O_2 concentration in the soil. Conversely the denitrification rate is primarily regulated by the soil O_2 level, secondly by the soil NO_3^- content and thirdly by the organic carbon concentration in the soil. It is likely, however, that the significance of specific factors are site specific.

1.8.1. Soil O_2 concentration and water content

The O_2 concentration of the soil controls both the processes of nitrification and denitrification. This O_2 concentration is immediately dictated primarily by the soil water content, but also by the soil type, (especially texture) temperature and microbiological activity.

Soil water content exerts an influence on the O_2 concentration in the soil via its effect on the total pore space available for gaseous diffusion through the soil matrix. This in turn is controlled by the water removal process of evapotranspiration and the water addition processes of precipitation and irrigation. Soil water content is

related to the texture and structure of the soil. Clays tend to have smaller pore sizes than sands, as do soils with a poor structure e.g. after cattle poaching. Water is held more tightly as pore size decreases, consequently pore damaged soil and clays tend to drain less easily than sands. The quantity of water filling the soil pores is extremely critical. A high percentage of water in a soil pore reduces the air space and hence gaseous diffusion is impeded, because gases diffuse through water about 10,000 times slower than in air (Davidson, 1993: Davidson & Schimel, 1995). The movement of oxygen from the atmosphere into the soil and subsequently to the sites of bacterial demand, and the removal of nitrogen oxides away from the production sites and out of the soil are, therefore, strongly regulated by the soil water content.

Anaerobicity can also develop even if there is only a thin water film restricting O_2 diffusion, due to high O_2 consumption rates (Parkin, 1987). Sites within the soil matrix may become anaerobic due to O_2 loss to plant roots or O_2 consumption by respiring heterotrophic micro-organisms (Bouwman, 1990). Consequently, the rhizosphere area surrounding plant roots, which is rich in heterotrophic bacteria, may frequently become O_2 deficient.

Denitrification is most frequently limited by the O_2 concentration of the soil. Low oxygen partial pressures stimulate anaerobic denitrification, whereas nitrification rates (particularly, the NH_4 oxidation rate) generally decline with decreasing soil O_2 levels (Firestone & Davidson, 1989). Conversely, high O_2 concentrations are likely to inhibit respiratory denitrification, but enhance nitrification rates.

Davidson (1993) suggests that non-linear responses of nitrogen gases to soil moisture (WFPS) is typical, with the optimum production of both NO and N_2O at midway values (*ca.* 60%) of WFPS, which in many soils is approximately equivalent to field capacity. This WFPS is roughly equal to the transition point between processes that operate aerobically and those which are anaerobic (Davidson, 1993). At a WFPS $\leq$ 60% NO and N_2O are predominantly released from autotrophic nitrification (Schuster & Conrad, 1992; Hutchinson *et al.* 1993; Williams *et al.* 1992), whereas a WFPS > 60% will emit NO/N_2O mainly from the denitrification process. The WFPS above which denitrification is the dominant process in the production of N_2O is not universally agreed upon and has also been estimated at 70%

(Dunfield & Knowles, 1999). Consequently, the optimum WFPS for nitrification is around 60%, while the maximum denitrification rate occurs at a WFPS > 80% when the main product is N_2 (Davidson *et al.* 1986; Davidson, 1993).

Commonly, aerobic biological activity is maximised around field capacity and above this level activity is inhibited, as a result of poorer diffusion of O_2 (Davidson, 1993; Killham, 1994). This helps to explain the decline in nitrification rates at higher soil water contents. Consequently, NO emission occurs over an extensive range of soil moisture conditions with NO production from anaerobic soils dominated by denitrification (Remde & Conrad, 1991a), but also N_2O generation in aerobic soils both from denitrification in anaerobic microsites and from nitrification (Baumgärtner & Conrad, 1992).

Commonly N_2O emissions from denitrification are short-term episodic events that develop with the onset of anaerobic soil conditions, as a result of an increase in soil water content. (Goodroad & Keeney, 1985; Byrnes *et al.* 1990). This association between soil moisture content and N_2O emissions may be observed after a precipitation or irrigation event, especially if the soil is relatively dry. Wetting of the soil stimulates the emission of N_2O from denitrification.

Partial pressures of oxygen affect the product formation from nitrification. If oxygen is limited, the production of N_2O is favoured over NO, which is favoured over NO_2^- and NO_3^- (Firestone & Davidson, 1989; Hutchinson *et al.* 1993). Commonly, NO is primarily produced during the autotrophic nitrification process and in considerably larger quantities than N_2O (Davidson & Schimel, 1995; Williams *et al.* 1998). Consequently the N_2O:NO_3^- ratio is generally < 1% (Firestone & Davidson, 1989) and the NO:N_2O ratio is normally greater than 1 (Anderson & Levine, 1986). As a result, N_2O emissions from autotrophic nitrification have been assumed to be insignificant (Umarov, 1990), although Klemedtsson *et al.* (1990) reported that the dominant production process of N_2O from soil fertilised with urea/NH_4^+ was nitrification.

Similarly, the product formed in denitrification is strongly dependent on the aeration status of the soil. In most soils, in completely anaerobic conditions N_2 is the main gaseous end product, but with more oxygen present the N_2O:N_2 ratios will rise (Klemedtsson *et al.* 1988b; Aulakh *et al.* 1984a; Firestone & Davidson, 1989; Arah

& Smith, 1990). This effect of the soil aeration status on the partitioning of denitrification products is primarily a result of the inhibition of N_2O reductase by O_2 (Aulakh *et al.* 1984a). The production of N_2O (particularly high fluxes) is predominantly associated with denitrification (Williams *et al.* 1998), whereas NO is generally not considered to be a key product (Firestone & Davidson, 1989), although rates of NO production from denitrification have, however, been shown to be greater than from nitrification. Consequently a greater proportion of N_2O to NO is likely from denitrification (Davidson & Schimel, 1995) resulting in a NO:N_2O ratio of approximately 0.01 (Anderson & Levine, 1987; Bouwman, 1990; Williams *et al.* 1988).

1.8.2. Soil mineral nitrogen content

Numerous researchers have achieved a strong correlation of NO and N_2O flux with soil mineral nitrogen; NH_4^+, NO_3^- or both. In anaerobic soils the availability of NO_3^- is the most important proximate controller over emissions. Indeed, Baumgärtner & Conrad (1992), reported that NO production via denitrification was limited by the availability of nitrate or nitrite. It is a common feature that at high soil NO_3^- levels the ratio of N_2O:N_2 formed from denitrification increases (Aulakh *et al.* 1984a; Arah & Smith, 1990; Van Breemen & Feijtel, 1990; Baumgärtner & Conrad, 1992) due to the inhibition of N_2O reduction (Aulakh *et al.* 1984a; Arah & Smith, 1989; Baumgärtner & Conrad, 1992). The rise in N_2O production is primarily the result of the preferential use of NO_3^- over N_2O as an electron acceptor (Aulakh *et al.* 1984a).

In a soil with a WFPS of < 60%, insufficient water can result in substrate unavailability (Davidson, 1993) and bacterial immobilisation, hence inhibition of the nitrification or denitrification process. Nonetheless, there are numerous reports in the literature of a rapid, large (1-3 orders of magnitude increase), but fairly short (< 24 h) burst of NO emitted from dry soil after rain events or irrigation of < 25 mm (Johansson & Granat, 1984; Slemr & Seiler, 1984; Anderson & Levine, 1987).

Mineralisation is a key process, whereby microbes utilise organically bound nitrogen yielding inorganic mineral nitrogen (NH_4^+ and NO_3^-) (Brady & Weil, 1999). This nitrogen may then be utilised by both nitrifiers and denitrifiers. It is possible

though that dormant nitrifying bacteria may remain in the carbon rich rhizosphere (the zone of soil adjacent to and strongly influenced by a plant root) of grassland plants and are reactivated during episodes of net mineralisation (Verhagen *et al.* 1992).

1.8.2.1. Nitrogen addition to soil

Globally crop production is frequently limited by the unavailability of nitrogen for plant growth. In the UK, approximately 1.4 million tonnes (Chalmers *et al.* 2000) and *ca.* 450,000 tonnes (Williams *et al.* 1999) of nitrogen is added as inorganic fertiliser and animal manure each year respectively.

Fertilisation of soil with mineral nitrogen has the capability to significantly adjust the global nitrogen cycle (Erickson & Keller, 1997). Undoubtedly nitrogen fertilisation has been demonstrated to exert a very strong stimulating effect on both NO and N_2O emissions (e.g. Slemr & Seiler, 1991; Shepherd *et al.* 1991; Skiba *et al.* 1993; Yamulki *et al.* 1995; Maggiotto *et al.* 2000).

Using several data sets, Veldkamp & Keller (1997) calculated the mean percentage of fertiliser lost as NO to be 0.5%, whilst Skiba *et al.* (1997) estimated that on average 0.3% of fertiliser nitrogen was lost as NO. Johansson & Galbally (1984) recorded only a 0.2% loss of nitrogen as NO from nitrate fertiliser from a fertilised soil in Sweden. This is comparable to the loss rate of 0.1-0.3% of the nitrate added, which Slemr & Seiler (1984) observed 2 weeks after fertiliser addition.

Furthermore, the percentage of fertiliser lost as NO was found to vary with fertiliser type. However, Veldkamp & Keller (1997) and Veldkamp *et al.* (1998) state that examining percentage of fertiliser nitrogen lost from different fertiliser compositions is ineffective, since environmental parameters are more significant. Certainly, reviewing the literature Harrison & Webb (2001) concluded that N_2O emissions in particular were critically dependent on the interaction between timing of fertiliser application and the weather.

Emissions of N_2O are similarly stimulated by the addition of nitrogenous fertilisers and the percentage of nitrogen lost as N_2O is of a comparable magnitude to that lost as NO. Total N_2O-N losses of applied nitrogen range from 0.4 to 7.0%

(Ryden, 1981; Anderson & Levine, 1987; Mosier & Hutchinson, 1981; Yamulki *et al.* 1995; McTaggart & Smith, 1996;. McTaggart *et al.* 1997a, Dobbie *et al.* 1999). Interestingly the magnitude of the percentage of added nitrogen lost appears to be influenced by the crop type. Dobbie *et al.* (1999) demonstrated that the percentage loss was greatest from brassicas > potatoes > spring barley and winter wheat. The method used by the Intergovernmental Panel on Climate Change (IPCC) to calculate emission inventories, estimates that the loss of N_2O emitted directly from synthetic fertiliser applied to agricultural soils is 1.25% of the added N (IPCC, 1997).

It is not just nitrogenous chemical fertilisers which are reported to have a stimulating effect on N_2O and NO_x emissions, organic fertilisers also have the potential to substantially enhance fluxes. Not only do grazing animals deposit highly localised inputs of carbon and nitrogen in the form of organic waste (dung and urine), but they also tend to compact the soil through trampling (poaching) and hence create ideal conditions for denitrification (Fowler *et al.* 1997; Velthof & Oenema, 1995b; Oenema *et al.* 1997). Similar to emissions from synthetic nitrogen fertilisers the percentage of nitrogen emitted as N_2O-N was recorded as 0.1-0.7% and 0.1-3.8% from total N applied as dung and urine respectively (Oenema *et al.* 1997) and the amount of nitrogen lost as NO-N from manure has been estimated at 0.3% (Paul *et al.* 1993). Watanabe *et al.* (1997) demonstrated comparable losses of NO-N of 0.48% and 0.45% from grassland amended with cattle and pig excreta respectively.

Although the percentage of NO_x and N_2O emitted from both organic and synthetic fertilisers is minimal representing an insignificant economic loss (Williams *et al.* 1992) and is of little consequence to the soil nitrogen budget (Johansson & Granat, 1984), the impact on global warming and local tropospheric chemistry (significantly O_3 pollution) could be substantial.

1.8.3. Soil organic carbon content

Denitrification by heterotrophic bacteria is strongly affected by the availability of soil organic carbon, which acts as an electron donor during the process. If the WFPS of a soil is < 60%, then the diffusion of carbon through water films is likely to be restricted, resulting in a decline of denitrification (Davidson,

1993). In anaerobic zones, in unfertilised soils denitrification may be governed by the availability of NO_3^-, but in nitrogen fertilised soils denitrification may be limited by carbon availability (Firestone & Davidson, 1989). Consequently, not all anaerobic soil aggregates will denitrify if they are carbon or nitrogen limited (Sexstone *et al.* 1985). If the supply of NO_3^-/NO_2^- greatly exceeds that of carbon, then the nitrogen oxide will be partially used generating N_2O (Firestone & Davidson, 1989). Consequently, as the availability of organic carbon decreases and that of the nitrogen oxide rises, the $N_2O:N_2$ ratio will increase (Firestone & Davidson, 1989; Arah & Smith, 1990; Van Breemen & Feijtel, 1990). Indeed, Arah & Smith (1990) observed that concentrations in soil of water soluble and mineralisable carbon are frequently significantly correlated with denitrification activity.

1.8.4. Soil pH

A soil which is acidic commonly results in a reduction of the emission production rate from both the microbial processes of nitrification (in particular the NH_4^+ oxidation rate) and denitrification (Firestone & Davidson, 1989; Killham, 1994). Indeed, it has been demonstrated that the production of N_2O from nitrification increased with a concurrent rise in pH from 4.7-6.7. This pH, just below neutral, corresponds to the optimum point for autotrophic bacteria (Van Breemen & Feijtel, 1990). However as the pH decreases the proportion of N_2O in the end products increases (Nägele & Conrad, 1990).

In a sequential process like denitrification, the lowered emission production rate can lead to the accumulation of intermediate substances, such as N_2O, so that the $N_2O:N_2$ ratio increases (Firestone & Davidson, 1989; Arah & Smith, 1990). This ratio rise is enhanced in acidic conditions (< pH 6), due to the acid sensitivity of the enzymes involved in N_2O reduction (Sahrawat & Keeney, 1986; Nägele & Conrad, 1990; Van Breemen & Feijtel, 1990). The optimum pH for denitrification is approximately at pH 7 (Parkin *et al.* 1985; Killham, 1994), but the process can still rapidly proceed at pH 4.7 (Bouwman, 1990). Both nitrifying and denitrifying bacteria within neutral microsites may continue to function shielded from the effect of the bulk soil pH, even if it has a pH < 5 and therefore not within the optimum pH range (Parkin *et al.* 1985; Bouwman, 1990).

1.8.5. Soil temperature

The effect of temperature on microbiological processes is well documented. A rise in temperature will enhance the rate of processes catalysed by enzymes (Davidson & Schimel, 1995), although a significant reduction in activity will begin to occur at several degrees higher than the optimal temperature (Killham, 1994). It is of widespread opinion that the relationship between NO and N_2O emissions and soil temperature is exponential (Williams & Fehsenfeld, 1991; Otter *et al.* 1999). In soil between 0 °C and 30/35 °C (Killham, 1994), for every 10 °C rise there is an approximate doubling of the biological processes of nitrification and denitrification, i.e. $Q_{10} = 2$. (Williams & Fehsenfeld, 1991; Meixner, 1994; Gödde & Conrad, 1999).

Denitrification is possible in the soil over a wide range in temperature (2-70 °C) (Firestone & Davidson, 1989; Bouwman, 1990), with the optimum temperature *circa* 25 °C (Bouwman, 1990). Rapid rates of denitrification can occur at temperatures as high as 60-70 °C (Bouwman, 1990; Meixner, 1994). Temperature influences the partitioning of the end products of denitrification. Between 6 and 8 °C, nitric oxide is the most dominant gas, which in turn is present in larger quantities than N_2 (Payne, 1981). Around 10 °C N_2O is the dominant product, whereas N_2 is the major end gas between 15 and 30 °C (Payne, 1981).

The most favourable temperature for nitrification is at approximately 30-35 °C (Bouwman, 1990), which is a similar temperature to when peak NO production develops (Meixner, 1994). Nitrification can, however, proceed at very low rates when soil temperatures are < 5 °C and > 40 °C (Bouwman, 1990).

Numerous field and laboratory studies report temperature exerting a strong control on the production of NO (Johansson, 1984; Johansson & Granat, 1984; Slemr & Seiler, 1984; Anderson & Levine, 1987). The influence which temperature has on emissions often results in marked seasonal and diurnal variations, the latter of which can be exaggerated with large fertiliser applications (Bouwman, 1990).

The temperature dependence of NO emissions is frequently described by the activation energy, which is calculated using the Arrhenius equation: ln NO emission = $E_a/R \times 1/T + A$, where Ea = the activation energy (J $mole^{-1}$), R = a gas constant (8.31 J $mole^{-1}$ K^{-1}), T = absolute temperature (K) and A = a constant. This dependence on temperature does however, weaken or entirely disappear at low soil

moisture contents (Meixner, 1994; Sullivan *et al.* 1996; Meixner *et al.* 1997), primarily as a result of moisture stress on the microorganisms (Galbally, 1989; Meixner *et al.* 1997). Furthermore, there may be an interaction of temperature with moisture, when an increase in temperature results in rapid drying of the soil, such that more NO is produced than N_2O, until the soil becomes too dry for further NO production (Pers. Comm., Dr. Ute Skiba, 2002).

1.8.6. Plant cover

Vegetation predominantly has the effect of reducing surface emission fluxes of both N_2O and NO_x (Conrad, 1990). Plants will alter both physical and biological parameters in the soil which strongly affect denitrification and nitrification processes, including temperature, moisture, nutrient levels (including carbon-rich material in the rhizosphere), microbial population sizes and activity (Meixner, 1994).

Conversely, however, Williams & Fehsenfeld (1991) suggested the presence of extra vegetation may be the cause of their larger observed NO fluxes. They proposed that plants may ventilate the soil aiding NO emission or that the larger microbial population associated with plant roots may have significance. Indeed from a wheat planted monolith, at an identical temperature Weber & Rennenberg (1996) detected a larger NO emission in the morning than in the afternoon, but not from bare soil. This phenomenon has been observed by other researchers and was accounted for by an increase in root exudation in the morning, which provided extra substrate for the microbiological production processes (Johansson & Granat, 1984).

1.8.7. Biomass burning

Burning of vegetation has frequently been documented to stimulate long-lasting emissions of both NO_x and N_2O from soil and is a highly significant process in tropical regions (Anderson *et al.* 1988; Johansson *et al.* 1988). The increase in NO and N_2O production is thought to evolve from a rise in soil inorganic nitrogen content brought about by a combination of the biological decomposition of amassed biomass and the release of nitrogen from soil minerals due to intense heat (Johansson

et al. 1988; Smith, 1997). Alternatively, the enhancement of soil nitrogen may be due to an increase in soil microbiological activity (Meixner, 1994). This soil microbial population is able to recover far faster following burning than plants and thus the availability of mineral nitrogen is higher as a result of reduced competition (Anderson *et al.* 1988). Research has also shown that fluxes following burning were considerably further enhanced when fertiliser or water was applied to the soil (Anderson *et al.* 1988; Johansson *et al.* 1988; Rondón *et al.* 1993; Levine *et al.* 1996).

1.9. Contribution of nitrification and denitrification to NO and N_2O production

The contribution of the specific processes, nitrification denitrification and chemodenitrification to NO and N_2O is influenced by both soil type and environmental conditions. Moreover, over very brief episodes the relative significance of nitrification and denitrification may vary hugely (Bouwman, 1990). It is generally assumed however, that if NO and N_2O fluxes correlate with NH_4^+ levels and the soil is well aerated the dominant production pathway is nitrification (Bouwman, 1990; McKenney & Drury, 1997). On the other hand, denitrification is considered to be the main production pathway in warm, moist soils with available organic carbon (Galbally, 1989), and when NO emissions correlate with soil NO_3^- and the soil is poorly aerated (McKenney & Drury, 1997).

The determination of the relative contribution of denitrification and nitrification to NO and N_2O production, however, can be achieved through the use of ^{15}N-labelled fertiliser or nitrification and/or denitrification inhibitors (Conrad, 1995). Acetylene (C_2H_2) is a versatile inhibitor since it can be used at different concentrations to suppress both the action of N_2O reductase during denitrification and NH_4^+ monooxygenase during autotrophic nitrification. Ammonium oxidation can also be blocked through the use of other inhibitors, e.g. nitrapyrin (N-serve) or dicyandiamide (DCD) and NO_2^- oxidation can be halted by the inhibitor, chlorate. It is however, advantageous to block the first step of nitrification rather than the second, so as to avoid the accumulation of NO_2^-, which is phytotoxic (Killham,

1994). It is difficult to differentiate between nitrification and denitrification because neither process is either wholly aerobic or anaerobic. Nevertheless through a combination of techniques/experiments, e.g. involving microbial stimulation through substrate addition, the use of metabolic inhibitors and varying the O_2 concentration, differentiation is generally possible (Conrad, 1990).

The contribution from chemodenitrification to NO production in theory can be determined through the establishment of a sterilised control (Conrad, 1990). A sterilised control destroys the NO_x producing microorganisms, e.g. by autoclaving, gamma irradiation or through the use of metabolic toxins, but it may also introduce bias to an experiment. Firstly, chemodenitrification relies upon biological reactions to generate its substrate, NO_2^- and secondly autoclaving can destroy NO_2^- itself. Both processes will result in a tendency to underestimate the contribution of chemodenitrification to the total production of NO.

In reviewing the literature it is clear that there are still large gaps in the knowledge with respect to the NO production processes and the influence of environmental factors. As a consequence of the fertiliser N input, agricultural soils can be a major source of NO and consequently contribute to tropospheric ozone pollution in rural areas. This research investigated various agricultural management practices that were likely to influence NO emissions. A broad measurement base of NO emissions is essential for incorporating into regional O_3 models and into inventories of NO required for governmental bodies. This study, therefore, aimed to increase the knowledge regarding the emission of NO from agricultural soils and to suggest mitigation practices.

Chapter 2. Materials and Methods

2.1. Gas sampling

2.1.1. Introduction

Two techniques are commonly used in the determination of trace gas fluxes; micrometeorology and enclosures. Both methods are reliable and precise and the method used depends on the design of the experiment and the questions being asked. If conditions allow micrometeorology can be used to complement results obtained through the enclosure technique (Williams *et al.* 1988; Fowler & Duyzer, 1989; Mosier, 1990). Experiments in central Scotland (Smith *et al.* 1994; Hargreaves *et al.* 1996) and in Denmark (Christensen *et al.* 1996) both demonstrated the value of using micrometeorology to measure N_2O fluxes at the field scale. Micrometeorology can overcome the problem of spatial variability frequently encountered with the use of enclosures.

Enclosures are an effective, low cost, easily operated method of determining trace gas fluxes. The technique is particularly efficient for the estimation of fluxes from scales below the resolution that is possible with micrometeorology. Consequently, enclosures are widely used in laboratory-based, in situ or plot studies, as well as in the examination of the physical, biological and chemical controls of trace gas exchange (Livingston & Hutchinson, 1995; Fowler *et al.* 1997). Typically, the surface area (often including low vegetation) enclosed by the chamber is less than 1 m^2. The operation of chambers are by 2 systems. The dynamic system is based on a forced air flow exchange through the chamber, while the static technique does not involve artificial air circulation.

Static chambers have the tremendous advantage that they are very simple to construct, install and remove. The principle of operation relies on the linear build up of the gas concentration. This makes the method particularly useful for detecting very small gas fluxes (Mosier, 1990; Livingston & Hutchinson, 1995). However, gas concentrations can build up sufficiently to inhibit the normal gas diffusion process. It is therefore essential that the gas collection period be terminated before this point is reached. Alternatively, a correction equation can be applied (Mosier, 1990).

Dynamic systems rely on the continuous pumping of external, often zero, air over the enclosed soil surface (Mosier, 1990). The gas flux is calculated from the concentration difference between the incoming and outgoing air, the flow rate and the surface area covered by the chamber (Mosier, 1990).

2.1.2. Field Chamber Measurements

2.1.2.1. NO

NO emissions from agricultural soil were determined in this study using a newly developed closed chamber method.

2.1.2.1.1. Method development

Chambers that had been used in former studies (Clayton *et al.* 1994; McTaggart *et al.* 1997a; Dobbie *et al.* 1999; Scott *et al.* 2000;) as part of a static system to estimate N_2O exchange, were evolved to measure NO fluxes. The pale grey, cylindrical, polypropylene chambers, dimensions 20-cm in length by 40-cm diameter, were used in a closed system covering a ground area of 0.126 m^2. Circumscribing the top of the enclosure a 4.5-cm wide, outward facing polyvinylchloride (PVC) flange was fitted. The lid was manufactured from an individual flange with a circle of rubber draft excluder attached to its underside. A circular sheet (diameter 70 cm), of clear, UV permeable plastic was attached to the upper side of the flange lid with heavy duty tape and prior to the placement of the lid over the chamber flange, domed upwards. The dome shape was essential to avoid pressure differences between the atmosphere inside and outside the enclosure during withdrawal of air when sampling. The lid was manually collapsed concurrently at the equivalent rate as sample removal. A gas tight seal was maintained between the 2 chamber sections by at least 4 metal clips exerting pressure. Nylon tubing fitted with a 3-way tap for sampling extended through a hole in the wall into the enclosure. Silicon sealant secured the sampling tube in place and prevented gas leaks.

Johansson & Granat, (1984), Anderson & Levine, (1987) Davidson *et al.* (1991) and Hutchinson & Brams, (1992) have all successfully measured NO flux

from soil under varying climatic regimes, using a static chamber system. In each case the accumulation of NO has followed the rapid removal of ambient ozone from the chamber through the chemical reaction below (Equation 2.1).

$$NO + O_3 \rightarrow NO_2 + O_2 \qquad (2.1)$$

All the researchers indicated that O_3 was effectively removed from inside the chamber with concentrations diminishing to < 2-3 ppb within the first 4 minutes of chamber closure. The NO flux was consequently estimated from the subsequent rise in the NO concentration.

Experiments were carried out in late January-mid February 1998 using our static chambers to corroborate the above conclusion. Two chambers were inserted into a sandy loam (Darvel Series), ungrazed, unfertilised grassland soil on the Bush Estate, approximately 10-km south of Edinburgh, Scotland. Concentrations of O_3 were measured for 2 min from inside the enclosed chamber following various closure times ranging from 3-45 min. The results demonstrate comparable findings to the previously mentioned research (Figure 2.1).

Following only a closure time of 3 min the O_3 concentration had decreased to approximately 3 ppb regardless of the ambient O_3 concentration, which ranged from 9-24 ppb. For this reason we did not find it necessary to employ an equation, which quantifies NO decay due to the presence of O_3. Several researchers (Aneja *et al.* 1995; Roelle *et al.* 1999) have used an equation to correct for bias due to the chemical reaction when NO is consumed by O_3 inside the chamber. In the calculation of NO emissions from forest soils using a dynamic chamber system, Van Dijk & Duyzer, (1999) applied such a correction equation based on an estimate of the average reaction rate in the chamber during monitoring.

The linearity of the increase in NO concentration during the collection period within the closed chamber was tested in the field site. At no time did the NO flux exceed the emission measured during the linearity test. Consequently the compensation point within the chamber would crucially not be reduced. The predetermined chamber closure time would therefore remain within the linear phase of NO increase.

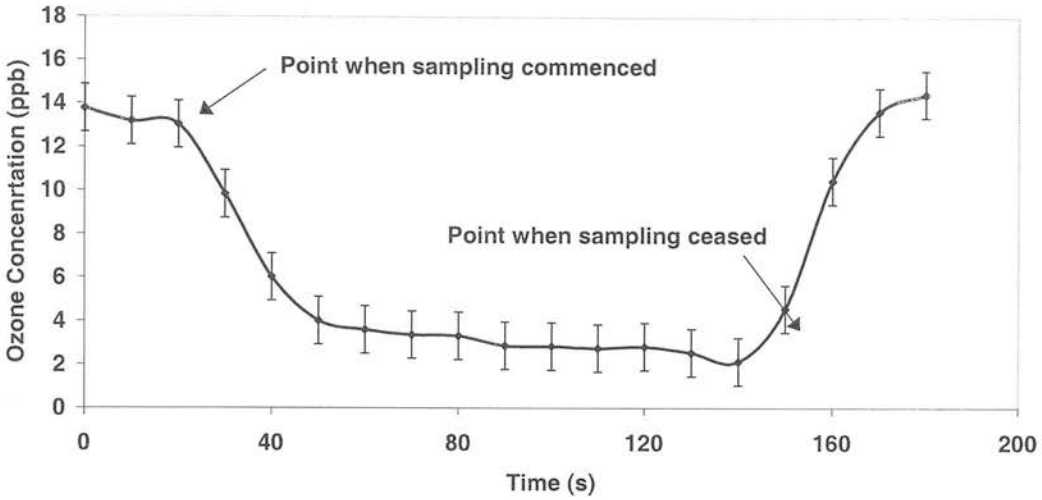

Figure 2.1 The mean ozone concentration sampled from a closed chamber after a 3 minute closure time. Error bars (n=1) represent one standard error.

The tests were performed on one chamber per plot in order to avoid spatial variability. Each chamber was sampled for NO using a chemiluminescent NO_x analyser after a closure time of 3 min. The procedure was repeated on the same chamber for closure times of 6, 10, 15 and 20 min. Between each closure period the chamber remained uncovered for 15 min. This allowed a recovery time so that the soil air could re-equilibrate with atmospheric ambient air.

The results are presented from an experiment, which was conducted on either fertilised (80 kg N ha^{-1} of NH_4NO_3) ploughed or direct drilled plots of a sandy clay loam soil (Winton series) located 10 km south of Edinburgh (Figure 2.2) illustrates that following fertilisation linearity can be expected up to a closure time of 20 min for measurements of NO emissions from a direct drilled plot. However, on the ploughed plots where emissions were larger, linearity can only be assumed up to a collection time of 15 min. The subsequent decline in NO concentration is likely to indicate the juncture at which the compensation point has been attained, resulting in the deposition rather than the emission of NO. Consequently, the chamber closure time for measurements taken on the Winton series of soil must be < 15 min.

Combined with a desire to keep the collection period to a minimum avoiding temperature differences between the inside and outside of the chamber and a requirement for linearity, 10 min was taken as the closure time.

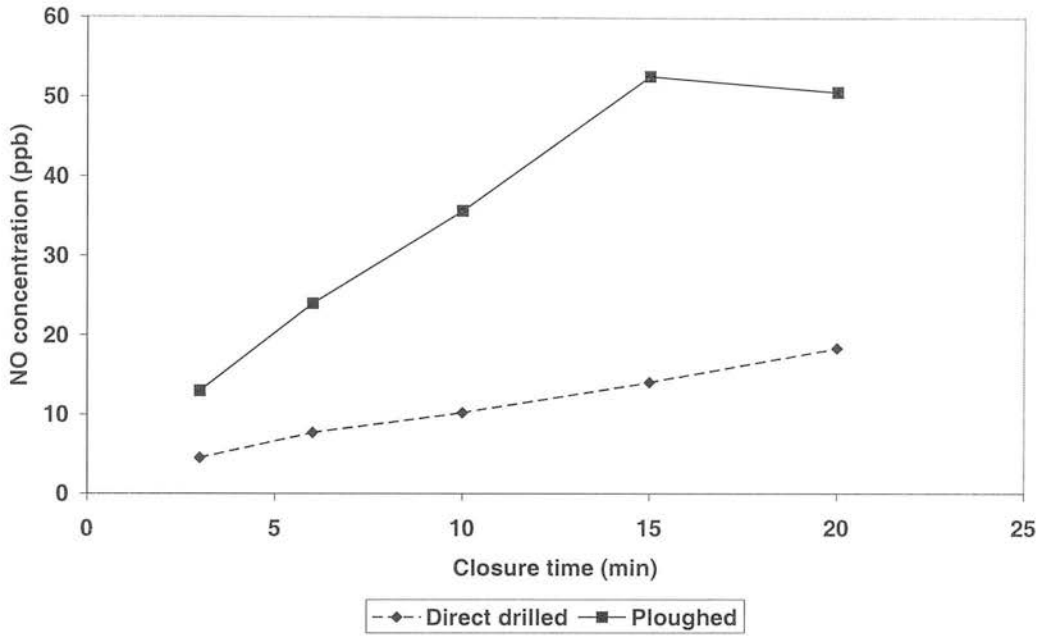

Figure 2.2 Cumulative NO concentration change in a closed chamber from a differently tilled plots, sampled after various closure times.

Previous research on NO flux determination (Anderson & Levine, 1987; Johansson & Granat, 1984) has involved the operation of fan fitted enclosures within a static system. The installation of a fan inside a chamber has been found to facilitate the mixing of the air resulting in the removal of a homogenous sample (Anderson & Levine, 1987; Johansson & Granat, 1984). Although Slemr & Seiler, (1991) estimated the error due to incomplete mixing as only *circa* 5% of the measured flux value. Laboratory experiments were performed to substantiate the effect of a fan within the chamber.

An identical chamber to those used in the field was fitted with a gas tight PTFE sheet at the chamber base. An additional sampling port was manufactured in the chamber wall approximately opposite the pre-existing air outlet point. Consequently the chamber could be used as a flow through system. The chamber was

initially flushed through with zero air for 10-min. Subsequently, NO was generated from a calibrator and was run for 10-min directly through the chamber. The incoming sample port was closed before the outgoing port to avoid over pressurisation of the chamber. Tests were performed with or without a fan working inside the chamber. The fan was either switched on during NO flow through or once the chamber was closed. Chamber closure time varied from 10-30 min. Figure 2.3 demonstrates the result of a test after the chamber had been closed for 10 min prior to sampling, following an input of approximately 30 ppb of NO.

The results of the experiments therefore established that in our design there was no difference in the output of the chamber if a fan was switched on or not. Consequently, it was not deemed necessary to fit fans to the chamber interiors.

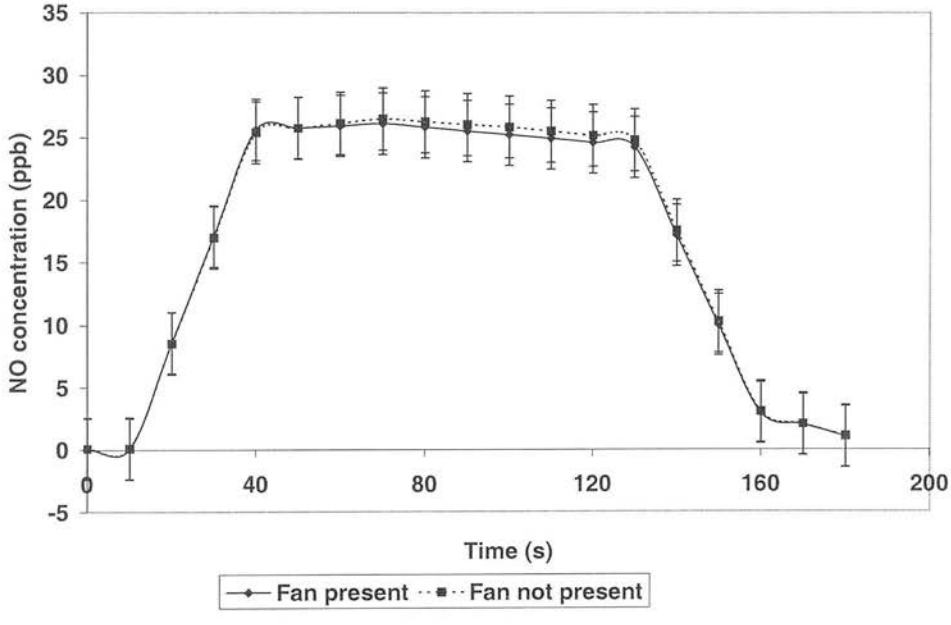

Figure 2.3 The NO concentration in a closed chamber with or without a fan fitted. Error bars represent the standard error of the mean (n=2).

2.1.2.2. N_2O

Throughout the field experiments (Chapters 3 and 4) N_2O fluxes were monitored using the static chamber technique. Emissions of N_2O were measured from identical chambers in some cases, the same chamber, as those used to monitor NO. The main difference was in the lids, which were either a flat sheet of aluminium (2 mm thick) or a plastic dome.

2.1.3. NO Analysis

The high chemical reactivity of NO has been previously discussed (Chapter 1). This instability of the gas requires that materials used in the sampling and analysing set up must be unreactive. Unlike N_2O, NO must be studied in situ rather than analysed from stored samples collected beforehand. In the first field season NO was analysed using a chemiluminescence analyser and in the second year of study a Scintrex LMA-3 analyser was used.

2.1.3.1. The NO_x chemiluminescence analyser

The NO_x analyser used to ascertain the trace gas concentrations in this study was a chemiluminescence analyser (Thermo Environmental Instruments, model 42C) fitted with a molybdenum converter for the determination of NO_2 (Equation 2.2). The converter requires heating to approximately 325 °C during the reaction.

$$3\ NO_2 + Mo \rightarrow 3NO + MoO_3 \qquad (2.2)$$

The NO_x analyser normally operates with the converter bypassed to generate the NO concentration. However, every 10-sec the instrument switches to route the sample through the converter producing a NO_2 reading. The sum of the NO and NO_2 concentrations give the NO_x measurement (Thermo Environmental Instruments Inc., 1994). The analyser does however suffer from interferences caused by other N trace gases e.g. PAN, which may result in an overestimation bias. Previous work has

indicated that during very polluted conditions a maximum error of 10% for NO was introduced by conversion of NO_y to NO_2 (Skiba *et al.* 1992).

The analyser employed has a lower detection limit of 0.30 ppb and a response time, over a 10 sec averaging time, of 40 sec (Thermo Environmental Instruments Inc., 1994). The stability of the analyser is excellent, < 0.40 ppb zero drift over a 24 h period and over the same time period a span drift of ± 1% full-scale (Thermo Environmental Instruments Inc., 1994). Calibration was performed by diluting 10.3 ppm NO in nitrogen by gas-phase titration using an Environics S100 calibrator. The instrument itself does not operate from a battery; requiring mains power (300 watts). This limits the mobility of the analyser, restricting its use to laboratory based experiments or those where it is possible to use a portable generator as a power source. In addition the instrument is problematical to transport due to its bulky nature, dimensions of 425 x 219 x 584 mm and a weight of 24 kg.

2.1.3.2. The Scintrex LMA-3 Analyser

The Scintrex portable analyser (Scintrex Unisearch, model LMA-3 luminox) has been widely used to determine atmospheric NO_x concentrations. Many researchers have reported its successful use in their studies (Yamulki *et al.* 1995; Watanabe *et al.* 1997; Hutchinson & Brams, 1992; Davidson *et al.* 1991). The analyser is small (38 x 20 x 22 cm), relatively light in weight (7 kg), battery operated and its carrying handle provides for ease of transportation. The instrument is consequently extremely practical for remote and lone fieldworking. Operating the instrument from its rechargeable battery did however place a time constraint on sampling. The analyser was capable of running for *circa.* 3 h, before problems ensued due to low battery power.

The Scintrex essentially analyses NO_2 via chemiluminescence produced when the gas encounters a surface (wick) wetted with a specially formulated luminol solution (5-amino-2,3-dihydro-1,4-phthalozinedione). For the purpose of fieldwork a sufficient volume of luminol solution was decanted into the external luminol reservoir of the Scintrex and this was placed inside a small cool bag containing freezer packs. As a contrast to the majority of chemiluminescent NO_x analysers, the

Scintrex does not require the prior conversion of NO_2 to NO, since it detects the NO_2 directly. NO can be monitored, however, via passing the air stream through a small cartridge of Chromium Trioxide (CrO_3) loaded on a substrate of Chromasorb A, which quantitatively converts the NO to NO_2. The principal advantage of the Scintrex is its high sensitivity. The analyser has a detection limit of 5pptv and a response time of 10s for a 100% change in the NO_2 mixing ratio (Scintex/Unisearch, 1987). The analyser was calibrated before and after each measurement occasion. NO_2 calibration gas was generated via a permeation tube contained inside an Environnement VE3M portable calibrator (Environnement S. A., Poissy, France).

Initially tests were performed to establish linearity in the response of the analyser detector to the reaction of NO_2 with the luminol II solution (Figure 2.4). NO_2 gas was generated as above. Various gas concentrations were drawn through the analyser and the response noted. Due to practicalities in generating a reliable gas concentration, linearity was ascertained only between 12-30 ppb NO_2. The slight variation between the 2 runs shown in Figure 2.4 can be attributed to the changes in the condition and composition of the luminol and the nature of the wick material (Clemitshaw *et al.* 1997; Nikitas *et al.* 1997).

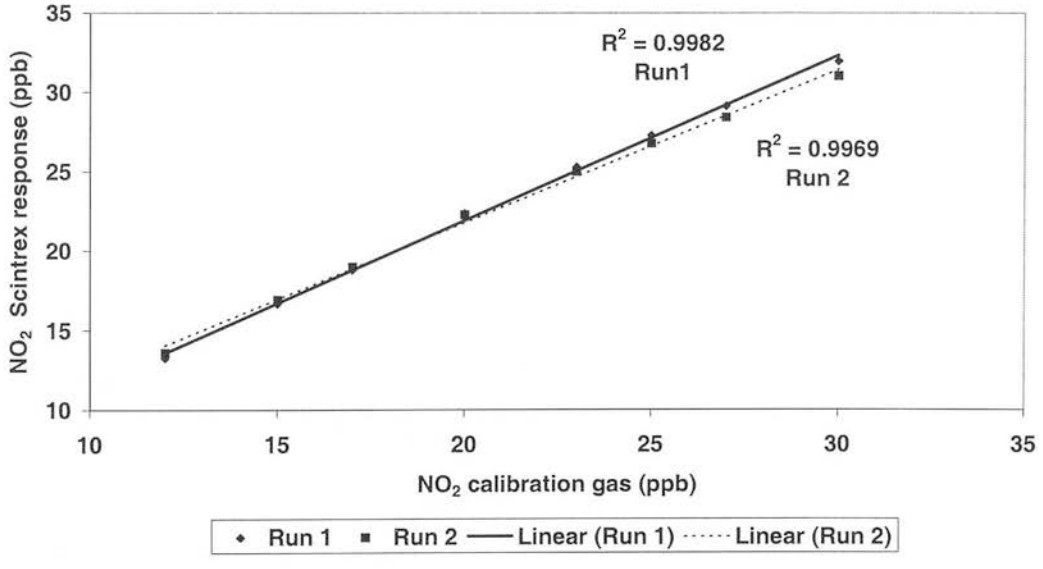

Figure 2.4 Linear response of the Scintrex analyser to NO_2 calibration gas.

Figure 2.5 demonstrates a laboratory test to determine the efficiency of the converter to be used during the field experiments. An airstream containing NO generated from a standard gas cylinder via an Environnement VE3M portable calibrator (Environnement S. A., Poissy, France) was divided to allow simultaneous analysis by a NO_x Analyser (Thermo Environmental Instruments, model 42C) and a Scintrex LMA-3 fitted with a CrO_3 converter.

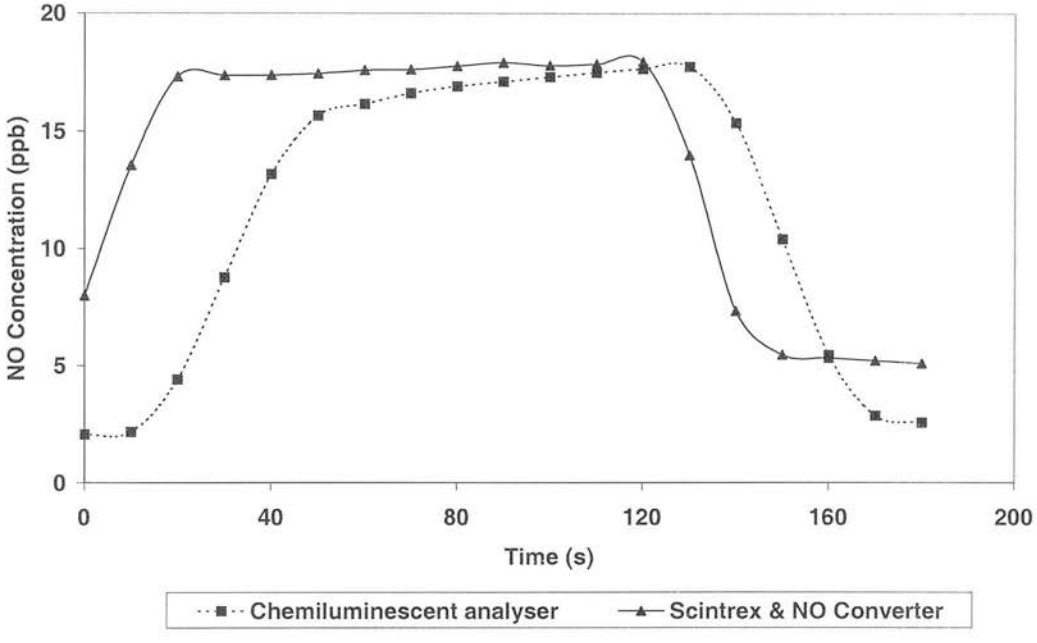

Figure 2.5 Similarity of NO concentration between the NO_x analyser and the Scintrex fitted with a CrO_3 NO converter.

The rapid response of the Scintrex to a change in gas concentration was immediately evident, with the NO_x analyser response lagging 30-40 s behind. There was however a strong agreement (< 0.5 ppb difference) between the 2 analysers after approximately 120 s of NO generation. This suggests that the converter was operating to its full potential and fully oxidising NO to NO_2.

2.1.4. N_2O Analysis

The standard analysis technique to determine the N_2O concentration in a sample is gas chromatography, although newer methods are becoming more

widespread e.g. the optical methods of tuneable diode laser absorption spectroscopy (TDLAS), Fourier transform infra-red spectroscopy (FTIR) and photo-acoustic infra-red spectroscopy (PAIRS).

The N_2O samples obtained during this study were analysed on a manually operated GC (Chrompack, CP 9000). The samples were physically syringe injected through the septa of the sample port and into the sample loop from either Tedlar bags (1 litre) or glass syringes (5 ml). The syringes had been pre-lubricated with silicone grease to maintain a gas tight seal. The deliverance of the gas from the sample loop into the carrier gas stream was achieved through the switching of a valve. The GC used N_2 as a carrier gas and the stationary phase separating column consisted of porous polymer beads (Porapak Q). A backflush facility was operated in order to separate the fast moving molecules of N_2 and N_2O from other gases e.g. H_2O, which could interfere with peak elution of subsequent analyses for N_2O. The detection limit for the GC was < 20 ppb and had a precision of 2% for N_2O determination. Prior to analysis of the actual samples however, at least 3 syringes containing a 1 ppm N_2O standard gas in a mixture of 20% O_2 and 80% N_2 (Air products) were injected into the instrument to check stability and to infer the concentration of the unknown samples. Previous tests had concluded that the response of the GC was linear up to at least 10 ppm. (Pers. Comm. U. M. Skiba, 2001). During sample analysis, every 12^{th} injection was a standard. After this injection and following any significantly large N_2O peaks, the sample port was flushed through with ambient air to avoid carry over between samples. The eluted peaks were automatically integrated and the peak area computed. The N_2O concentration was calculated with reference to the average of the peak areas obtained from the standards injected.

2.1.5. Laboratory Chamber measurements

The chambers used in the laboratory experiments consisted of a 20 cm high and 5.5 cm diameter plastic cylinder continually sealed at the base with a plastic lid fitted with an airtight rubber sleeve. During gas measurement the top of the column was also capped with a plastic lid and rubber seal. Gas entry and exit ports consisting of tubing and 3-way taps were located on opposite sides of the column. Soil cores

were repacked into the columns, such that every soil core was encased in its own cylinder.

2.1.5.1. NO

NO flux was measured from repacked agricultural soil cores using a gas flow-through system and subsequent analysis by chemiluminescence (42C NO-NO_2-NO_x analyser, Thermo Environmental Instruments Inc.) (Figure 5.1 and Plate 5.1). The experimental system was installed in a walk-in temperature controlled room, adjusted as required.

The NO_x analyser requires an air flow of *ca.* 1100 ml min^{-1}. However, in order to ensure an airflow over the soil surface and not through the soil, this air stream was split, so that only 40 ml min^{-1} was pulled over the surface of the soil column and the remaining air flow was provided by ambient air. This resulted in an approximate 27 fold dilution of the air sampled from the soil column. Despite the dilution, the analysers' detection limit of 0.3 ppb was capable of measuring fluxes. Flow rates through the column and into the analyser were measured using flow meters (Aera FC 7700C). NO concentrations and flow rates were logged (Campbell Scientific 21x) at 10 s intervals.

Ambient air and column inlet air were scrubbed free of O_3 and NO_x using charcoal and purafil filters respectively. Throughout the measurement period (15 min duration) the scrubbed air flowed continuously through the 10 cm high column headspace. At the start and end of each experimental run, NOx measurements were determined from empty columns, to quantify NO_x dynamics of the chamber walls. The NO flux was calculated as the product of the air stream (40-ml min^{-1}), the increase in NO concentration above ambient (empty column) and the dilution constant rate (27) divided by the area of the soil surface (23.8 cm^2).

2.1.5.2. N_2O

N_2O fluxes were determined using the static chamber technique. Following the measurement of NO, the lid from each soil column was removed to re-establish free gaseous exchange between the soil surface and the room atmosphere. The

concentration of N_2O in the columns was subsequently assessed after sealing the columns with plastic lids fitted with an airtight rubber sleeve for 15 min. Air samples (5 ml) were withdrawn from the column headspace by syringe from the outlet tap. Prior to closure, an ambient room air sample was also collected using a 5 ml syringe from directly above the open column. Samples were immediately analysed for N_2O by gas chromatography (Chrompack CP9000 fitted with an Electron Capture Detector (ECD). The N_2O fluxes were calculated as the product of the height of the headspace above the repacked soil core and the increase in N_2O concentration divided by the time the column was sealed.

2.2. Soil Sampling

Throughout the field experiments, accompanying the measurement of gas fluxes, soil samples were taken in order to establish relationships between NO fluxes and several key environmental variables within the soil matrix. Gravimetric moisture content and exchangeable soil ammonium and nitrate concentrations were established from each soil core. Additionally, the crop height within the chamber was determined, as well as the soil temperature at the soil surface and at a depth of 50 mm.

2.2.1. Design of the Sampling Strategy

The sampling strategy was developed to be as statistically viable as possible in order to represent the true values within the field, but to be practically feasible. Each plot was randomly sampled in triplicate avoiding possible 'damaged' areas; a 50 cm wide strip inside the plot perimeter, the immediate area surrounding the sampling chamber and any tractor wheelings.

2.2.2. Sampling Protocol

A simple c-shaped gouge auger (Internal diameter = 32 mm), marked at 10 cm intervals, was used to extract the soil from the ground. For the duration of the first field season (1997 – 98), samples were taken to a depth of 20 cm, but due to

time practicalities during the second field season (1998 – 99), samples were only obtained down to a depth of 10 cm. The soil was carefully removed from the auger at increments of 10 cm and if vegetation was present at the top of the core it was discarded. Care was taken not to eliminate 'A' horizon soil together with the sward. The core was deposited into a pre-labelled polythene grip-seal bag (125 x 187 mm) and the majority of the air was expelled and the bag sealed. The auger was cleaned between samples to avoid contamination.

The height of the crop was measured using a tape measure 5 times within the chamber; one measurement in the middle and 4 equally spaced around the perimeter. It should be noted that within the chamber, garden shears were periodically used to cut the crop (in the case of grass) to a height of approximately 10 – 15 cm. This action was necessary to prevent the chamber from becoming too full of vegetation and restricting the free gas. All cut vegetation was removed from inside the chambers. Any crop cutting was performed at least 2 days before gas measurements were sampled to avoid any associated effects on gaseous exchange from both the plants and soil.

The temperature at the soil surface and at a depth of 50 mm was taken in triplicate using a temperature probe (RS components). The readings were taken concurrently with the gas sampling, whilst the instrument was shielded from direct sunlight.

2.2.3. Sample Storage

All soil samples were directly placed in a cool box containing freezer packs and were transported to the laboratory for immediate analysis if time permitted. Alternatively, the samples were stored over night (still in the cool box) in a cold room (< 4 °C) to await analysis the next morning.

The soil samples were not frozen after collection, i.e. prior to analysis. Freezing samples is undoubtedly convenient allowing analysis at a suitable time, but subjects the soil sample to well-documented physical and biological changes that take place in the soil upon thawing. Increased rates of nitrogen mineralisation, denitrification and nitrification have all been reported from thawing soil (Christensen & Tiedje, 1990; DeLuca *et al.* 1992; Edwards & Cresser, 1992). Such enhanced

microbial activity will inevitably result in the transformation of N species. The thawed sample is likely to posses different properties to that of the original soil sample.

2.3. Soil Mineral N (NH_4/NO_3) Determination

To calculate the exchangeable soil ammonium and nitrate concentrations, soil samples were shaken in an excess of potassium chloride (KCl) solution. This is to ensure that K^+ and Cl^- ions bring all the NH_4^+ and NO_3^- ions into solution through replacement in the soil matrix.

Firstly, each soil sample was well mixed by hand (within its polythene bag) to break up any large aggregates and to ensure that the soil was homogeneous. Latex gloves were worn for safety purposes and to avoid contamination of the samples.

From each sample, approximately 20 g of soil was weighed on a 4 decimal place balance (Oertling) into a pre-weighed and labelled polystyrene pot and the readings noted. Added to this 100 ml of 1M KCl was automatically dispensed (Brand Dispensette) in two 50 ml aliquots and shaken on an orbital shaker (Gallenkamp) for 1 hour. All pots were randomly positioned on the shaker. In order to calculate the exchangeable soil ammonium and nitrate concentrations, it is necessary to determine the gravimetric moisture content from the same soil sample. This method is discussed in section 2.4. The shaken extracts were left to filter (Whatman 42) unaided through polypropylene funnels into polypropylene sample bottles. The resultant filtrate was briefly shaken by hand and a portion was decanted into a pre-labelled 30 ml glass vial and stored in the fridge (< 4 °C) until analysis. The samples were stored for no longer than 14 days prior to analysis.

Prior to use all plastic and glassware had been thoroughly cleaned using Decon (Decon Ltd), rinsed with deionised water (Purite) and dried in a heating cabinet (Victor) set to 107 °C.

2.3.1. The Auto Analyser

The exchangeable soil ammonium and nitrate concentrations in each sample were determined by continuous flow analysis (Chemlab Instruments Ltd). Initially,

the auto-analyser was warmed up for 1 hour and simultaneously the instrument was washed through with the standard reagents (Chemlab 1982; Chemlab, 1988) appropriately mixed with 1 ml of bridging solution (30%).

All samples were removed from the fridge and checked for breakages. If any of the vials had cracked then the sample was discarded. Each vial was briefly shaken by hand and a measure was poured into a polystyrene sample cup (2 ml) and placed into the sample turntable of the auto–analyser. A set of 6 standards (0 – 2.5 ppm NH_4:N; NO_3:N) was analysed twice at the beginning of the sample run and once at the end. Additionally, after every 12^{th} sample a blank and a standard were run. The samples were automatically aspirated from the sampling cups into the continuous-flow system. The peaks computed as a result of the analysis were checked and manually manipulated as necessary. The attached software automatically integrated the peak to give a concentration of mineral N (ppm). This was expressed on a dry-weight basis ($\mu g\ N\ g^{-1}$ dry soil) using a calculation based on the accompanying gravimetric water content.

2.4. Soil Moisture Determination

Gravimetric moisture content is the standard direct measurement of soil moisture and is commonly expressed as the mass of water per unit mass of oven-dry soil (Brady & Weil, 1999). It is a simple technique that is routinely used, although it is highly labour intensive and is destructive, removing a quantity of soil at each sampling.

The volumetric water content of the soil is the volume of water per unit volume of soil. This is a more relevant expression than gravimetric moisture content as it allows direct comparisons between different soil samples from various sites. Volumetric water content requires either the volume from which the sample was taken to be known or the bulk density of the soil from independent measurements in the surrounding area (Gardner, 1986).

Water filled pore space (WFPS) is a useful measure when relating the soil water content to gases as it encompasses the texture of the soil, via the soil porosity

and hence the air space in the soil. Porosity is a term which describes the Volume % of the total soil bulk not occupied by solid particles (Brady & Weil, 1999).

2.4.1.Gravimetric Moisture Content

Approximately 30 g of soil was weighed into a pre-weighed and labelled aluminium dish (diameter, 650 mm), the values noted, and placed in an oven (Gallenkamp) at 105 °C. After 24 hrs the sample was cooled in a desiccator, reweighed and the new weight recorded. The final value was calculated (Equation 2.3) and expressed on a g g^{-1} of dry soil basis.

$$m = \frac{\text{Mass of water lost}}{\text{Mass of oven dry soil}} \qquad (2.3)$$

Where, m = gravimetric water content

2.4.2. Volumetric Moisture Content

Volumetric water content is defined as:

$$\theta = m * \gamma_d \qquad (2.4)$$

Where, θ = Volumetric water content

γ_d = Dry bulk density

m = gravimetric water content

The accuracy of volumetric water content as a measure of soil moisture content depends on the exactness of the bulk density value obtained. This is likely to be erroneous to a certain degree, attributable to the intrinsic heterogeneity of the soil in the area being sampled. However, the error involved is probably no more critical then the inaccuracy from representing the water content at depth (Gardner, 1986).

2.4.3. Water Filled Pore Space (WFPS)

WFPS is expressed on a percentage basis and defined as, the ratio of volumetric soil water content to total porosity (Meixner, 1994):

$$WFPS\ (\%) = \frac{\theta}{n} * 100 \qquad (2.5)$$

Where, WFPS = Water filled pore space

θ = Volumetric water content

n = porosity

and porosity is defined as:

$$n = 1 - \frac{\gamma_d}{\gamma_s} \qquad (2.6)$$

Where, n = porosity

γ_d = Dry bulk density

γ_s = particle density (taken as 2.65)

Chapter 3. NO emissions from agricultural soils following various tillage treatments

3.1 Introduction

The most widespread method of soil tillage in the UK is conventional mouldboard ploughing to a depth of *ca.* 200 mm. Deep ploughing to a depth of *ca.* 300 mm and zero-tillage (or direct drilling) are techniques used to a lesser extent. Ploughing to depth permits organic matter to be mixed over a greater area. As a result, immobilisation and nitrogen fixation in mineral sub-soils is increased along with a rise in CO_2 emissions (Nieder *et al.* 1995; Reicosky, 1997; O'Sullivan *et al.* 2001). Conventional tillage disturbs the soil less and so stimulation of CO_2 emissions is reduced (Ball *et al.* 1999b; O'Sullivan *et al.* 2001). Direct drilling involves even less soil disturbance as the seed is directly sown into ground that has been left uncultivated since the previous harvest. Zero-tillage therefore, may result in considerable accumulation of soil surface organic matter and sequestration of carbon (Ball, 1994; O'Sullivan *et al.* 2001).

A further consequence of reduced soil disturbance is the associated rise in the populations of both beetles and earthworms, which are beneficial to the crop and soil respectively (Ball, 1994). Beetles are known to consume aphids, which are capable of cereal disease transmission (Ball, 1994) and earthworms greatly enhance both soil fertility and productivity (Brady & Weil, 1999), particularly through the improvement in soil structural stability (Ball, 1994; Rasmussen, 1994). Additionally, direct drilling has the potential to allow a larger area of autumn crops to be sown as a result of a more rapid seed bed preparation and a reduction in machine passes, therefore cutting fuel usage (Christian, 1994).

In the highly arable region of east England direct drilling of cereal crops into previously burnt stubble was widespread from 1970-1980 (Spoor, 1994). In Scotland the technique was less common, predominantly because the cool, wet climate induced excess soil wetness that limits the timing and effectiveness of field operations (e.g. decreasing the efficiency of pesticides and increasing the potential of soil structural damage) and because direct drilling was commonly viewed as an

adverse risk to crop yield (Ball, 1994). The practice of zero-tillage, however, virtually ceased in the UK following the straw stubble burn ban of 1992 and as a result of the significant problems encountered in controlling grass weeds e.g. *Bromus sterilis* (perennial grass weed) (Ball, 1994; Christian, 1994; Spoor, 1994).

One of the most important roles of ploughing soil is considered to be its effectiveness in controlling weeds (Christian, 1994). Following conventional ploughing the majority of weed seeds are distributed in soil at a depth of 100-200 mm (Melander, 1994). On the other hand, in soil subjected to direct drilling the bulk of weed seeds are commonly found in the top 50 mm (Melander, 1994). Consequently, crops sown by direct drilling have a higher degree of weeds present (Christian, 1994; Melander, 1994) and it is therefore necessary to apply a larger quantity of herbicide (e.g. Paraquat, Glyphosate) than would be required under conventional tillage practices (Ball, 1994). The use of more efficient herbicide strategies within crop rotations has the potential to cut the substantial weed problem (Christian, 1994).

Although the use of direct drilling in Scotland is decidedly limited (Ball, 1994), it is hoped that through encouragement by EU and government policy direct drilling will be used in the future here, and in other temperate climate countries, in rotation with conventional ploughing (Christian, 1994; Rasmussen, 1994). This would enable a move to a more extensive and sustainable system of agriculture where a good soil structure and the natural ecology of the soil could be maintained (Christian & Ball, 1994).

Interest in zero-tillage as a possible abatement strategy to cut greenhouse gas emissions of CO_2 through carbon sequestration has recently been growing (Lemke *et al.* 1999). The lack of soil disturbance associated with zero-tillage may, lead to an increase in the quantity of water holding pores (< 80 μm in effective diameter) following drainage and a subsequent reduction in aeration (Aulakh *et al.* 1984a; Rasmussen, 1994; Brady & Weil, 1999). The soil conditions generated are likely to favour denitrification and hence have the potential to enhance N_2O emissions. Alternatively, conventional ploughing of soil is known to stimulate NO emissions due to mineralisation of organic nitrogen. This increase has been estimated to be as much as a factor of 2-7 (Skiba *et al.* 1997). The use of direct drilling rather than

conventional ploughing may thus have the potential to reduce NO and CO_2 emissions, but to increase emissions of N_2O.

The objectives of this study were to compare NO emissions from soil amended with differing rates of mineral nitrogen fertiliser and under various tillage treatments; direct drilling, deep ploughing and conventional ploughing.

3.2 Sites and treatments

The Beechgrove (BG) experimental site (Plate 3.1) is located on the Bush Estate, *ca.* 15 km south of Edinburgh (NT243628), at an altitude of 185 m and on former 12/14 year old grass and grass-clover swards that had previously been managed for a grasslands systems trial (Swift & Vipond, 1991; Swift *et al.* 1993; Vipond *et al.* 1997). Following a pilot study in 1991-1992 (Davies *et al.* 2001), a 3 year field trial was established in 1995 by the Scottish Agricultural College (SAC) to quantify the agronomic and environmental effects of ploughing out grass and grass-clover swards (O'Sullivan *et al.* 2001; Vinten *et al.* 2001). This experimental design was primarily blocked by sward type i.e. grass and grass-clover, but preliminary data showed that by 1998 there was no residual effect of sward type on the nitrogen cycle (O'Sullivan *et al.* 2001). My PhD research partially utilised plots from the above ploughing experiment, although my work did not commence until the final year of the parent project.

The soil at this site is represented by two soil series, both of which are imperfectly drained brown earths and are characteristic of a large area of the mixed agricultural land in the Midland Valley of Scotland (Vinten *et al.* 2001). Treatment blocks 1-8 (Figures 3.1 and 3.2) were typified by the Macmerry series, a sandy loam topsoil over a partially sorted sandy clay loam/clay loam subsoil, whilst treatment blocks 9-16 (Figures 3.1 and 3.2) were principally of the Winton series, a clay loam top soil overlying a silty clay loam/clay loam subsoil. The land capability classification for agricultural is 3.1, limited by wetness (Bibby *et al.* 1982).

The prevailing climate is moist and cool with an average annual rainfall at the site of 870 mm and an annual potential evapotranspiration rate of 450 mm. The site mean monthly temperature fluctuates between 2.5 °C in January and 14.8 °C in July. The experimental plots studied were sown to *Delibes* variety spring barley (*Hordeum vulgare L.*) and the timings of the field operations on these plots are displayed in Table 3.1. All plots were part of a two treatment factor split-plot design with randomised blocks. Tillage technique was the whole-plot factor with nitrogen fertiliser level as the sub-plot factor, each treatment was replicated three times. The site plan for 1998 is illustrated in Figure 3.1 and for 1999 in Figure 3.2.

Plate 3.1 Beechgrove field site

Plate 3.2 Chamber situated in an unfertilised direct drilled (DD N-) plot

The 12 m long and 10 m wide plots were either deep mouldboard ploughed to 300 mm (DP), conventional mouldboard ploughed to 200 mm (CP) or not ploughed i.e. direct drilled (DD) into straw stubble using a single-disc drill that generated seedling slits of *ca.* 5 cm in depth. The DP treatment was discontinued after the first experimental year (1998).

Table 3.1 Timings of field operations on spring barley plots at Beechgrove field, 1998-1999

Experimental Year	Cultivation	Crop sown	Fertiliser application	Harvest
1998	20/04/98	28/04/98	04/05/98	16/09/98
1999	10/03/99	09/04/99	27/04/99	06/09/99

Plots were either amended with 80 kg N ha^{-1} in 1998 or 70 kg N ha^{-1} in 1999 of ammonium nitrate (NH_4NO_3) as 'Nitram' (N+) or had no additional nitrogen added (N-). The quantity of added fertiliser varied between the two study years as a result of inaccurate calibration of the spreading equipment. Fertiliser was applied to the spring barley (*ca.* crop growth stage [GS] 5) in early May and measurements continued at weekly to fortnightly intervals between mid May and July in 1998, and in 1999 from April until the end of June. Measurements ceased at GS > 57 (during ear emergence) when gas sampling was unfeasible due to the height of the crop (> 600 mm)

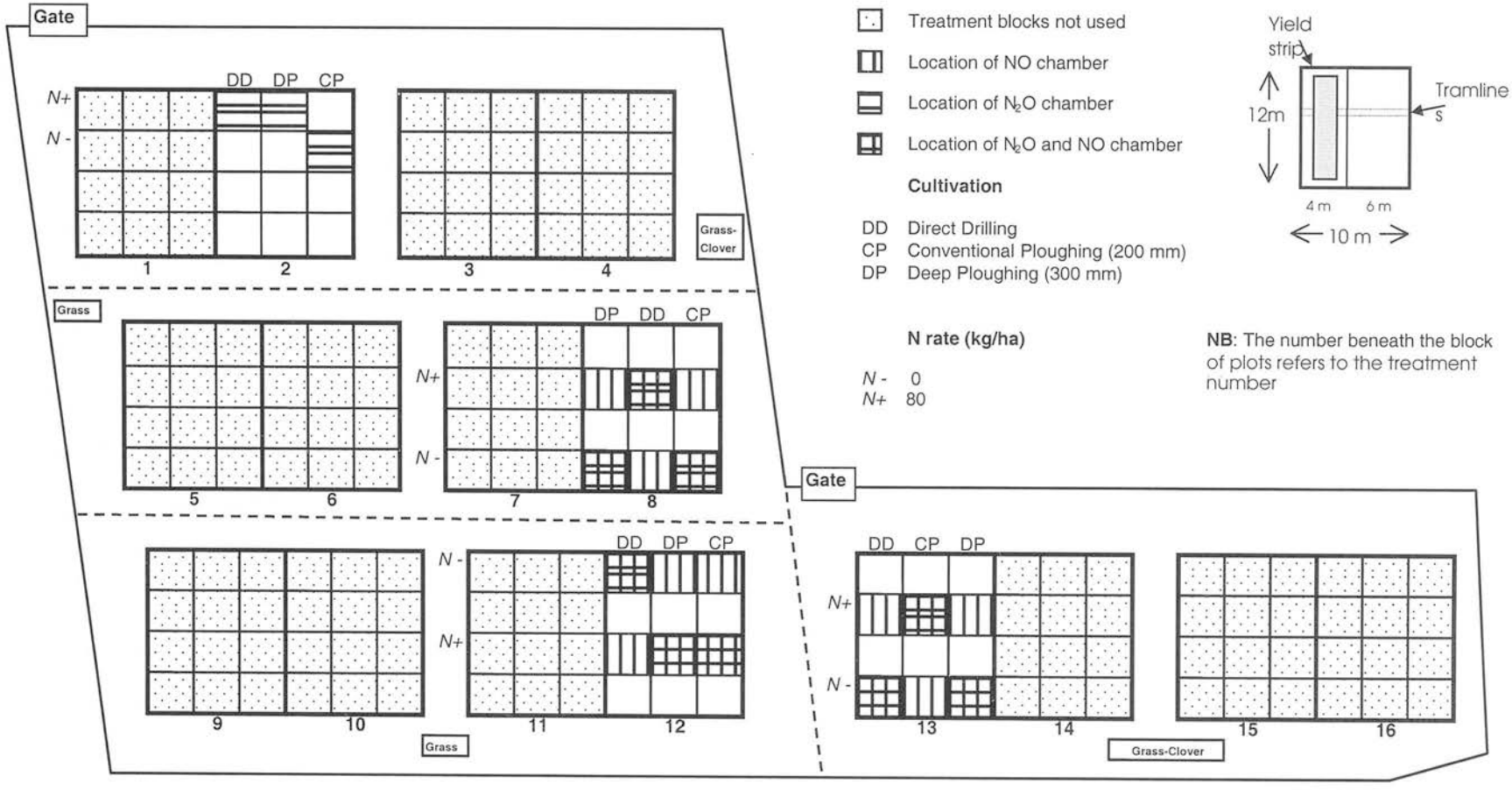

Figure 3.1 Beechgrove Field Experiment (Spring Barley) Site Plan 1998

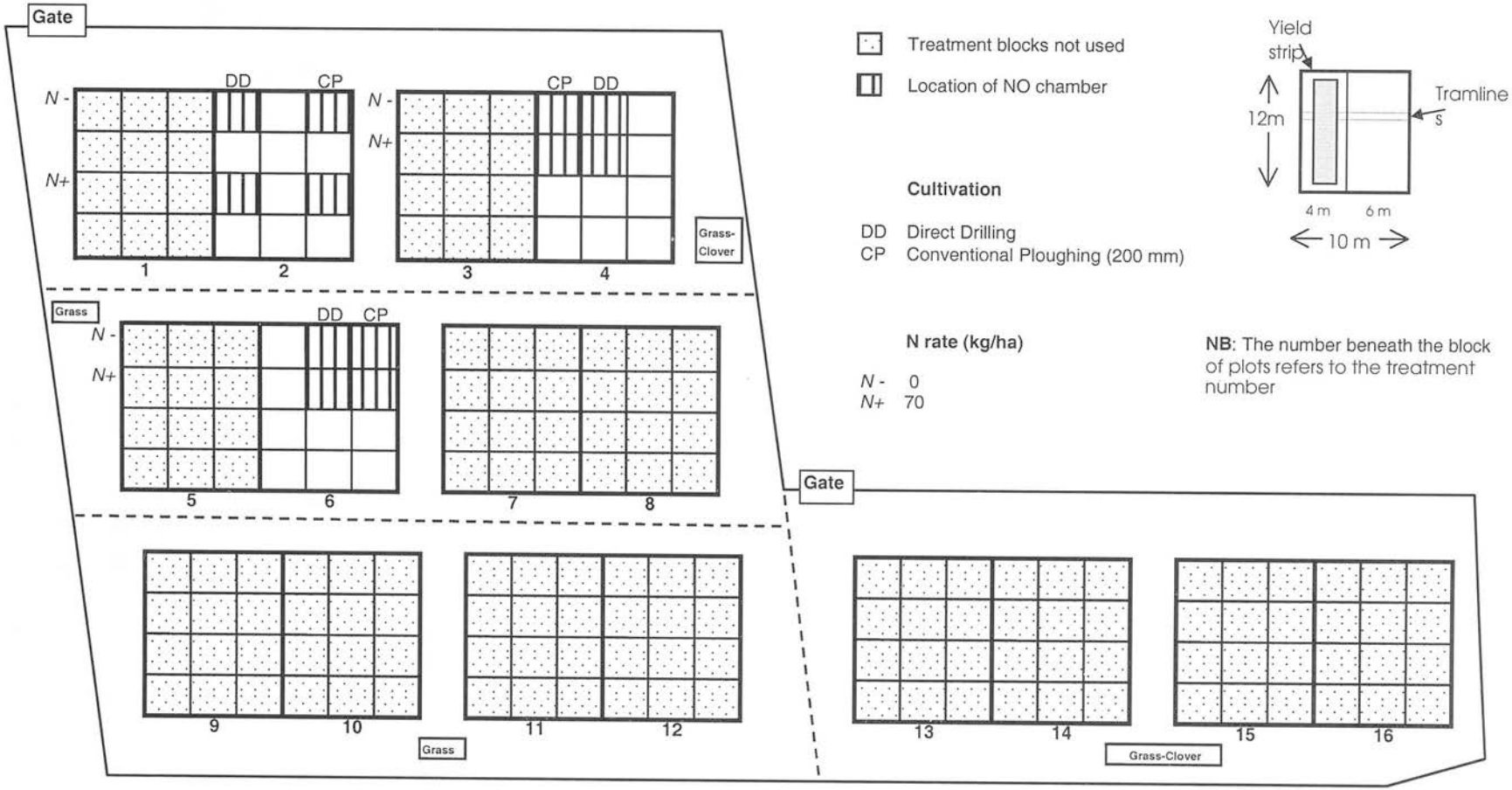

Figure 3.2 Beechgrove Field Experiment (Spring Barley) Site Plan 1999

3.3 Materials and Methods

A closed system was used to measure NO emissions. Polypropylene cylindrical chambers (20 cm high, 40 cm diameter) were inserted into the soil to a depth of not less than 30 mm. The chambers remained in place throughout the experiment, with the exception of their necessary removal at harvest. However, it was impractical to replace the chambers in exactly the same position as prior to harvest. An ambient air sample was withdrawn for 1 min from inside the open chamber, via tubing fitted with a three-way tap and inserted through a hole in the chamber wall. Subsequently, the chamber was sealed for a 10 min period with a collapsible, clear, plastic, dome shaped lid. After the 10 min interval to permit the accumulation of NO, the atmosphere inside the chamber was again sampled for the duration of 1 min. In order to avoid problems with pressure differences during sampling, the lid was slowly pushed down, collapsing at a rate equivalent to that of the sample removal.

The gas samples were pulled through a PTFE line using the chemiluminescent NO_x analyser (Thermo Environmental Instruments, model 42C) in 1998 and the chemiluminescent Scintrex analyser fitted with a CrO_3 converter in 1999. The NO concentrations detected by the analysers were recorded on a data logger (Campbell Scientific, model 21x). In 1998 the analysing equipment was transported around the field plots in a handcart and powered by an uninterruptable power supply (3 KVA) located in the boot of a four-wheel drive vehicle. However, in 1999 the smaller dimensions of the internally battery powered Scintrex meant that an external power source was no longer required and only a small sack-trolley was needed to transport the equipment between plots. Furthermore, the use of the Scintrex analyser fitted with the CrO_3 converter extended both the chamber ambient and headspace sampling times from 1 min to 2 min, such that the time was equally divided for sampling with and without the converter switched on.

NO concentrations were additionally measured from a chamber fitted its base with a sheet of PTFE, in order to account for any losses of NO to the chamber wall.

In 1998 N_2O emissions were routinely taken from static chambers as part of the parent experiment and the method is fully described in Clayton *et al.* (1994).

Sampling for NO continued further into the field season, therefore N_2O measurements were subsequently taken from the same chambers and using the same lids as those used for NO measurements. Measurements of N_2O were taken following NO sampling with at least a one hour interval between chamber closures.

The chambers were closed for approximately one hour before sampling. The samples were collected in tedlar bags and analysed using ECD gas chromatography (Chrompack, CP 9000).

In each plot, soil temperature was measured in triplicate at the soil surface and at a depth of 50 mm. After each gas measurement three soil samples (0-10 cm) were taken from each plot, were kept at 4 °C and analysed the following day for moisture and available mineral nitrogen (ammonium and nitrate) content, as described in detail in Chapter 2.

3.4 Results

The experiment was set up as a two treatment factor split-plot design with randomised blocks, three replicates per treatment (Figures 3.1 and 3.2). The NO and N_2O emission data from the trial were analysed using a split-plot, repeated measures analysis of variance (ANOVA) (Genstat release 4.2). The available soil NH_4^+-N, soil NO_3^--N and soil WFPS data were analysed using a split-plot analysis of variance (ANOVA) (Genstat release 4.2).

Cumulative losses were calculated for each plot (replicate) by linearly interpolating the data points and integrating the underlying area over a 75 d period following fertilisation. Nitrogen loss due to application of fertiliser was estimated via the subtraction of the cumulative NO emission from the unfertilised treatments from the corresponding cumulative emissions of the fertilised treatments and the differences were related to the amount of nitrogen applied.

3.4.1 Experimental year 1998

The daily precipitation distribution and daily mean air temperature (April-July) recorded at the meteorological station located at the same altitude and approximately 300 m north-west of the field site are shown in Figure 3.3.

The site was much wetter than usual with a total annual precipitation during 1998 of 1056 mm, which is 186 mm greater than the average amount. Over the 3 month summer (May to July) sampling period the site received 42% of the 1998 annual quantity at 443.5 mm of rain. Rainfall over the preceding winter months (October to January) amounted to 338.2 mm. The mean daily air temperature for the month of July ranged from 7.4 °C to 20.5 °C with an average of 13.5 °C, which was 1.3 °C colder than normal (14.8 °C).

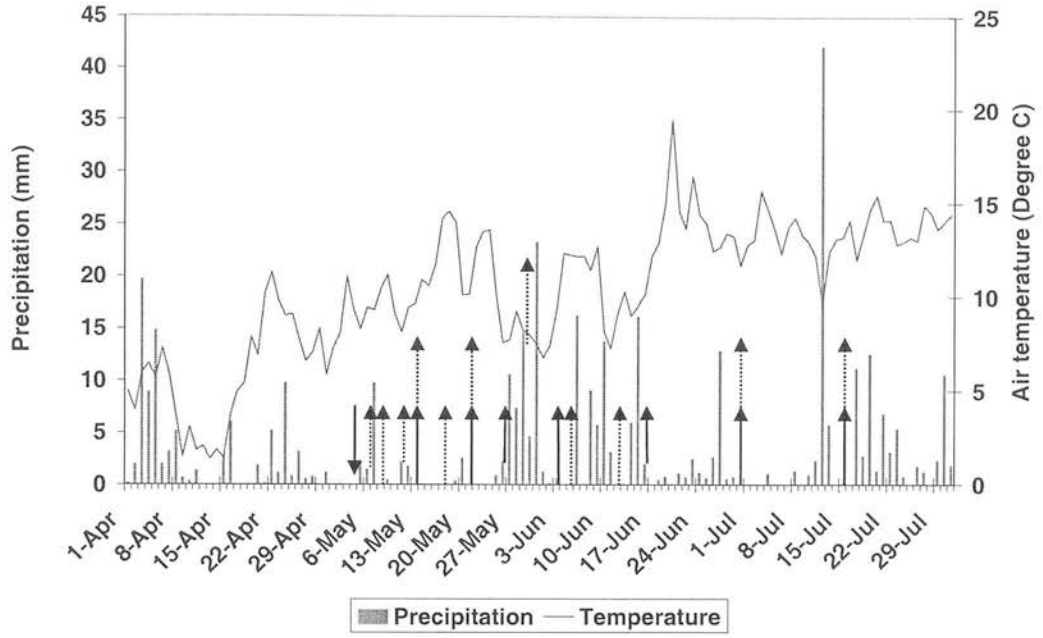

Figure 3.3 The precipitation and air temperature recorded from April through July 1998 (downward arrow indicates application of nitrogen fertiliser; upward solid arrow indicates NO measurement taken; upward dashed arrow indicates N_2O measurement taken)

3.4.1.1 Gaseous emissions NO fluxes

3.4.1.1.1 NO emissions

The NO fluxes measured from the soil to atmosphere at this arable site varied from 0.1 to 49.5 μg NO-N m^{-2} h^{-1} for nitrogen (N) fertilised plots and up to 20.2 μg NO-N m^{-2} h^{-1} for unfertilised plots (Figure 3.4). There was a highly significant ($P < 0.001$) effect of time on the NO flux (Table 3.2). Peaks of NO emission occurred on 9 d after fertilisation from all the ploughed treatments and 22 d after fertilisation for both of the no tillage treatments. All treatments showed a substantial drop in NO emission between 22 and 30 d after fertilisation. This coincided with a sharp increase in the water filled pore space (WFPS) of between 14 and 25% (Figure 3.5) and a large drop in soil NO_3^--N concentration of 14.8-29.2 μg g dry $soil^{-1}$ (Figure 3.6).

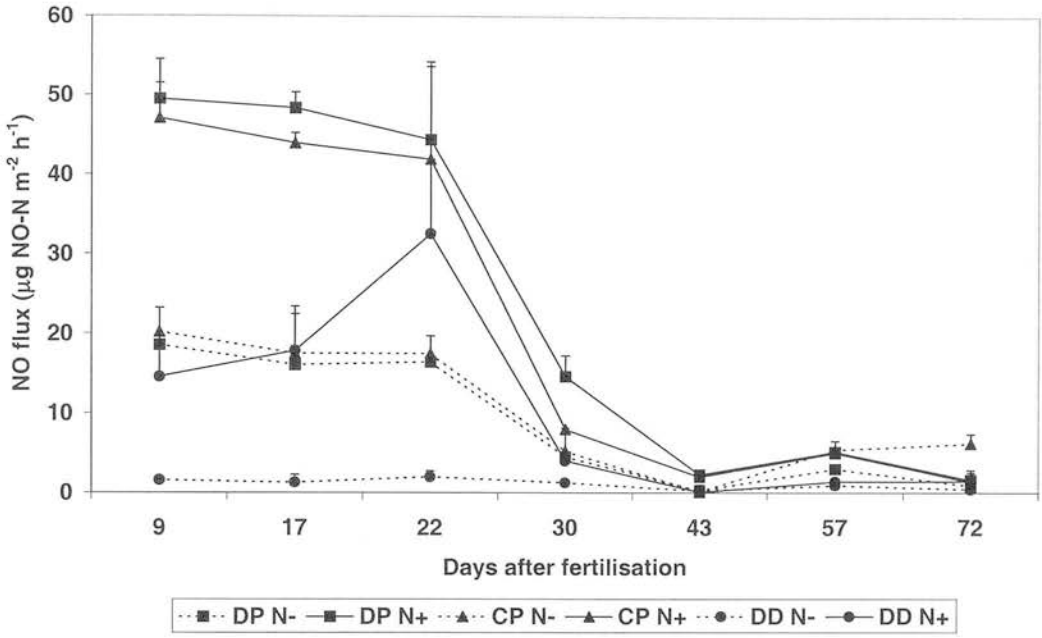

Figure 3.4 Mean NO flux measured in 1998 from deep ploughed (DP), conventional ploughed (CP) and direct drilled (DD) spring barley field plots with (N+) and without N fertiliser (N-). Fertiliser application was on the 4th May. Error bars represent one standard error c.

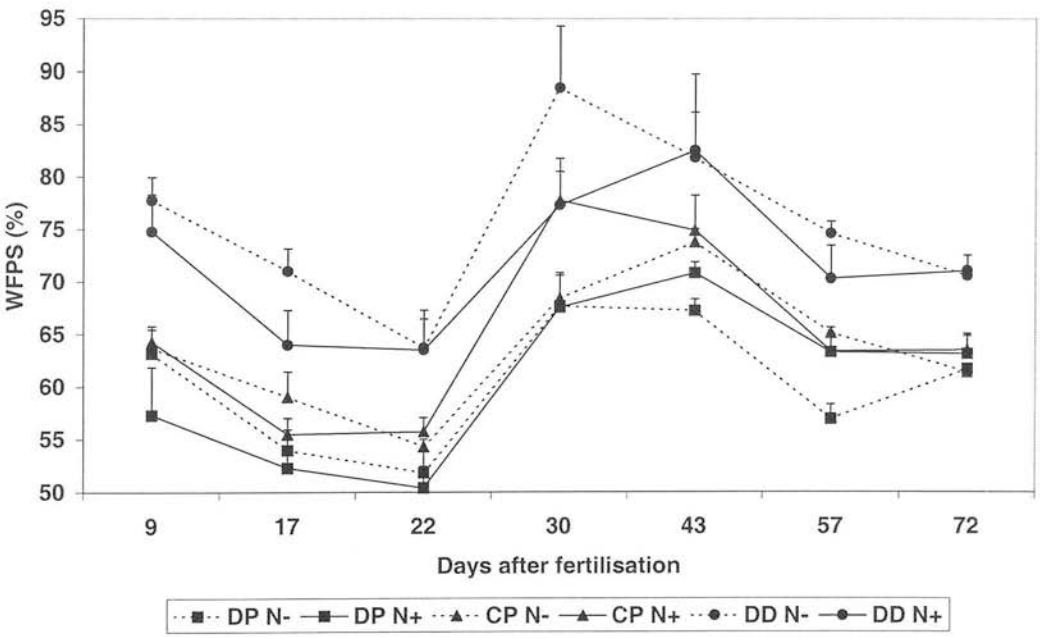

Figure 3.5 Mean WFPS measured in 1998 from deep ploughed (DP), conventional ploughed (CP) and direct drilled (DD) spring barley field plots with (N+) and without N fertiliser (N-). Fertiliser application was on the 4th May. Error bars represent one standard error (N=3).

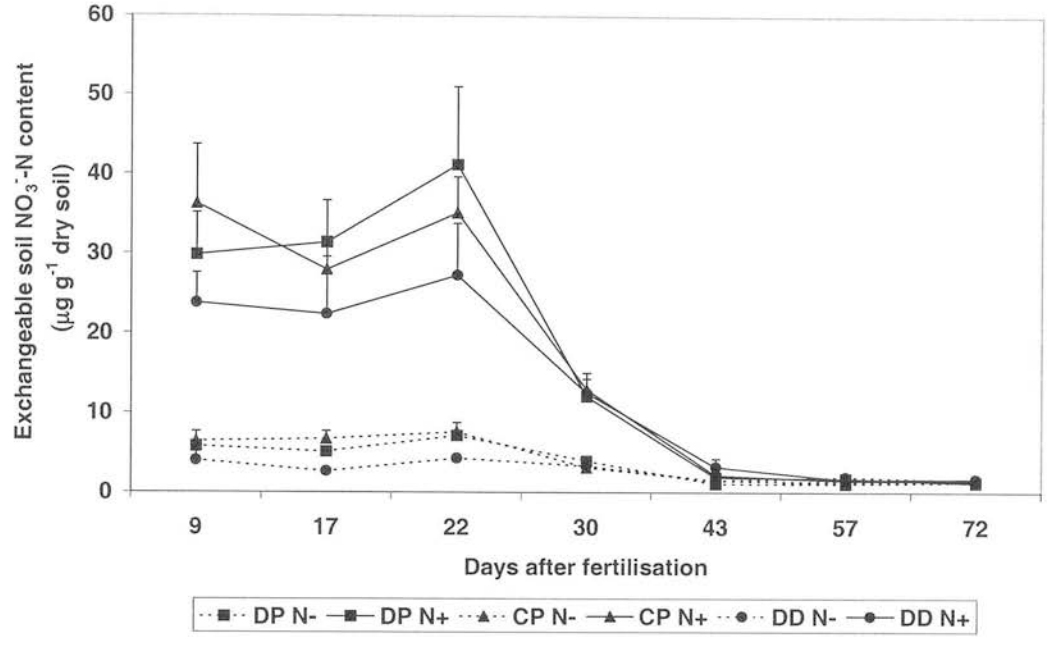

Figure 3.6 Mean soil NO_3^--N measured in 1998 from deep ploughed DP), conventional ploughed (CP) and direct drilled (DD) spring barley field plots with (N+) and without N fertiliser (N-). Fertiliser application was on the 4th May. Error bars represent one standard error (N=3).

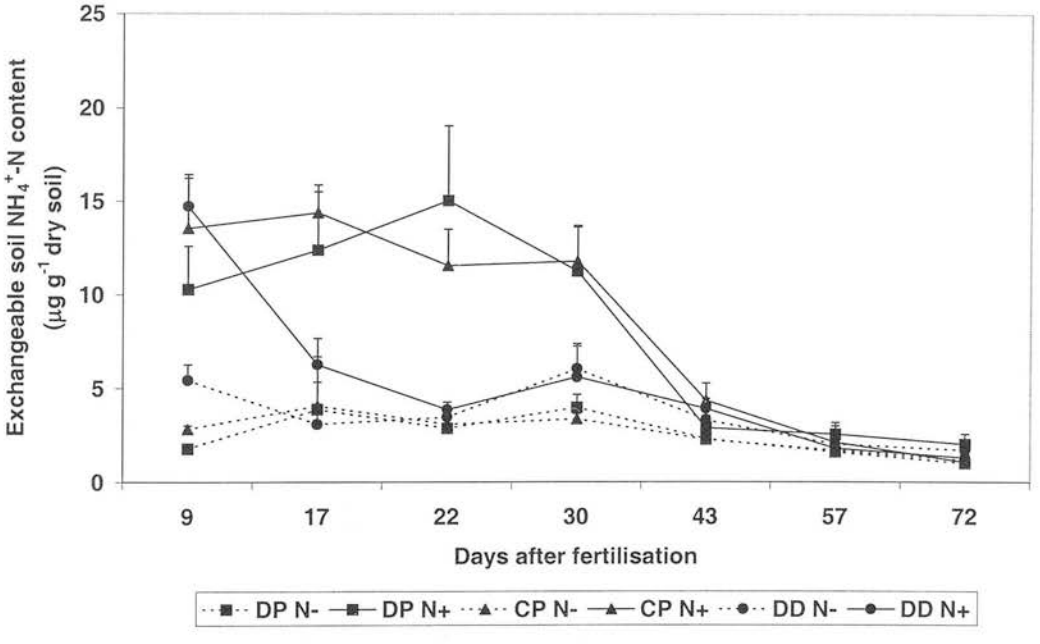

Figure 3.7 Mean soil NH_4^+-N measured in 1998 from deep ploughed DP), conventional ploughed (CP) and direct drilled (DD) spring barley field plots with (N+) and without N fertiliser (N-). Fertiliser application was on the 4th May. Error bars represent one standard error (N=3).

Emission minima were measured 43 d after fertilisation with the exception of the two ploughed fertilised treatments when the lowest fluxes were measured 72 d after fertilisation (Figure 3.4). Negative NO fluxes was observed once- 43 d after fertilisation from five individual plots (DD N-, CP N- and DD N+) and coincided with low soil available N (< 4 μg g^{-1} NH_4^+-N; *ca.* < 3.0 μg g^{-1} NO_3^--N) and a high WFPS (*ca.* >70%). A secondary emission peak (< 5.5 μg NO-N m^{-2} h^{-1}) was measured 57 days after fertilisation concurrently with a drop in WFPS of between 7 and 12%.

Table 3.2 Table of mean values (tillage, nitrogen and time) produced as a result of repeated measures ANOVA of NO flux (μg NO-N m^{-2} h^{-1}) in 1998.

Tillage method			Degrees of freedom	Standard error of differences of means
Deep plough (DP)	Conventional plough (CP)	Direct drill (DD)		
16.3	15.6	5.7	4	1.0

Nitrogen level		Degrees of freedom	Standard error of differences of means
No N fertiliser (N-)	N fertiliser at 80 kg N ha^{-1} (N+)		
6.5	18.6	6	2.1

Time - Days after fertilisation							Degrees of freedom	Standard error of differences of means
9	17	22	30	43	57	72		
25.2	25.1	25.4	6.2	1.1	2.0	2.6	47	1.8

Measured NO fluxes were used to calculate potential cumulative losses over a 75 d period following fertilisation (4th May-18th July) (Figure 3.8).

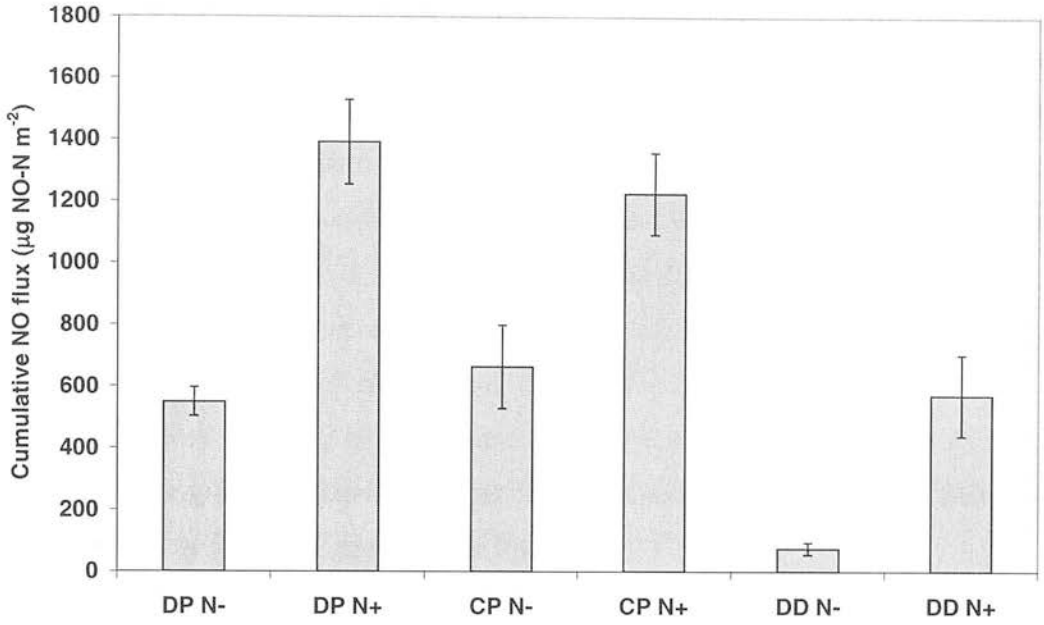

Figure 3.8 Cumulative NO emission over a 75 d period following fertilisation in 1998 from deep ploughed DP), conventional ploughed (CP) and direct drilled (DD) spring barley field plots with (N+) and without N fertiliser (N-). Error bars represent ± one standard error (N=3).

Table 3.3 Table of means (tillage and nitrogen) produced as a result of ANOVA for cumulative NO (µg NO-N m^{-2}) emitted over a 75 day period following fertilisation in 1998.

Tillage method			Degrees of freedom	Standard error of differences of means
Deep plough (DP)	Conventional plough (CP)	Direct drill (DD)		
971	944	323	4	50.4
Nitrogen level			Degrees of freedom	Standard error of differences of means
No N fertiliser (N-)	N fertiliser at 80 kg N ha^{-1} (N+)			
429	1063		6	101.5

There was a highly significant effect of both method of tillage ($P < 0.001$, d.f = 2) and N fertilisation ($P < 0.001$, d.f = 1) on the cumulative emission of NO from the soil surface. Losses of NO were higher from the ploughed plots and from those with added N fertiliser. (Table 3.3). Cumulative NO loss ranged from 75 to 1400 μg NO-N m^{-2}, with the lowest emission measured from DD N- and the highest from DP N+. The cumulative emission from the DP and the CP N+ plots were 2.5 and 1.8 times the value from the corresponding N- plots respectively. The DD N+ and N- plots differed by a factor of 7.6 (Figure 3.8).

There was a significant difference ($P < 0.05$; s.e.d = 0.0008; df = 4) in the percentage of applied fertiliser N lost as NO, with smallest loss from the DD plots and the largest from the DP plots (Table 3.4).

Table 3.4 The percentage of fertiliser nitrogen lost as NO-N in 1998 over a 75 d period following fertilisation

Treatment	Fertiliser nitrogen lost as NO-N (%)
DP N+	0.011
CP N+	0.007
DD N+	0.006

a) Comparison of N fertiliser treatments

Repeated measures ANOVA showed that there was a statistically significant ($P < 0.01$) effect of N fertilisation on the emission of NO and a highly significant ($P < 0.001$) interaction between time and N fertilisation. Fertilisation stimulated emissions such that the fertiliser treated plots emitted significantly more NO than the unfertilised plots, however the effect of time was different for the two levels of N fertilisation (Table 3.3).

Generally the NO fluxes from the plots that received no fertiliser were approximately half the magnitude of the emissions from plots that were amended with fertiliser. Emissions of NO from the DD N- treatment were as low throughout the period of the experiment as the fluxes measured on the N amended ploughed plots 43 and 72 d after fertiliser applications.

Table 3.5 Summary of ANOVA results (*P* values and associated level of significance) for each measurement occasion (days after fertilisation) in 1998 from soil NO_3^-, soil NH_4^+ and soil WFPS data (N=3).

Data	Treatment	Days after fertilisation						
		9	17	22	30	43	57	72
Soil available NO_3^-	Nitrogen	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	0.029 *	NS	NS
	Tillage	NS	NS	NS	NS	NS	NS	NS
	Nitrogen x Tillage	NS	NS	NS	NS	NS	NS	NS
Soil available NH_4^+	Nitrogen	<0.001 ***	0.003 **	0.007 **	0.008 **	NS	NS	NS
	Tillage	NS	NS	NS	NS	NS	NS	NS
	Nitrogen x Tillage	NS	NS	NS	0.026 *	NS	NS	NS
WFPS	Nitrogen	NS	NS	NS	NS	NS	NS	NS
	Tillage	0.015 *	0.017 *	0.011 *	0.029 *	0.023 *	0.002 **	0.019 *
	Nitrogen x Tillage	NS	NS	NS	NS	NS	NS	NS

*** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$; NS = not significant

NA = not available - too many missing values for ANOVA.

There was a strongly significant ($P < 0.05$ to $P < 0.001$) effect of N fertilisation on the soil available mineral N up to at least 43 days after fertilisation (Table 3.5). Significantly higher soil N concentrations were reported in the fertilised plots compared to unfertilised. A significant interaction ($P < 0.05$) between tillage method and N fertilisation on soil NH_4^+-N was shown 30 days after fertilisation, such

that N fertilisation had a larger effect on the ploughed plots than the direct drilled plots.

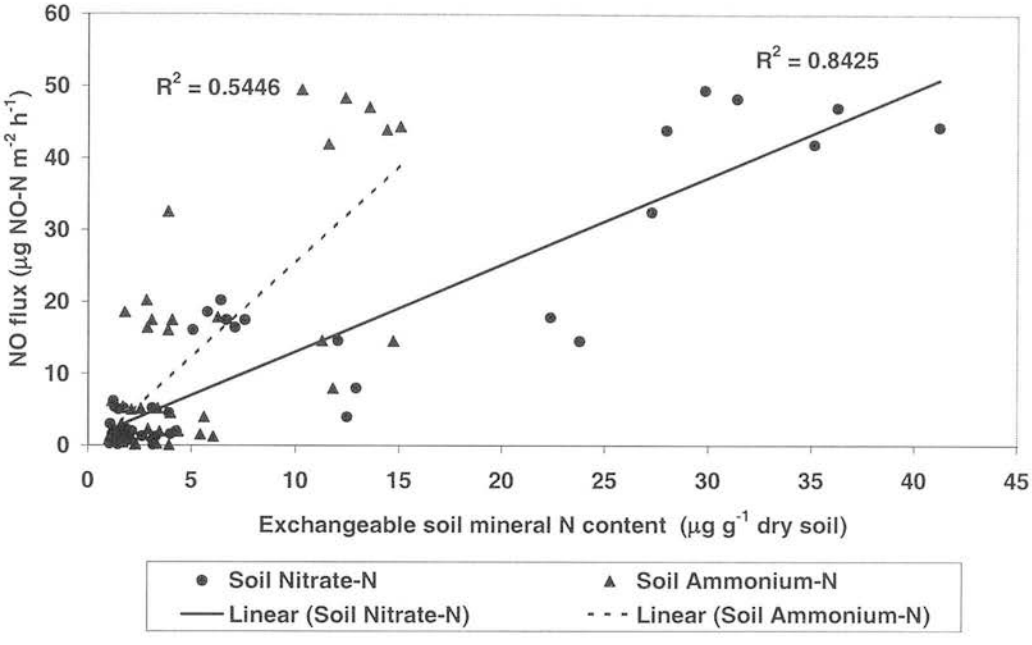

Figure 3.9 The relationship between mean NO emission and exchangeable soil NO_3^--N and soil NH_4^+-N concentration measured in 1998 from all tillage treatments (N=3).

A highly significant ($P < 0.001$) positive relationship between NO emissions from all treatments and both soil available NH_4^+-N ($r^2 = 0.54$) and NO_3^--N ($r^2 = 0.84$) was observed (Figure 3.9). Significant relationships ($P < 0.05$ to $P < 0.001$) between NO emissions and soil NO_3^--N for each individual treatment ranged from $r^2 = 0.82$ to $r^2 = 0.94$. Soil NH_4^+-N appears to impose less influence on NO emissions than soil NO_3^--N, with significant ($P < 0.05$) relationships (r^2 =0.72 and $r^2 = 0.69$) from the two fertilised ploughed plots.

b) Comparison of tillage treatments

There was a highly significant ($P < 0.001$) effect of both tillage method and the interaction between time and tillage method on the emission of NO, such that the effect of time on NO emission was different for the three tillage methods. There was

a marked difference in the magnitude of NO fluxes between tillage treatments with virtually consistently smaller fluxes observed from the DD plots. Emissions from the DP and CP plots were not significantly different. This difference in NO loss from the three tillage treatments was reflected in the mean NO fluxes for each tillage technique produced as a result of the repeated measures ANOVA and shown in Table 3.3.

Tillage technique did not have a significant effect on either soil NH_4^+-N or NO_3^--N concentrations (Table 3.5). Tillage method showed a strong relationship to WFPS, with a statistically significant ($P < 0.05$ to $P < 0.01$) effect noted on each measurement occasion (Table 3.5). WFPS was significantly greater in the DD plots than the DP and CP plots, frequently by more than 7% and is a reflection of their higher soil water contents and/or bulk densities. The dry bulk density from the soil surface (0-5 cm) was calculated in May 1998 and in increased in the order; 1.05 g cm^{-3} from the DD treatment, 1.13 g cm^{-3} from the CP treatment and 1.25 g cm^{-3} from the DP treatment.

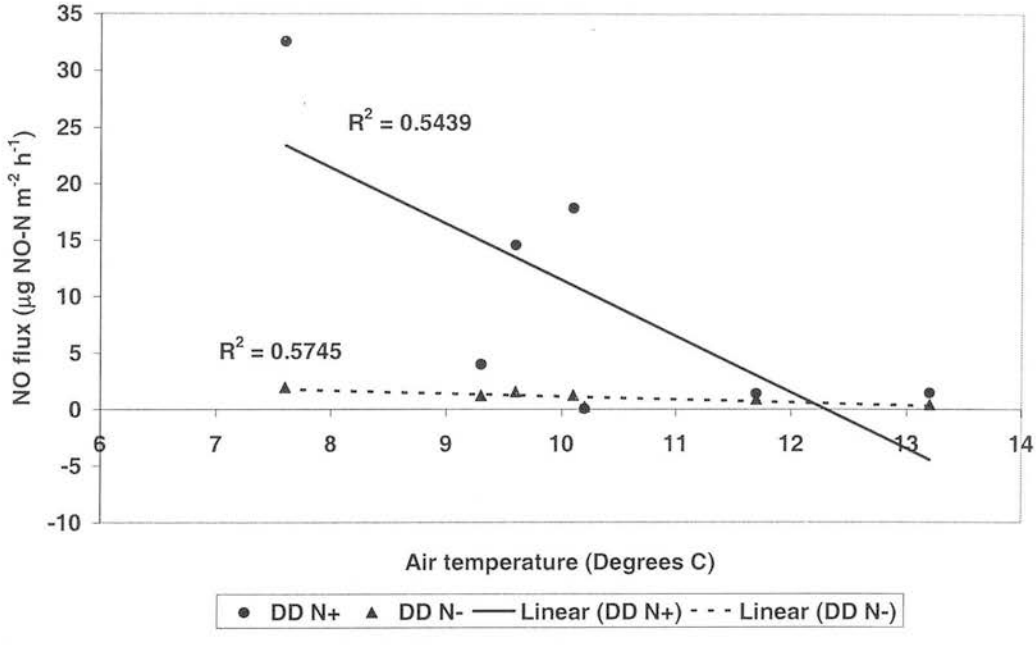

Figure 3.10 The relationship between mean NO emission and air temperature measured in 1998 from the direct drilled plots with (DD N+) and without (DD N-) added fertiliser (N=3)

Despite the apparently clear influence of tillage method on the WFPS, a significant ($P < 0.05$) negative relationship ($r^2 = 0.73$) between NO emission and WFPS was only observed from one treatment, DP N+. Furthermore, WFPS itself was able to explain only a relatively small proportion of the variation in the NO emission data from all treatments ($r^2 = 0.37$; $P < 0.001$). A combination of both soil NO_3^--N and WFPS was able to explain more of the variation in the NO data, producing a highly significant ($P < 0.001$) relationship ($R^2 = 0.91$).

The only relationship between temperature (air, surface or at a depth of 5 cm) and NO emission was a statistically significant negative correlation ($r^2 = -0.57$, $P = 0.048$ and $r^2 = -0.54$, $P = 0.059$) between air temperature and NO emission from the DD N+ and DD N- treatments respectively (Figure 3.10). It was possible to establish a significant negative relationship ($P < 0.05$) for all treatments between NO emission and cumulative precipitation that had fallen over the previous 4 to 7 days before sampling.

3.4.1.1.2 N_2O emissions

Measurements of N_2O were primarily taken as part of the parent experiment and these results are shown by kind permission of Dr. Bruce Ball (SAC, Edinburgh). The last measurement was taken on the 12th of June, therefore, on the last two NO samplings N_2O fluxes were also monitored from the same chambers used for NO measurements, but different to the ones previously used for N_2O monitoring. The N_2O fluxes from all of the measurement occasions are shown in Figure 3.11 and varied from 2 to 3500 μg N_2O-N m^{-2} h^{-1} for fertilised plots and up to 1000 μg N_2O-N m^{-2} h^{-1} for unfertilised plots.

Typically the direct drilled soil produced substantially more N_2O than the equivalent conventionally or deep ploughed soil. Variability between replicate plots was inconsistent, although the average coefficient of variation (CV) was 82%. Variability in N_2O emissions at this site were partially attributed to topographic differences influencing the hydrology (O'Sullivan *et al.* 2001). Despite the variability, a statistically significant effect of time ($P < 0.05$), nitrogen ($P < 0.001$), tillage ($P < 0.01$) and interaction between tillage and nitrogen ($P < 0.001$) was observed over the first nine of the eleven sampling dates. Larger N_2O emissions were

measured from the direct drilled plots and from those with added N fertiliser. There was also a significant effect of tillage method ($P < 0.01$), but none of N fertilisation over the last 2 sampling occasions.

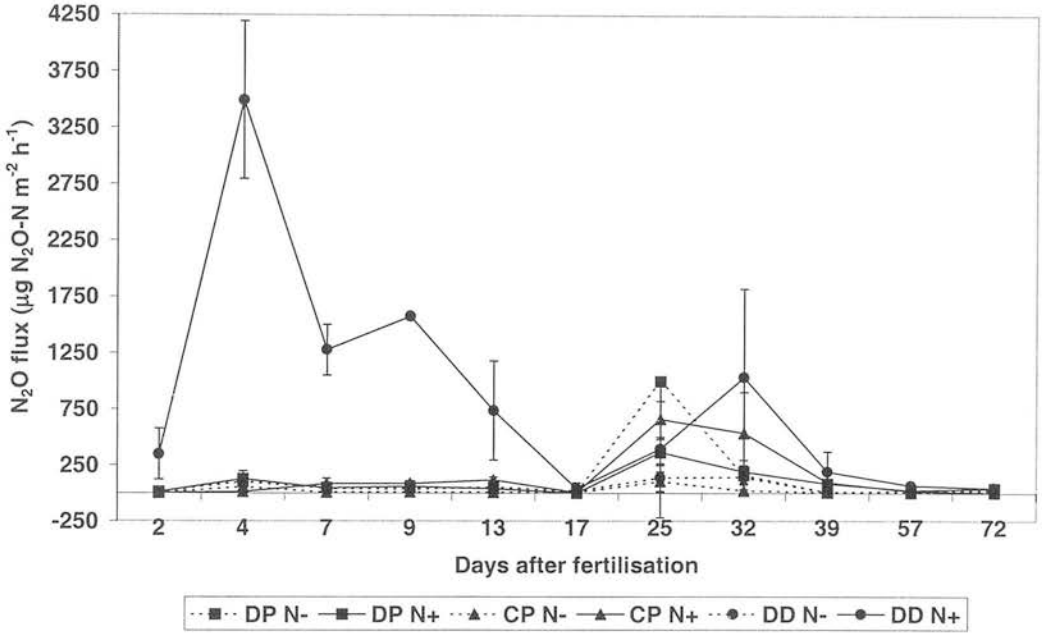

Figure 3.11 Mean N_2O flux measured in 1998 from deep ploughed (DP), conventional ploughed (CP) and direct drilled (DD) spring barley field plots with (N+) and without (N-) added nitrogen fertiliser. Error bars represent ± one standard error (N=3).

3.4.2 Experimental year 1999

The daily mean air temperature and daily precipitation patterns for April-July 1999 recorded at the meteorological station previously described are illustrated in Figure 3.12. During 1999 the site was 54 mm drier than average with a total annual precipitation of 816 mm. Over the 3 month summer (May to July) sampling period the site received less than half of the 1998 quantity of rain at 194.4 mm, although during the preceding winter months (October 1998 to January 1999) the recorded rainfall (420.7 mm) was 82.5 mm more than in 1998.

The mean daily air temperature for the month of July of 14.5 °C was similar to the mean value (14.8 °C) and ranged from 9.9 °C to 19.5 °C, but was 1 °C colder than the value recorded in 1998. The average temperature of the two previous months of May and June were also approximately 1 °C colder than in 1998.

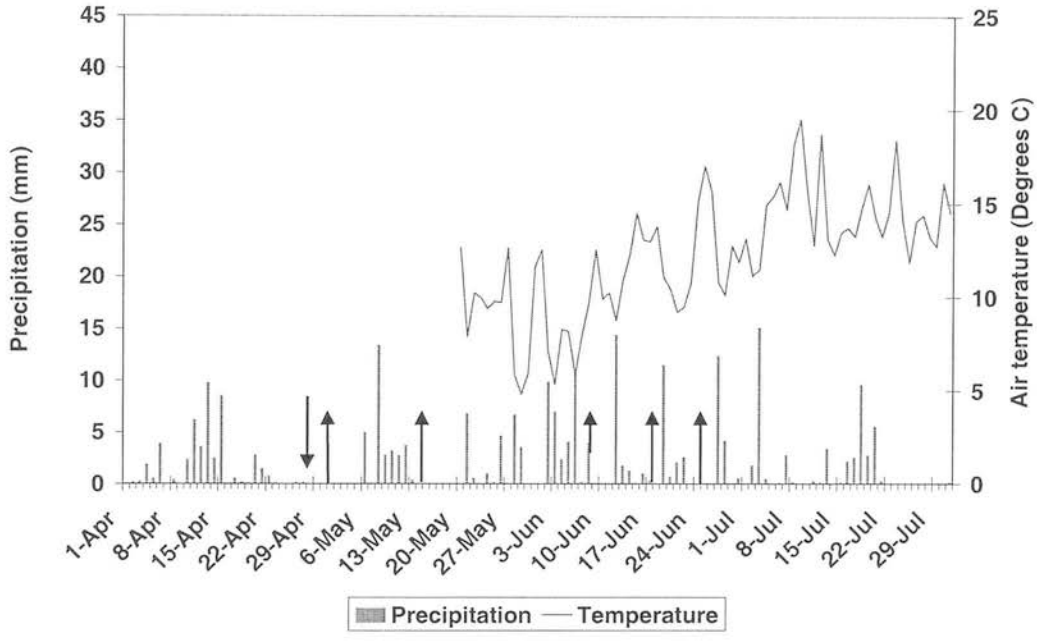

Figure 3.12 The precipitation and air temperature recorded during April to July 1999 (downward arrow indicates application of nitrogen fertiliser; upward arrow indicates NO measurement taken). Air temperature was not recorded until late May due to equipment failure.

3.4.2.1 Gaseous emissions

3.4.2.1.1 NO emissions

The NO fluxes measured from the soil surface at this site in 1999 ranged from -2.6 to 16.5 μg NO-N m^{-2} h^{-1} for fertilised plots and from -2.0 up to 7.7 μg NO-N m^{-2} h^{-1} for unfertilised plots (Figure 3.13). Fluxes were considerably lower than those measured in 1998 with maximum emissions approximately three times smaller. In 1998, however, two more sampling events occurred in the first 6 weeks following fertilisation than in 1999 when larger fluxes would have been expected.

There was a significant ($P < 0.05$) effect of time on the emission of NO at this site (Table 3.6), although there was little consistency in the timing of recorded peaks.

Table 3.6 Table of means (tillage, nitrogen and time) produced as a result of repeated measures ANOVA of NO flux (μg NO-N m^{-2} h^{-1}) in 1999.

Tillage method		Degrees of freedom	Standard error of differences of means
Conventional plough (CP)	Direct drill (DD)		
5.1	0.5	2	0.7

Nitrogen level		Degrees of freedom	Standard error of differences of means
No N fertiliser (N-)	N fertiliser at 80 kg N ha^{-1} (N+)		
1.0	4.6	4	0.3

Time - Days after fertilisation						Degrees of freedom	Standard error of differences of means
3	17	42	51	58	161		
4.6	6.8	2.3	1.6	2.5	-1.2	37	1.1

Despite the fact that the largest values of soil mineral N were observed 3 d after fertilisation (Figures 3.14 and 3.15), the maximum flux from the CP N+ treatment was measured 17 d after fertilisation. Following this peak emissions decreased *ca.* four fold and thereafter remained constant at 6-7 μg NO-N m^{-2} h^{-1} until the last measurement after harvest. In contrast, NO emissions from the DD N+ treatment approximately halved after the measurement peak and continued to decline for the remainder of the experiment. NO emission from the CP N- treatment peaked 3 d after fertilisation and subsequently, in common with all the measurements taken from the DD N- plots, was measured at ≤ 1.6 μg NO-N m^{-2} h^{-1}. Low NO emissions (≤ 2.0 μg NO-N m^{-2} h^{-1}) from all treatments coincided with very small soil available NO_3^--N (< 3.0 μg g^{-1} NO_3^--N) and NH_4^+-N (< 4.0 μg g^{-1} NH_4^+-N) concentrations (Figures 3.14 and 3.15).

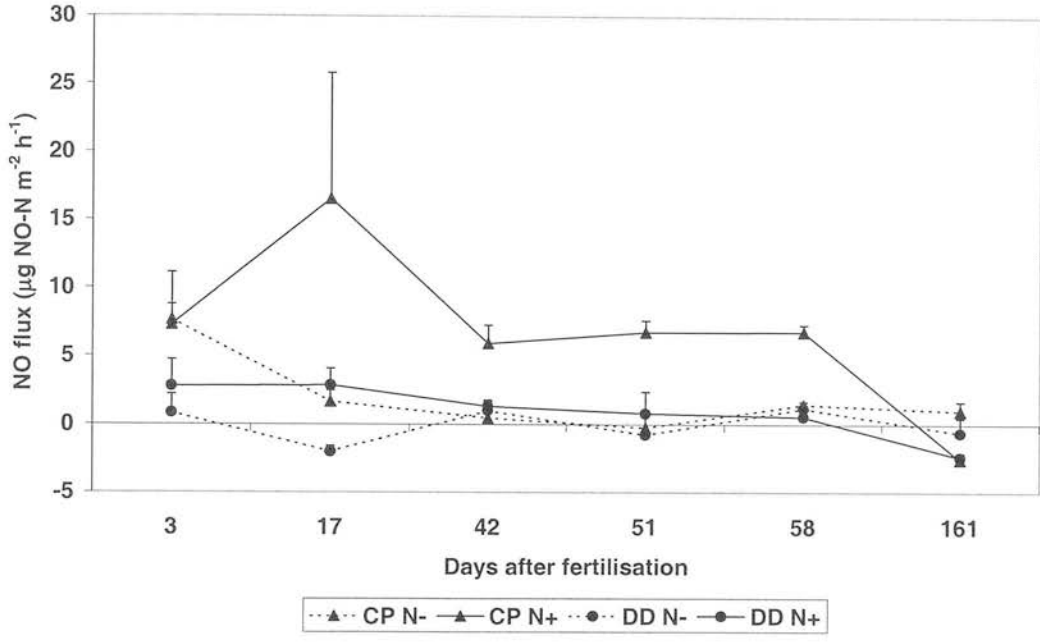

Figure 3.13 NO flux measured in 1999 from conventional ploughed (CP) and direct drilled (DD) spring barley field plots with (N+) and without (N-) added nitrogen fertiliser. Fertiliser application was on the 27th April. Error bars represent the standard error (N=3). Note the different scale to that in 1998.

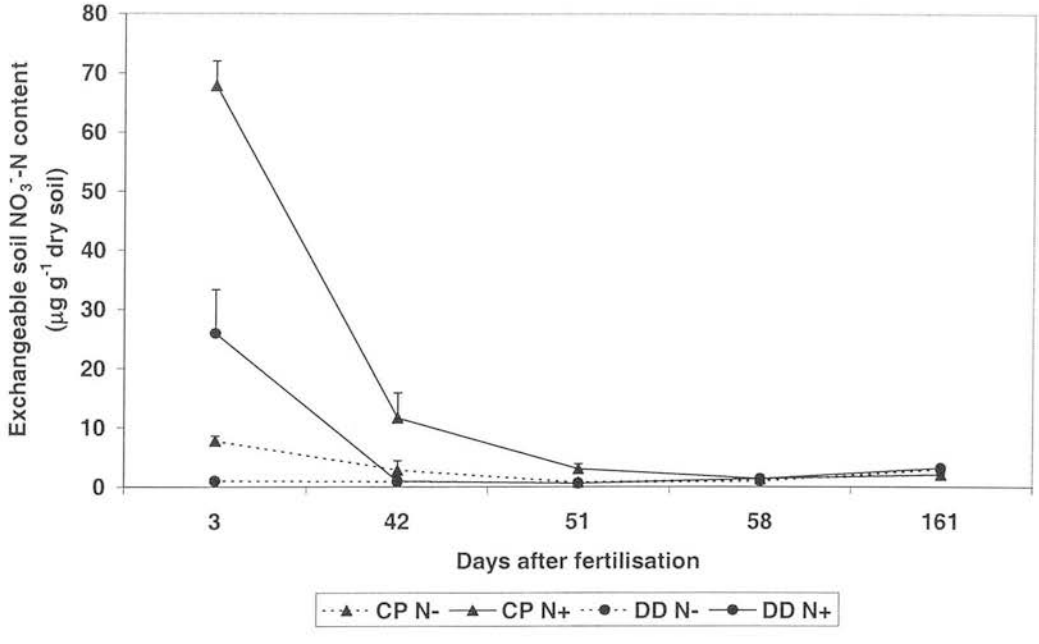

Figure 3.14 Soil NO_3^--N measured in 1999 from conventional ploughed (CP) and direct drilled (DD) spring barley field plots with (N+) and without (N-) nitrogen fertiliser. Fertiliser application was on the 27th April. Error bars represent the standard error (N=3).

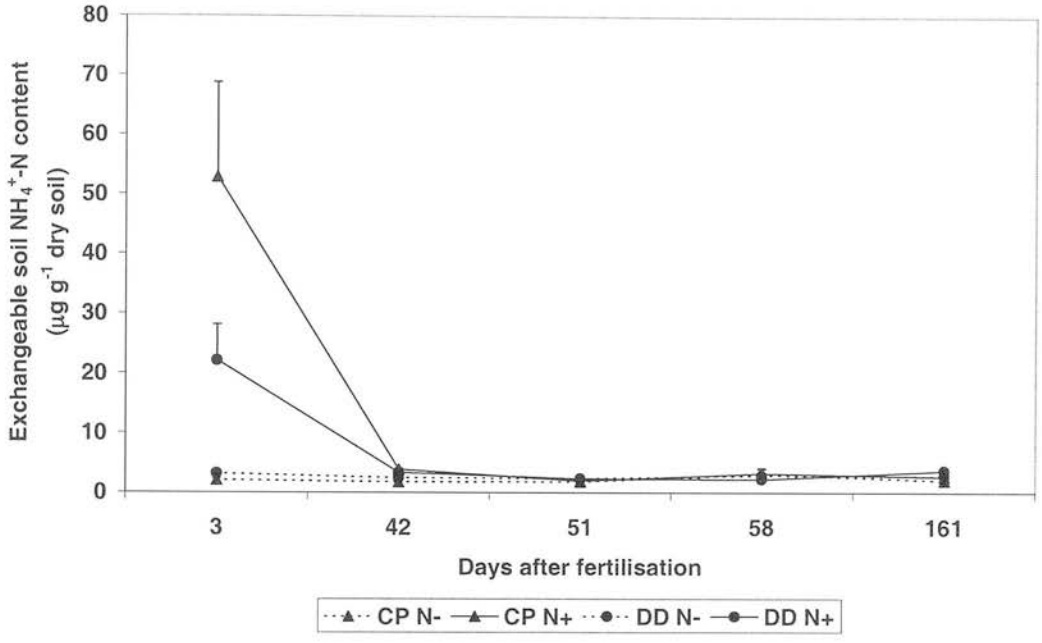

Figure 3.15 Soil NH_4^+-N measured in 1999 from conventional ploughed (CP) and direct drilled (DD) spring barley field plots with (N+) and without (N-) nitrogen fertiliser. Fertiliser application was on the 27th April. Error bars represent the standard error (N=3).

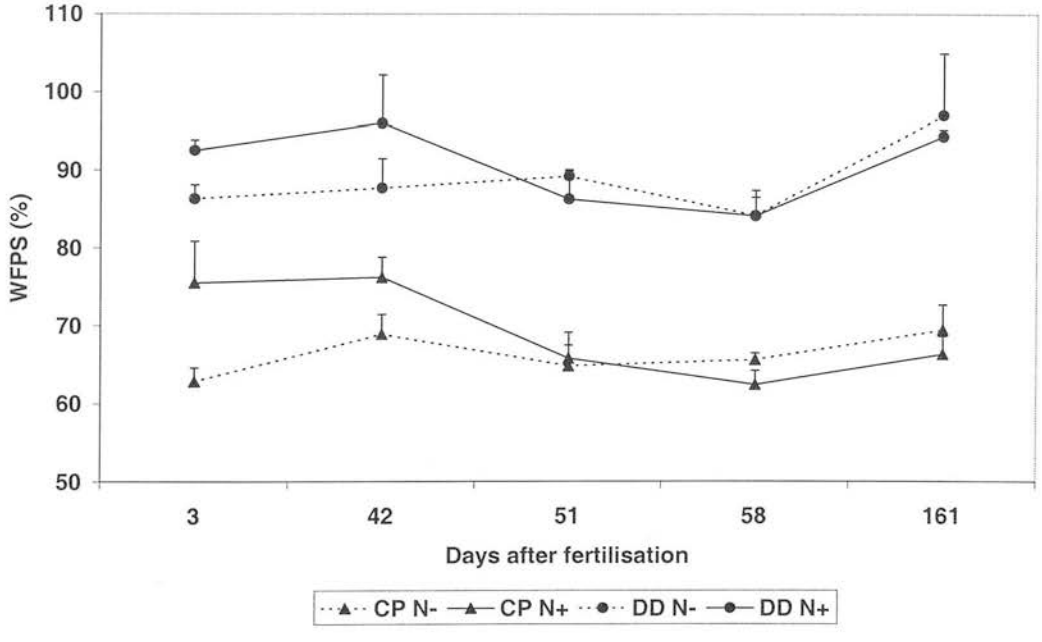

Figure 3.16 WFPS measured in 1999 from conventional ploughed (CP) and direct drilled (DD) spring barley field plots with (N+) and without (N-) added nitrogen fertiliser. Fertiliser application was on the 27th April. Error bars represent the standard error (N=3).

Emission minima were measured 161 d after fertilisation for both the N+ treatments, whereas the lowest fluxes were observed 17 and 51 d after fertilisation from the DD N- and CP N- treatments respectively. Negative NO fluxes were observed on six occasions, which is likely to reflect the visibly wetter soil conditions in this year (Figures 3.5 and 3.16).

Measured NO fluxes from all but the last sampling occasion following harvest (161 d after fertilisation) were used to calculate potential cumulative losses over a comparable 75 d period following fertilisation (27th April-7th July) to that in 1998. Cumulative NO loss ranged from 0.5 – 700 μg NO-N m^{-2}, with the lowest emission measured from DD N- and the highest observed from CP N+ (Figure 3.17). Losses of NO were considerably higher from the CP treatments and from those with added N fertiliser (Table 3.7).

Table 3.7 Table of means (tillage and nitrogen) produced as a result of ANOVA for cumulative NO (μg NO-N m^{-2}) emitted over a 75 day period following fertilisation in 1999.

Tillage method		Degrees of freedom	Standard error of differences of means
Conventional plough (CP)	Direct drill (DD)		
477	66	2	38.4
Nitrogen level		**Degrees of freedom**	**Standard error of differences of means**
No N fertiliser (N-)	N fertiliser at 80 kg N ha^{-1} (N+)		
72	470	4	24.7

The cumulative emission from the CP N+ treatment was *ca.* 6 times the amount from the corresponding N- treatment. As a result of the extremely low emissions and frequent negative fluxes from the DD N- treatment, the DD N+ and N- treatments differed by a factor of 255. (Figure 3.17). The fact that the addition of N fertiliser stimulates NO emissions to a much greater extent on the DD treatment than

on the CP treatment caused a statistically significant ($P < 0.01$) interaction between cultivation technique and level of N fertilisation.

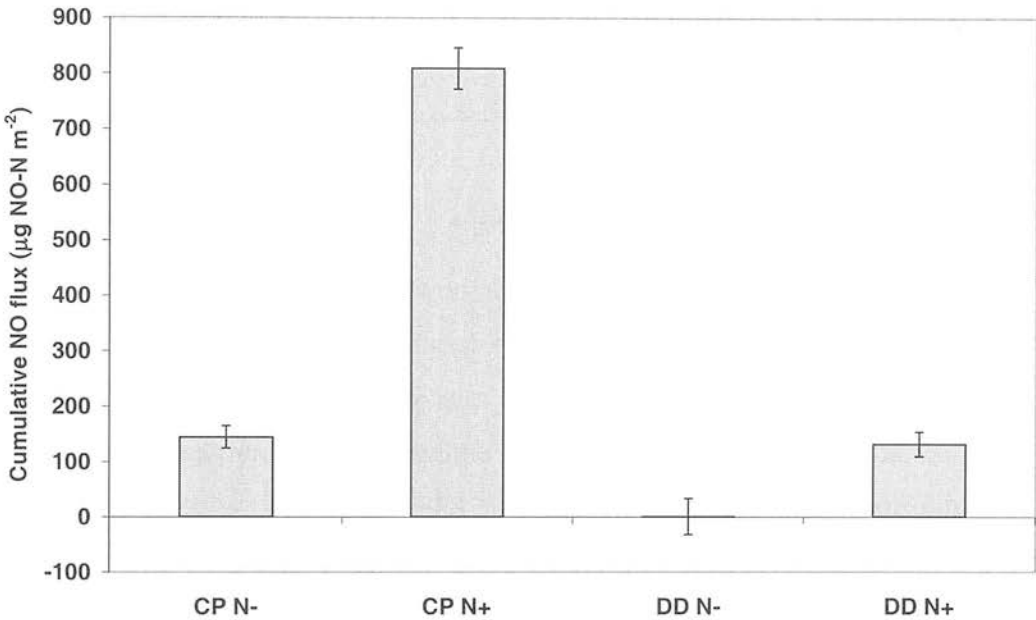

Figure 3.17 Cumulative NO emission over a 75 d period following fertilisation in 1999 from conventional ploughed (CP) and direct drilled (DD) spring barley field plots with (N+) and without (N-) added nitrogen fertiliser. Error bars represent one standard error (N=3).

There was a significant effect of both method of tillage ($P < 0.01$, d.f = 1) and N fertilisation ($P < 0.001$, d.f = 1) on cumulative NO emission from the soil surface (Figure 3.17). Cumulative losses per treatment were substantially lower in 1999, with emissions from the two CP and the DD N+ treatments 1.5-5 times larger in 1998 and the DD N- treatment 146 times larger (Figures 3.8 and 3.17). There was a significant difference ($P < 0.01$) in the percentage of applied fertiliser N lost as NO from the two treatments with the largest loss recorded from the CP plots (Table 3.8). The percentage of applied fertiliser lost as NO from the CP plots in 1999 was *ca.* 1.5 times more (0.010%) than in 1998 (0.007%), conversely the percentage loss of NO from the DD plots was 3 times larger in 1998 (0.006%) than in 1999 (0.002%).

Table 3.8 The percentage of fertiliser nitrogen lost as NO-N in 1999 over a 75 d period following fertilisation

Treatment	Fertiliser nitrogen lost as NO-N (%)
CP N+	0.010
DD N+	0.002

a) Comparison of N fertiliser treatments

Over the experimental period there was a highly significant effect of N fertilisation ($P < 0.001$) on the emission of NO with larger emissions from the N fertilised plots (Figure 3.7). There was also a significant interaction between time and nitrogen, such that the effect of time on NO emission was different at the 2 levels of nitrogen addition. The soil WFPS measured 3 and 42 d after fertilisation was higher from the N+ treatments, than from the corresponding N- treatments, although the effect was only significant ($P < 0.05$) 3 d after fertilisation (Figure 3.16 and Table 3.9).

A significant effect of nitrogen treatment on the soil exchangeable mineral N was rarely observed in this experiment, probably as a result of extremely low concentrations of both NO_3^--N (< 3.0 μg g^{-1} NO_3^--N) and NH_4^+-N (< 4.0 μg g^{-1} NH_4^+-N) reported in all treatments 51 d after fertilisation onwards (Figures 3.14 and 3.15). Significantly higher soil NO_3^--N in the N+ plots was recorded twice, 3 ($P < 0.01$) and 51 d ($P < 0.05$) after fertilisation and a significant difference in soil NH_4^+-N between the two levels of N fertilisation was also observed twice 3 ($P < 0.05$) and 42 ($P < 0.01$) days after fertilisation (Table 3.9).

Despite no significant effect of tillage technique on the concentration of soil NH_4^+-N for the sampling event carried out 42 days after fertilisation and on the concentration of soil NO_3^--N 51 days after fertilisation, a significant interaction ($P < 0.05$) between the level of N fertilisation and cultivation method was established. Fertiliser N consequently, had a much greater effect on soil NH_4^+-N and soil NO_3^--N on plots which have been conventionally ploughed than those which were direct drilled (Table 3.9 and Figure 3.15).

Table 3.9 Summary of ANOVA results (*P* values and associated level of significance) for each measurement occasion (days after fertilisation) in 1999 from NO emission, soil NO_3^-, soil NH_4^+ and soil WFPS data

Data	Treatment	Days after fertilisation					
		3	17	42	51	58	161
Soil available NO_3^-	Nitrogen	0.007 **	NA	NS	0.049 *	NS	NS
	Tillage	0.018 *	NA	NS	NS	NS	NS
	Nitrogen x Tillage	0.037 *	NA	NS	0.040 *	NS	NS
Soil available NH_4^+	Nitrogen	0.036 *	NA	0.001 **	NS	NS	NS
	Tillage	NS	NA	NS	NS	0.015 *	NS
	Nitrogen x Tillage	NS	NA	0.027 *	NS	NS	NS
WFPS	Nitrogen	0.021 *	NA	NS	NS	NS	NS
	Tillage	0.038 *	NA	NS	0.013 *	0.024 *	0.037 *
	Nitrogen x Tillage	NS	NA	NS	NS	NS	NS

*** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$; NS = not significant

NA = not available - due to time constraints soil samples not taken.

There were no significant relationships or relationships that were not strongly controlled by 1 data point between NO emissions from either all treatments or individual treatments and the measured soil exchangeable NH_4^+-N or NO_3^--N variables.

b) Comparison of tillage treatments

There was frequently a substantial difference in the size of fluxes between the two tillage treatments, particularly from the N+ treatments, with smaller emissions measured from the DD plots. Emissions of NO from the CP plots were 1.3 to 13 times larger than from the DD plots (Figure 3.13).

There was a statistically significant ($P < 0.05$) effect of the tillage method on NO emission as well as a significant interaction between time and tillage, such that the effect of time on NO emission was different for the two tillage techniques. As in 1998, emissions were larger from the CP treatments than from the DD treatments (Table 3.6). Furthermore, a significant interaction ($P < 0.01$) between nitrogen and tillage was observed, as fertiliser N had a far greater influence on emission of NO from ploughed soil than from soil that had undergone direct drilling.

There was a significant ($P < 0.05$) effect of tillage method 3 days after fertilisation on soil NO_3^--N, such that the concentration was larger from the CP plots (Table 3.9). Nitrogen addition, however, had a much greater effect on the CP plots which resulted in a significant interaction ($P < 0.05$) between fertiliser N and tillage method 3 and 51 days after fertilisation (Table 3.9). There was also a significant effect of tillage technique on soil NH_4^+-N 58 days after fertilisation, such that the concentration was larger in the CP plots.

As in 1998, the cultivation method exerted a strong control over the WFPS, with a statistically significant ($P < 0.05$) effect noted on each measurement occasion, except 42 days after fertilisation (Table 3.9). In the DD soil WFPS was never measured at less than 84% and was significantly greater, by more than 17%, than that of the CP plots.

WFPS itself was able to explain a small amount of the variation in the NO emission data from all treatments at 22% ($P < 0.05$) (Figure 3.18), although none of the variation from individual treatments or in combination with soil mineral N. Due to a malfunction at the meteorological station, air temperature was not available for the majority of the experiment, therefore the relationship between this and NO emission could not be explored. Again there was no significant effect of soil temperature either at the surface or at a depth of 50 mm. The only significant relationship ($P < 0.05$) that could be found in the CP N- treatment was between NO

emission and cumulative precipitation that had fallen over the previous 5 days before sampling.

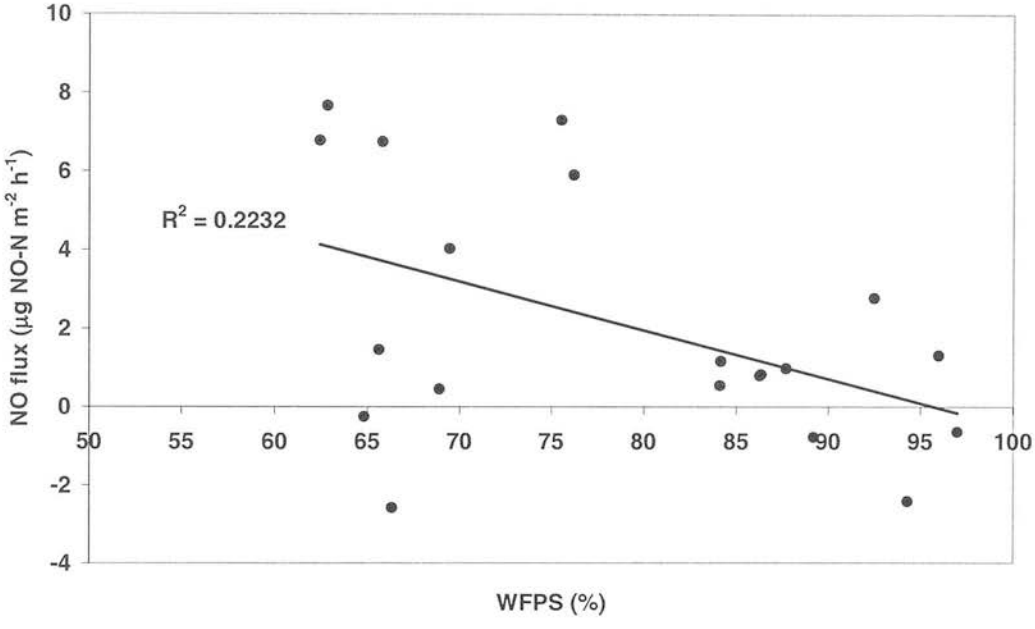

Figure 3.18 The relationship between mean NO emission and WFPS (%) measured in 1998 from all treatments (N=3)

3.4.2.1.2 N_2O emissions

Measurements of N_2O were not taken in 1999. The parent experiment had finished and due to time constraints it was not possible to continue N_2O sampling.

3.4.3 Agronomic properties

All agronomic data was obtained for the parent project and use of such data is gratefully acknowledged (Dr. Bruce Ball, SAC Edinburgh). Spring barley grain yields and total N uptake were calculated from a specifically designated 12 by 4 m strip within each plot (Figures 3.1 and 3.2).

In 1998, there was an apparent effect of nitrogen fertilisation and cultivation technique, but due to lost data it was not possible to carry out any statistical operations. The addition of nitrogen fertiliser enhanced the yield as did the use of a plough, although there appeared to be no difference between CP and DP treatments. In 1999 both cultivation techniques demonstrated a response to the added nitrogen fertiliser such that yields were significantly ($P < 0.01$) greater from nitrogen amended plots than from those with no nitrogen added. There was also a significant difference ($P < 0.01$) between the two cultivation methods with the greater yield measured from the CP treatments rather than from the DD plots (Figure 3.19). The yield was essentially zero from the DD N- plots, since the majority of the crop had failed and grass had grown in its place (Plate 3.2).

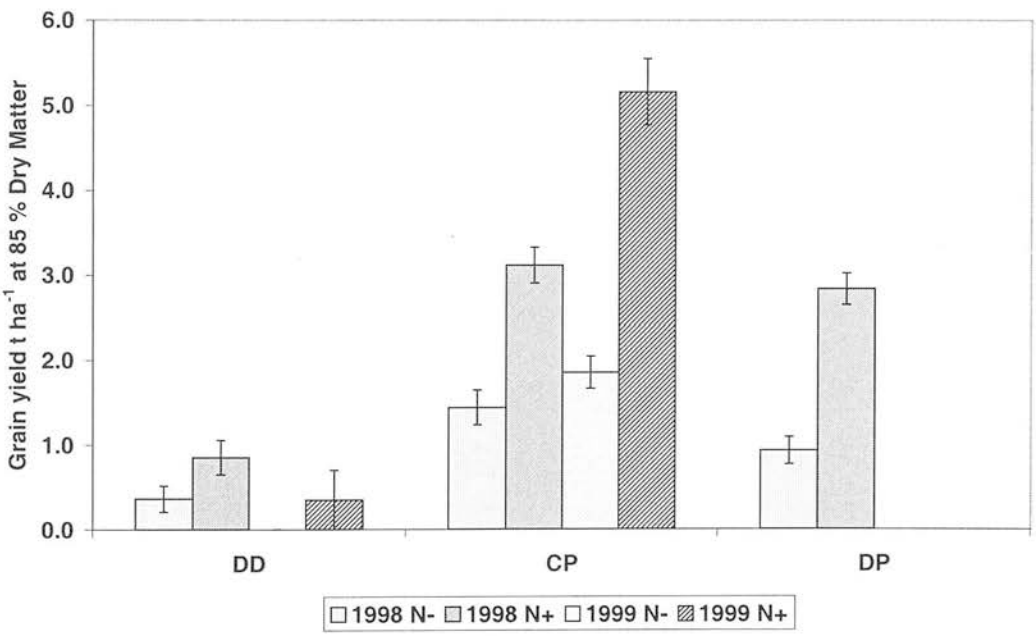

Figure 3.19 Spring barley yield measured in 1998 and 1999 from all treatments; direct drilled (DD), conventionally ploughed (CP) and deep ploughed (DP) and in 1999 from direct drilled (DD) and deep ploughed (DP). Error bars in 1998 represent ± one standard error between calculated mean yield values from the grass and grass-clover plots. Error bars in 1999 represent the standard error of the mean (N=3).

Smaller yields were measured from the DD treatments in 1999 than in 1998, but higher yields were collected from the CP treatments in 1999 compared to 1998. The larger yield obtained from the CP treatments is reflected in the measured crop

height (Figure 3.20). Crop height measured *ca.* weekly on the CP N+ plots was consistently taller in 1999 than in 1998.

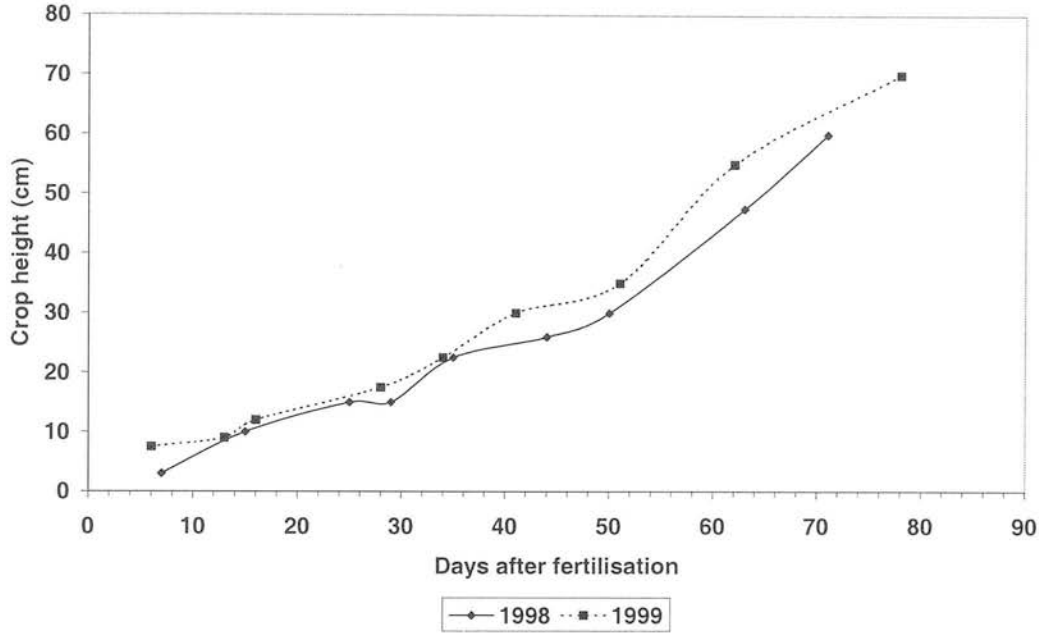

Figure 3.20 Crop height of spring barley measured in 1998 and 1999 from the CP N+ treatment (Courtesy of Dr. Bruce Ball, SAC Edinburgh)

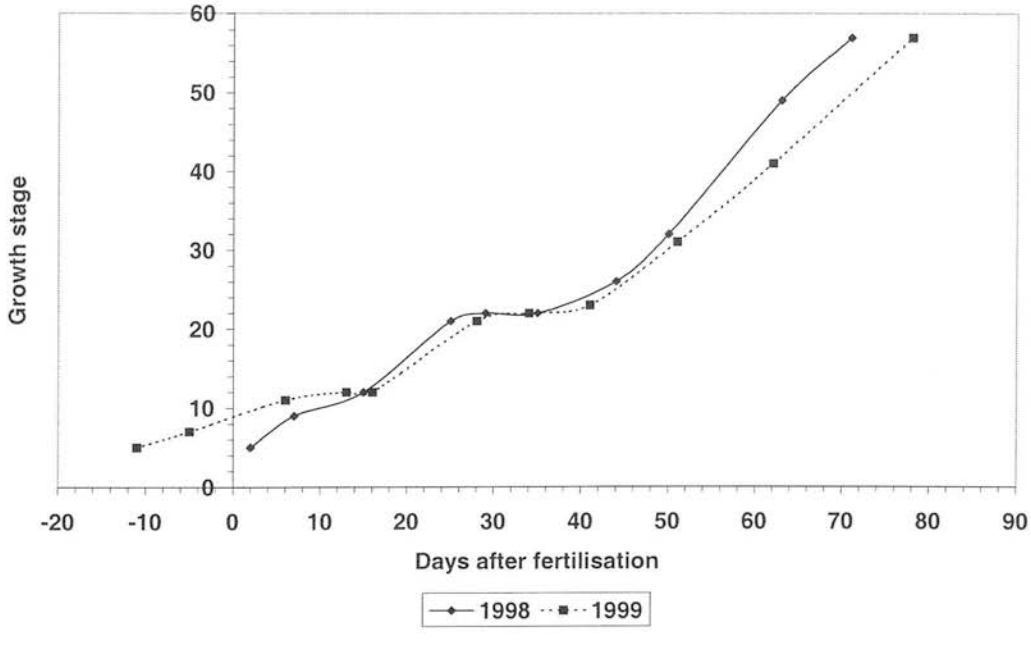

Figure 3.21 Growth stage of spring barley measured in 1998 and 1999 from the CP N+ treatment in relation to days after nitrogen fertilisation (Courtesy of Dr. Bruce Ball, SAC Edinburgh)

The growth stage of the spring barley was also recorded *ca.* weekly on the CP N+ plots. The crop was better established in 1999 at the time of fertilisation with a growth stage of *ca.* 9/10, which corresponds to the last stage of germination (leaf just at coleoptile tip) and the first stage of seedling growth (first leaf unfolds) (Tottman, 1987) (Figure 3.21). This echoes the earlier sowing date of 1999.

At the time of fertilisation in 1998 the crop was still germinating with a growth stage of < 5 (Tottman, 1987). However, after about growth stage 35 the crop in 1998 was consistently more advanced at a particular time following fertilisation.

3.5 Discussion

3.5.1 Effects of nitrogen fertilisation on NO emission

The addition of NH_4NO_3 fertiliser to all treatments at this site enhanced NO emissions in 1998 and 1999. The empirical results do not give a reason for this, but it is probably due to the stimulation of NO production processes, as a result of the increase in the soil available N (NH_4^+ and NO_3^-) content. In 1998 this is clearly seen in the significant relationships found between NO emissions and soil NO_3^--N and NH_4^+-N. This relationship was much less clear in 1999, probably due to limited data or other environmental constraints. Similarly, Linn & Doran (1984) observed that regardless of the tillage technique, considerably greater amounts of N_2O were produced from soils fertilised with N.

In both years, not only did the addition of fertiliser have a statistically significant effect on NO emission, but it also increased, as expected, the soil exchangeable mineral N, particularly NO_3^-. The effect of N fertilisation on NO emission and soil exchangeable mineral N did, however, diminish through the growing season as the actively growing spring barley created a sink for inorganic N and reduced that remaining in the soil which was at risk of loss via NO emission.

Overall, emissions of NO following fertilisation (Figures 3.4 and 3.13) were generally of a similar magnitude to those measured in 1997 from deep ploughed and direct drilled plots at the same field site in a short term experiment (Skiba *et al.* 2002). However, in 1998 peak emissions from the deep ploughed plots were more than double those in 1997 and in 1999 emissions from the direct drilled treatment were much lower. Emission peaks were, however, much smaller than previously observed e.g. Yamulki *et al.* (1995) detected fluxes from a UK wheat field up to 150 μg NO-N m^{-2} h^{-1} following the addition of more than double the amount of NH_4NO_3 fertiliser used in our experiment at 150-200 kg N ha^{-1}, although generally fluxes were measured below 14.4 μg NO-N m^{-2} h^{-1}.

Veldkamp & Keller (1997) estimate that *ca.* 0.5% of applied N fertiliser is lost as NO during the crop growing season. This is 1-2 orders of magnitude greater than that measured at Beechgrove (0.002-0.011%) over the 75 d period following fertilisation in both 1998 (Table 3.4) and 1999 (Table 3.8). However, Slemr & Seiler

(1991) reported a similar fertiliser loss rate from a sandy loam soil fertilised with 100 kg N ha^{-1} of $NaNO_3$ to that obtained at this field site at 0.003%. It should be noted though that this fertiliser loss related to a period of only 19 d and not the 75 d used in this study.

The disparity in the percentage loss of fertiliser N calculated in this study and that of other values recorded in the literature may be on account of the cool (average July temperature 5-15°C less than in most other studies e.g. Anderson & Levine, 1987; Hutchinson & Brahms, 1992), moist climate of Scotland, which is likely to favour denitrification and hence N_2O and N_2 production over that of NO. Furthermore, most previous studies were carried out using sand or a sand loam (e.g. Anderson & Levine, 1987; Hutchinson & Brahms, 1992; Skiba *et al.* 1993), which would encourage gaseous diffusion and reduce the loss of NO to consumption during denitrification and increase the production of NO from nitrification. Conclusions about the treatment differences are likely to be accurate, although a sampling regime of a higher frequency e.g. continuously or at least weekly throughout the year would have permitted a more reliable estimate of NO flux.

Regardless of the difference in cumulative NO fluxes measured between 1998 (Figure 3.8) and 1999 (Figure 3.17), the percentage loss of fertiliser N from conventionally ploughed plots were similar at 0.007 and 0.008% respectively (Tables 3.4 and 3.8). In 1998 the NO loss from direct drilled plots was not significantly different to that from conventionally ploughed plots, primarily due to the enhanced NO emissions in the unfertilised treatment due to ploughing. However, in 1999 there was a significant difference in the percentage loss of fertiliser N as NO between conventionally ploughed and direct drilled treatments. The very wet soils (WFPS > 80%) and hence highly anaerobic soil conditions under the direct drilled regime resulted in very low emissions of NO and, upon occasion, negative fluxes (Figure 3.13), presumably because of a lower soil compensation point induced by a high NO consumption rate. Consequently, losses of NO were relatively insignificant.

3.5.2 Effects of tillage on NO emission

In both years the comparison between direct drilled and both the conventionally and deep ploughed soils demonstrated a marked difference in the

magnitude of NO emissions (Figures 3.8 and 3.17). Fluxes from the 2 ploughed soils were not significantly different, but were significantly larger than from the direct drilled soil. The lower NO emissions were primarily a result of the higher WFPS induced by the direct drilling tillage method (Figures 3.5 and 3.16). The bulk physical soil parameters e.g. soil moisture and bulk density, however, may not have been measured at a high enough resolution in order to observe a strong statistically significant relationship between WFPS and NO emission.

Direct drilling has a large influence on the structure of the soil, particularly over the size distribution of soil pores. The bulk density of surface soil under direct drilling is frequently reported to be higher with smaller sized soil pores compared to that which has been conventionally ploughed (Ellis *et al.* 1979; Doran, 1980). Pore dimension is crucial to the amount of water held within the soil, small pores (< 80 μm in effective diameter) will retain more water and thus preclude oxygen. The accumulation of surface organic matter inhibits evaporation of soil water and further increases the soil moisture content (Rice & Smith, 1982).

The results obtained at this site from the parent experiment, 'the agronomic and environmental effects of ploughing out grass and grass-clover swards,' (O'Sullivan *et al.* 2001) demonstrated that the dry bulk density of the soil surface (0-5 cm) in the direct drilled plots (1.05 g cm^{-3}) was lower than in the conventionally ploughed plots (1.13 g cm^{-3}) (Pers. Comm. Ed Robertson, 2000). This difference in the bulk density, combined with considerably lower air permeability and mean relative diffusivity observed in the direct drilled plots (Pers. Comm. Ed Robertson, 2000) suggest that diffusion of O_2 was restricted to a greater extent and hence anaerobic conditions are more likely, compared to the adjacent conventionally ploughed treatments.

In both years there was a statistically significant effect of tillage on soil moisture content, such that the WFPS was consistently higher in the direct drilled soil compared to the ploughed soil (Tables 3.5 and 3.9). This is supported by both Ellis *et al.* (1979) and Doran (1980) who observed that WFPS was significantly higher in the compact surface (0-10 cm and 0-7.5 cm respectively) soil under zero-tillage than soil under conventional tillage.

Doran (1980) suggested that the water content of the soil is the principal factor in affecting soil microbial populations and together with soil temperature, aeration and the location of microbial substrates, controls the type and dominance of active soil microorganisms. Research has indicated that higher microbial populations and enzyme activities are present in surface soil under direct drilled as compared to conventionally ploughed soil (Doran, 1980; Doran, 1987). The larger microbial population in the zero-tillage system has a greater potential to remove, via immobilisation, mineral N that has been added as surface applied fertiliser. Nitrogen immobilisation will limit the availability of substrate to the nitrifying microorganisms and may contribute to the lower NO fluxes observed from the DD plots.

The reduced O_2 diffusion as a result of a high soil WFPS will encourage the development of anaerobic conditions and therefore stimulate denitrification. In an experiment using intact soil cores from zero-tilled and conventionally ploughed systems, denitrifying activity was significantly correlated with soil moisture (Rice & Smith, 1982). This is consistent with reported increased activity from denitrifiers in direct drilled soils compared to ploughed soils (Rice & Smith, 1982). The importance of denitrifying microorganisms to zero-tillage was reinforced through the work of Doran (1980), who demonstrated that the denitrifier population in the top 7.5 cm of soil was seven times as large as that in the same soil which had been conventionally ploughed. Rice & Smith (1982) propose that microorganisms present in zero-tillage soils are adapted to denitrifying conditions to a greater extent than those from conventionally ploughed soils.

It is not only a high WFPS which promotes denitrification in the surface of DD soils. Organic matter within the soil profile will strongly affect biological activity and consequently may also strongly influence the differences in denitrification seen between tillage techniques (Doran, 1980; Rice & Smith, 1982; Cameira *et al.* 1996; Young & Ritz, 2000). The wet, more anaerobic soil surface conditions in zero-tillage are also associated with an organic matter layer, which provides a readily accessible supply of organic substrates for microbial denitrification. Conversely, organic N and carbon are present in larger quantities at greater soil depths in conventionally tilled system than under zero-tillage (Doran,

1980; Dick, 1983) and are associated with significantly higher populations of aerobic bacteria and autotrophic nitrifiers (Doran, 1980). The action of ploughing in crop residues physically disturbs the soil and increases aeration so that compared to soil in a zero-tillage system biological oxidation (e.g. nitrification and mineralisation) is greater (Dowdell & Cannell, 1975; Doran, 1980; Dick, 1983; Cameira *et al.* 1996; Young & Ritz, 2000). In a conventionally tilled system the capacity for maximum aerobic microbial activity, therefore, was observed to continue to a greater depth, whereas in a zero-tillage system microbial populations were found to decline rapidly below the 7.5 cm depth (Doran, 1980).

The potential rate of denitrification in soil is, therefore, considerably higher in a zero-tillage system, whereas the potential rate of mineralisation and nitrification in soil is higher in a ploughed system (Doran, 1980). This explanation helps to explain the differences in NO emission detected between the three different tillage regimes; direct-drill, conventional plough and deep plough. It is widely recognised that the aerobic process of nitrification is the dominant process in the production of emitted NO (Davidson & Schimel, 1995; Williams *et al.* 1998), although in most soils both denitrification in anaerobic microsites and nitrification will occur simultaneously (Arah & Smith, 1990; Davidson, 1993). In this study the higher NO fluxes were emitted from the more oxic ploughed soils, whereas smaller emissions were associated with the more anaerobic direct drilled soils (Figures 3.4 and 3.13). The larger soil pores in the ploughed soil would also increase the probability of NO produced from either nitrification or denitrification escaping from the soil surface prior to consumption, primarily during denitrification. Conversely, the higher denitrification potential associated with smaller soil pores and a higher WFPS would considerably enhance the NO consumption rate in the direct drilled plots. This explanation of the observed variation in NO emission between the tillage treatments is supported by the N_2O fluxes measured during 1998 in both this study and predominantly in the parent experiment (Figure 3.11). Production of N_2O is commonly associated with denitrification (Firestone & Davidson, 1989) and consequently N_2O losses were substantially greater from the direct drilled plots than from the conventionally or deep ploughed plots (Vinten *et al.* 2001). Cumulative N_2O emissions calculated in the parent project over the sampling period of 18th

March to 12th June 1998 were 3 and 5.7 times larger from the direct drilled soil compared to the conventionally and deep ploughed soil respectively (Vinten *et al.* 2001). Similarly, in the first two years of the parent project N_2O fluxes were far greater from the direct drilled soils (Ball *et al.* 1999b; O'Sullivan *et al.* 2001; Vinten *et al.* 2001). The effect of tillage technique on the emission of N_2O/N_2 has been studied by several researchers who also reported that fluxes were considerably larger from zero-tilled soils as compared to those that were conventionally-tilled (Aulakh *et al.* 1982; Rice & Smith, 1982; Aulakh *et al.* 1984a; Aulakh *et al.* 1984b; Linn & Doran, 1984).

In the first experimental year (1998), when both conventionally ploughed and deep ploughed treatments were examined, it appeared that the deep ploughed treatments generally emitted more NO than conventionally ploughed, although this was never statistically significant (Figure 3.4). There may be an expectation that deep ploughed soil would generate more NO, primarily because of the greater mixing of the soil and the subsequent increase in top soil air-filled porosity. The action of ploughing to a greater depth would be to expose more organic N substrate to the microbial population and hence to increase mineralisation, providing a larger quantity of mineral N for denitrification, and specifically, nitrification. This effect will be short-lived i.e. will cease shortly after ploughing (Dowdell & Cannell, 1975) and may explain the measured similarity between the two ploughing techniques, since the first NO measurement occasion was not until 23 d after cultivation. Nonetheless, even at the first measurement occasion there still appears to be an effect of ploughing, as previously discussed in relation to NO stimulation on unfertilised plots.

In both experimental years, the first three sampling dates showed higher soil NO_3^--N from the ploughed treatments than the direct drilled treatments, although the differences were rarely significant (1999- 3 d after fertilisation) (Figures 3.6 and 3.14). Furthermore, twice in 1999 an interaction between nitrogen fertiliser and tillage treatment was recorded, such that the effect of fertiliser N had a much greater effect on the increase in soil mineral N in the ploughed treatment than the direct drilled (Table 3.9). This effect of tillage method on the soil NO_3^--N level has led several researchers to conclude that stimulation of denitrification in the more

anaerobic zero-tillage regime may partially explain the reduced soil NO_3^--N concentrations (Rice & Smith, 1982). Following the first 3 sampling dates the trend in soil NO_3^--N levels between tillage regimes was frequently reversed, such that higher concentrations were present in the direct drilled soils, although again the relationship was not statistically significant (Table 3.9). It may be possible that the barley plants in the less wet, aerobic soils in the ploughed systems grew more vigorously, removing more N from the soil. The substantially larger crop yields from the ploughed systems support this, although previous research has indicated that N uptake by crops does not greatly differ between the different tillage systems (Carter & Rennie, 1984).

Additional explanations have been suggested by several authors to account for the smaller NO_3^--N concentrations frequently observed in zero-tillage systems as compared to conventional tillage. The larger soil water content and lower evaporation of zero-tilled soils will encourage a higher leaching rate and subsequent loss of the highly mobile NO_3^- anion (Carter & Rennie, 1984). Results from the parent experiment, however, found that in 1997/98 at the Beechgrove field site winter (October to March) leaching from the direct drilled plots was actually significantly less than from the deep and conventionally ploughed plots (O'Sullivan *et al.* 2001). Alternatively, Vlasenko *et al.* (2001) suggested that a possible explanation for the reduction of soil NO_3^- under reduced tillage was the result of uptake by the larger weed population, although with adequate application of herbicide this potential problem should be minimised.

In 1998 and also observed by Cameira *et al.* (1996), the concentration of NH_4^+-N was not significantly affected by the tillage treatment, although it may have been expected that the NH_4^+-N concentration would be greater in the direct drilled soils due to reduced nitrification (Tables 3.5 and 3.9). Conversely in 1999 soil NH_4^+-N was larger in the CP plots than the DD plots, although the measured concentrations from all plots were small at < 3.5 μg g^{-1} NH_4^+-N.

3.5.3 Variation in NO emission over the sampling period

Similar to other studies, the pattern of NO emission in both years of the experiment demonstrated peak emissions during the first few days of fertilisation

(Veldkamp & Keller, 1997). In the 1998 field season, despite the first NO measurement not being taken until 9 days after fertilisation, peak emissions on both the fertilised and unfertilised, deep and conventionally ploughed plots occurred at the start of the measurement period (Figure 3.4). The stimulation of NO fluxes has frequently been observed following the addition of microbial substrate in the form of N fertiliser (Slemr & Seiler, 1991; Shepherd *et al.* 1991; Skiba *et al.* 1993a; Yamulki *et al.* 1995; Maggiotto *et al.* 2000). The peak fluxes also measured from the unfertilised plots may be due to the stimulating effect of ploughing. Ploughing disrupts soil aggregates and exposes organic N to microorganisms ultimately for use in nitrification and denitrification (Dowdell & Cannell, 1975; Paustian *et al.* 1997).

In 1998, the lowest fluxes from the fertilised ploughed plots were detected on the last measurement day, 72 days after fertilisation (Figure 3.4). This decline in the NO emission over the growth season is consistent with a concurrent decrease in the plant available soil mineral N levels, presumably as a result of uptake by the growing barley crop.

A different pattern in the timing of the peak emission was monitored from the direct drilled plots in 1998 (Figure 3.4). There was a general rise in emissions from 9 to 22 days after fertilisation, which may be a reflection of the corresponding decline in WFPS from > 75% to 64% (Figure 3.5). At a WFPS > 70% the dominant microbial production process is assumed to be denitrification, with N_2O as the largest proportion of end products (Davidson, 1991). As the WFPS drops the NO:N_2O ratio from denitrification will rise and therefore the consumption rate of NO will simultaneously decrease. Additionally, NO is likely to be produced from nitrification as well as denitrification taking place in anaerobic microsites.

In the 1999 field season the peak flux measured from the fertilised ploughed treatment did not occur until the second measurement occasion, 17 d after fertilisation (Figure 3.13). This may be explained by the fact that only 0.2 mm of rain fell between fertiliser application and the first NO sampling, 3 days after fertilisation. This small amount of rainfall was probably insufficient to fully dissolve the fertiliser prills and subsequently to wash the N into the soil to become available to the soil microbial population. An emission peak of a larger magnitude may have been missed due to the length of time between the first and second measurement occasions,

although sampling occurred as soon as possible after the end of the first spell of rain following fertiliser application.

The extremely low fluxes (< 1.5 μg NO-N m^{-2} h^{-1}) measured from the unfertilised direct drilled plots in 1999 fluctuated between a minimal emission and negative fluxes (Figure 3.13). It is likely that without N fertilisation, the high WFPS (Figure 3.14) and subsequent anaerobic nature of the soil environment exerted a dominant control over the NO emission, so that NO consumption during denitrification was highly significant.

The substantial reduction in NO emission monitored in 1998 between 22 and 30 days after fertilisation corresponded to a considerable drop in the soil NO_3^--N concentration, even in the unfertilised plots (Figure 3.6). The decrease in substrate available for denitrification may have contributed in part to the lower NO fluxes and the emission minima observed in all but the fertilised, ploughed plots. However, in response to the heavy precipitation (> 60 mm) which fell between 22 and 30 days after fertilisation, the WFPS increased sharply between 14 and 25% (Figure 3.5). This rise in WFPS to *ca.* 70% and above will have greatly increased the denitrification potential and reduced gaseous diffusion with the result that any NO produced would have a much greater chance of being consumed and therefore not be lost to the atmosphere.

It is however, possible that the rapid rise in the soil moisture content may have strongly influenced the NO_3^--N concentration. The expected increase in the denitrification rate due to the more intense anaerobic conditions may itself have reduced the NO_3^--N concentration via the use of NO_3^- as a terminal electron acceptor. Alternatively, it may be assumed that the wet soil conditions would have induced leaching, consequently removing the mobile NO_3^- anion. It is interesting to note that the concentration of the much less mobile NH_4^+-N did not significantly decline until between 30 and 43 days after fertilisation (Figure 3.7). On the other hand the reduction of NO_3^--N may have been due to plant uptake, although the most rapid crop growth occurs around growth stage 31 (Pers. Comm., John Garstang, 2001), which in this study corresponds to *ca.* 50 days after fertilisation. Indeed, Williams *et al.* (1998) attributed a rapid decline in observed NO and N_2O fluxes to a concurrent decrease in soil mineral N as a result of leaching and plant uptake.

In 1998, a secondary emission peak was measured following the general emission minima (Figure 3.4), which probably reflected the 7 to 12% drop in WFPS (Figure 3.5) rather than the level of inorganic N in the soil. The reduced anaerobic nature of the soil would decrease NO loss via consumption and increase nitrification, whereas both the soil NH_4^+-N and NO_3^--N concentrations remained virtually unchanged or slightly reduced.

3.5.4 Variation in NO emission between field seasons

The much larger NO emissions in 1998 (0.1-49.5 μg NO-N m^{-2} h^{-1}) (Figure 3.4) compared to 1999 (-2.6-16.5 μg NO-N m^{-2} h^{-1}) (Figure 3.13) can be explained by the variation in the timing of the field operations, the pattern of precipitation at fertilisation and potentially in WFPS. It is unlikely that the slightly lower application of N fertiliser (70 kg N ha^{-1} instead of 80 kg N ha^{-1}) contributed to this difference.

In 1999 the plots were ploughed 30 days before the crop was sown and it was a further 18 days until fertiliser application, whereas in 1998 the crop was sown only 8 days after ploughing and just a further 6 days until the addition of fertiliser (Table 3.1) . In 1998 it is suggested that the microbial population may be actively utilising the freshly exposed substrates and therefore stimulating fluxes even in the unfertilised plots. The length of time elapsed between cultivation and measurements in 1999 may have failed to measure any such activity. Furthermore, the crop was better established in 1999 prior to fertiliser application and may already have depleted the mineral N concentration available for use by the soil microorganisms.

The pattern of precipitation around fertilisation greatly differed between the two years (Figures 3.3 and 3.12). In 1998, the surface applied fertiliser was immediately washed into the soil matrix by *ca.* 14 mm of rain in the 3 days after fertilisation and then there was < 5 mm of rain between the next few measurement occasions. This rainfall pattern would have brought available N to microbiologically active sites and stimulated NO production. In contrast, in 1999 in the 8 days following fertilisation there was no precipitation to transport the fertiliser to the soil microbial population and to permit enhanced NO production. Subsequently, there was *ca.* 30 mm of rain in 8 days, which is likely to have increased the anaerobicity of

the soil and to reduce NO fluxes. Unfortunately, there were no supporting soil moisture and N_2O flux measurements taken at this time.

In 1999, despite a smaller recorded rainfall the soil was generally wetter than in 1998, particularly following fertilisation and from the direct drilled plots, which consistently had a WFPS > 80% (Figure 3.16). Diffusion of NO will be much slower in a soil with a high water content, therefore there is a greater opportunity for its consumption during denitrification and a greatly diminished NO emission. The wetter soil in 1999 may be the consequence of the change in chamber position from higher up a sloping field to further down slope. The down slope soil texture is, however, classed as sandier and this would suggest better drainage and improved gas exchange. Over winter rainfall was greater preceding the 1999 field season than 1998 and with little evaporation early in the year, the newly sited chambers may have been much closer and more affected by the ground water table.

The high soil WFPS recorded in the direct drilled plots in 1999 may have been partly due to the lower water use by the lack of crop growth on this treatment and may have been exacerbated due to the prolific growth of moss (absent in 1998) on the soil surface reducing evaporation. Furthermore, the moss may have formed a relatively impermeable barrier and reduced the chance of NO diffusion from the soil surface to the atmosphere.

The contrast in measured NO emission from the two years may simply be the result of inadequate sampling in 1999 and hence missed peak emissions. In the six weeks following N fertilisation NO was sampled more frequently in 1998 than in 1999 and in this time period measured high NO emissions. Nonetheless measurements taken in both years 17 days after fertilisation demonstrate that this may not be the case, since NO emissions from the CP N+ plots were *ca.* double those in 1998 compared to 1999 and more than four times the magnitude of emissions from DD N+ plots in 1998 compared to 1999.

3.5.5 Factors Controlling NO emission

It is suggested from the data recorded in both years that the availability of mineral N strongly influenced NO emission. The addition of NH_4NO_3 fertiliser clearly stimulated NO production. Not only was there a statistically significant effect

of N fertilisation both in 1998 and 1999, significant positive relationships were established between NO emission and soil mineral N (NO_3^--N and NH_4^+-N) in 1998. This suggests that mineral N influenced the emission of NO. Significant relationships between NO and soil NO_3^--N were observed on both fertilised and unfertilised soils. The relationship between NO emission and soil NH_4^+-N, however, was much less apparent with only a significant correlation from the fertilised ploughed soils. The lack of the strong relationships in 1999 as compared to 1998 may be the result of fewer measurements taken within the first month following fertiliser application and the subsequent smaller soil mineral N concentrations measured after the 2nd measurement occasion.

Despite the apparently obvious effect of the tillage technique on the WFPS and subsequent NO emission in both 1998 and 1999, on a treatment basis over the whole measurement period WFPS was only able to account for 37% and 22% of the variation in the NO emission data, respectively. To evaluate the combined effect of soil mineral N and WFPS, a stepwise multiple regression analysis was performed. In 1998 combining WFPS and NO_3^--N resulted in maximising the R^2 value at 0.91, however in 1999 the combination was very strongly influenced by one data point and was therefore rejected. A re-run of the stepwise regression without the outlier was also rejected. Thus, this study suggests that both mineral N (particularly NO_3^--N) and WFPS are important factors affecting NO losses.

It is apparent from the data obtained in both years that losses of NO were not strongly influenced either by soil temperature or air temperature. In 1998 there was a relationship between air temperature and the losses of NO from the direct drilled plots. In the wetter direct drilled soils, it is more probable that there is a larger percentage of NO contained within soil water than in the ploughed soils. The subsequent release of NO from direct drilled soils is, therefore, more likely to be influenced by temperature than from the ploughed soils. Certainly the observed relationship indicated that as the air temperature rose, the release of NO diminished, which is consistent with the theory of gas solubility in water (Goodroad & Keeney, 1984b).

Temperature is frequently correlated to NO emission, although other researchers have reported a lack of a relationship e.g. Slemr & Seiler, (1991). At the

same field site in 1997, periodically a relationship was established between NO emission and the diurnal temperature variation (Skiba *et al.* 2002). However, such a correlation was absent at low emission rates and at a high WFPS (Skiba *et al.* 2002), which may explain the lack of an observed relationship in this study in 1999. Similarly, Johansson (1984) proposed that where emissions were low (e.g. on unfertilised soils) a relationship between temperature and emission was not evident. Conversely, it has been suggested that the strongly controlling variable of soil mineral N may override other soil variables such as temperature (Yamulki *et al.* 1995). The observed strong relationships between soil mineral N and NO emission in 1998, therefore, may mask the effect of temperature.

The results from both years show that at higher NO fluxes the influence of the controlling environmental factors are more clearly seen, with both soil WFPS and mineral N levels evidently affecting the magnitude of NO losses. In fact the dominance of these two soil variables appear to mask other parameters e.g. temperature, which have previously been recorded as highly influential in affecting the size of NO fluxes. In contrast, at low NO emission rates the controlling factors were much harder to establish. This was evident in the data obtained from the 1999 field season. More subtle environmental parameters, e.g. soil carbon, plant cover, air temperature etc. or combinations of parameters may have been important, which were not fully taken into account.

3.5.6 Source of NO emission

In an agricultural soil, as studied in this project, it is reasonable to assume that the bulk soil pH is not consistently low enough for heterotrophic nitrification to dominate or to encourage chemodenitrification at a significant rate (Killham, 1986; Skiba *et al.* 1997). The dominant production of NO is liable to arise from microbial autotrophic nitrification and/or anaerobic denitrification.

In this study, the fertiliser was applied as NH_4NO_3 and thus it was not possible to differentiate between the effect of the individual components of the fertiliser (i.e. NH_4^+ and NO_3^-) on NO emission. Either purely NH_4^+-based or NO_3^--based fertilisers and their relationship with the NO flux have been used in previous

studies to indicate whether the emission source is from nitrification or denitrification (Sanhueza *et al.* 1990; Hutchinson & Brams, 1992).

A positive relationship between NO emission and soil NO_3^--N has been used to suggest denitrification, since emissions are highest when the concentration of the primary substrate for denitrification, NO_3^-, is at its peak (Sanhueza *et al.* 1990). However, the presence of NO_3^--N may indicate the end product of nitrification and hence a large NO flux may have arisen from nitrification. Similarly, a positive correlation between NH_4^+-N and NO emission may signify production by nitrification whereby the high levels of NH_4^+ substrate yield large NO emissions. A further complication arises due to the fact that nitrification and denitrification can occur simultaneously with any NO_3^- produced during nitrification consumed during denitrification.

A relationship between soil NH_4^+-N and NO flux from the ploughed treatments in 1998 indicating nitrification may be expected due to a higher degree of aeration and larger pore sizes, which favour nitrification over denitrification and subsequent loss of NO from the soil surface. The observed relationship between soil NO_3^--N and NO emission from the ploughed treatments in 1998 may be the result of nitrification, but it is more likely that as in the direct drilled plots and in 1999 the relationship reflects the use of NO_3^--N as a substrate in denitrification. Therefore in these soils it is probable that both nitrification and denitrification occur simultaneously and that both contribute to the production of NO.

Secondary relationships between NO emission and other soil parameters (e.g. soil moisture) which strongly influence nitrification and denitrification may additionally provide a suggestion of the dominant production process. In the range of water contents typically present in agricultural soils, both nitrification and denitrification can occur simultaneously (Linn & Doran, 1984). The relative significance of each process is primarily governed by the O_2 concentration in the soil, which in turn is strongly influenced by the soil water content. Thus the percentage of WFPS may be an effective indicator of the potential for aerobic and anaerobic microbial activity in the soil (Linn & Doran, 1984).

In 1998 the WFPS spanned the proposed transition point of 60% between processes which operate aerobically and anaerobically (Davidson, 1993) (Figure

3.5). This is reflected in Figure 3.22, which displays a distinct shift at a WFPS equivalent to field capacity, similar to that suggested by Davidson, of *ca.* 65% between NO emissions up to 50 μg NO-N m^{-2} h^{-1} and those < 15 μg NO-N m^{-2} h^{-1}. At a WFPS > 80% emissions of NO in 1998 were consistently < 2 μg NO-N m^{-2} h^{-1}. The larger of the NO fluxes all occur between a WFPS of 50-65%, which is generally within the quoted range (WFPS ≤ 60%) whereby NO is predominantly released from autotrophic nitrification (Schuster & Conrad, 1992; Hutchinson *et al.* 1993b; Williams *et al.* 1992). The highest measured fluxes are generally from the more aerated ploughed treatments, which would favour the escape of NO from the soil surface and which also yielded a positive relationship with soil NH_4^+-N.

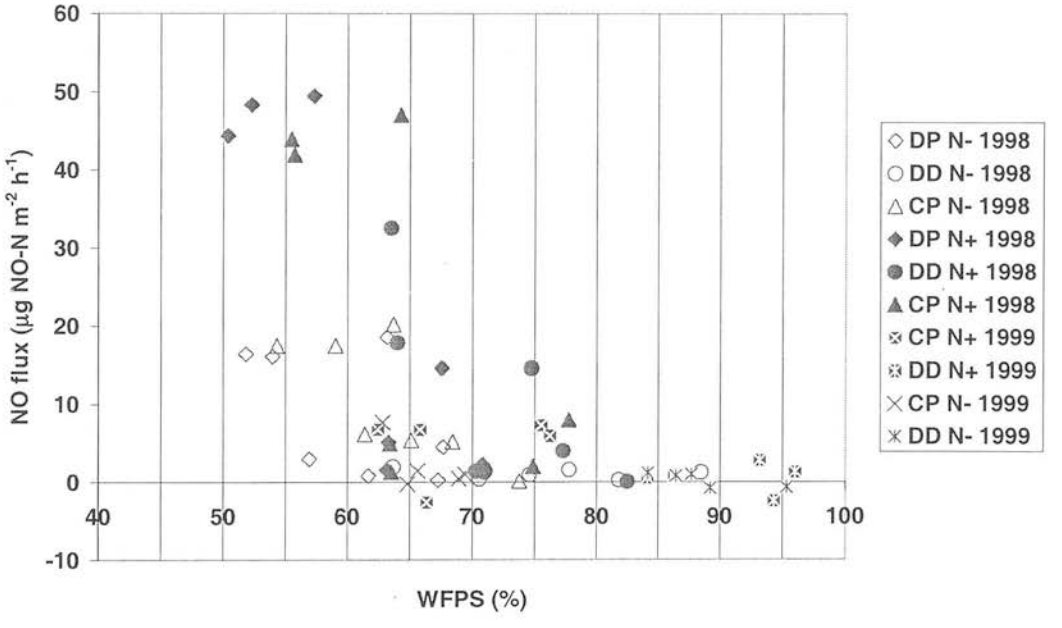

Figure 3.22 Relationship between soil WFPS and NO emission measured in 1998 and 1999 from all treatments

On the other hand, the small NO fluxes emitted from soil with a WFPS > 65%, including the majority of emissions from the direct drilled treatments, are predominantly expected to be released from denitrification (Schuster & Conrad, 1992; Hutchinson *et al.* 1993b; Williams *et al.* 1992). It is, therefore, likely that at this site both nitrification and denitrification are responsible for the production of

NO, although under specific conditions either nitrification or denitrification may predominate.

In 1999, however, the WFPS of the soil was consistently calculated as > 60%, furthermore the WFPS in the direct drilled soils was > 80% (Figure 3.14). Thus, it is reasonable to assume that the majority of the NO emitted was produced from denitrification. This helps to explain the low emissions measured (< 8 μg NO-N m^{-2} h^{-1}), particularly from soil with a WFPS > 80% when a high NO consumption rate would be present and the majority of N loss would be in the form of N_2 (Davidson et al. 1986; Davidson, 1993).

The relative emission of NO and N_2O represents a potential method to distinguish between nitrification and denitrification as the source of gaseous production (Williams *et al.* 1998). The theory relies upon the hypothesis that NO is primarily produced during autotrophic nitrification and in considerably larger quantities than N_2O (Davidson & Schimel, 1995; Williams *et al.* 1998). The production of N_2O, however, is predominantly associated with denitrification (Williams *et al.* 1998), and NO is generally not considered to be a key product (Firestone & Davidson, 1989).

In 1998 there were only four measurement occasions when both NO and N_2O samples were taken on the same day; 9 (13/05), 17 (21/05), 57 (30/06) and 72 (15/07) d after fertilisation (Table 3.10). However, these two gas measurements were not taken from the same chamber or at precisely the same time of day, and hence any relationships should be treated with caution. The NO:N_2O ratio was consistently < 1, except from the CP N- treatment 9 d after fertilisation and the P N-, CP N+ and DP N+ treatments 17 d after fertilisation. A NO:N_2O ratio > 1 has been estimated to indicate a dominant production process of nitrification (Anderson & Levine, 1986) and, therefore, these results support the previous data, which suggests that the NO emission from these plots at this time is likely to have at least partially arisen from the nitrification process. The variation in the NO:N_2O ratio appears to be primarily controlled by the magnitude of the N_2O flux, since it is a decrease in the N_2O emission, rather than a rise in the NO emission, which permits the increase in the NO:N_2O ratio to > 1.

The NO:N_2O ratio has been estimated to be *ca.* 0.01 if the primary source of N gas production is from denitrification (Anderson & Levine, 1987; Bouwman, 1990; Williams *et al.* 1988). In this experiment on days when both NO and N_2O measurements were taken, the ratios calculated from the direct drilled plots were typically in the range 0.01-0.06 i.e. very similar to that reported for denitrification. This data corroborates previously discussed information that has suggested that denitrification is the major production process in the direct drilled soils. Moreover, ratios obtained using data from the 5th NO measurement occasion (16/06/98 - 43 d after fertilisation) ranged from 0.0003-0.04, although the N_2O data used in the calculation had been collected 4 d previously.

Table 3.10 NO:N_2O ratios for losses measured from spring barley field plots in 1998. The sampling date in parentheses represents the date when N_2O was measured.

Treatment	Sampling Date						
	13/05 (13/05)	21/05 (21/05)	26/05 (29/05)	03/06 (05/06)	16/06 (12/06)	30/06 (30/06)	15/07 (15/07)
DP N-	0.40	0.84	0.02	0.03	0.04	0.77	0.17
DD N-	0.05	0.06	0.01	0.01	0.04	0.03	0.01
CP N-	2.11	3.04	0.17	0.24	0.01	0.36	0.25
DP N+	0.84	22.62	0.12	0.08	0.03	0.42	0.46
DDN+	0.01	0.45	0.08	0.004	0.0003	0.02	0.03
CP N+	0.54	6.11	0.06	0.01	0.02	0.27	0.25

The NO:N_2O ratios derived from the remaining ploughed treatments lie within the range 0.17-0.84, neither large enough to be predominantly from nitrification nor small enough to be principally from denitrification (Table 3.10). It is suggested, therefore, that both nitrification and denitrification are operating simultaneously and contribute to the production of NO.

3.6 Conclusion

- In this study we have discovered that direct drilling of soil significantly reduces NO emission over conventional ploughing and according to our best estimate (albeit limited), it reduces it in fertilised soils by 55-85% and in unfertilised soils by 85-99%. However, from fertilised soils the 'price' is an increase in the emission of the potent greenhouse gas N_2O of 300-450%.
- The results show that direct drilling significantly decreased the percentage of fertiliser N lost as NO over conventional ploughing by 45-80%.
- In both field seasons direct drilling at this site significantly increased the WFPS of the soil by more than 7% compared to the ploughed plots. The reduced O_2 diffusion as a result of the high WFPS will encourage the development of anaerobic conditions, which would favour denitrification. The smaller NO fluxes consistently measured from the direct drilled plots are likely to be the result of this higher denitrification potential coupled with the smaller soil pores, which is a common effect of the direct drilling system. Consequently, NO loss via consumption during denitrification will be encouraged and the ease of gaseous diffusion to the soil surface will be reduced.
- The study showed that there was no significant difference in the NO emission from conventional and deep ploughed soil, but that our estimate is that deep ploughing of soil significantly reduces N_2O emission over conventional ploughing by 45%.
- The addition of 70-80 kg N ha^{-1} of NH_4NO_3 fertiliser provided mineral N substrate that could be used by soil micro-organisms during nitrification and/or denitrification and subsequently stimulated NO fluxes by a factor of 2-255 above those measured from unfertilised plots.
- In both years of this study the cool, wet climate and/or clay loam soil may explain the low percentage of fertiliser nitrogen lost as NO over the 75 d period following fertilisation at, 0.002-0.011%, compared to that previously reported.
- The difference in the cumulative total of NO between the two field seasons and the variation in timing of peak NO emissions throughout the growing season appears to be influenced by several factors. The change in chamber location,

perhaps to a wetter area, and the timing in field operations e.g. earlier ploughing and sowing allowing for a more established crop and increased competition for mineral N, may have contributed to the lower fluxes seen in the 2nd year. The increase in WFPS in the 2nd year to consistently above 60% is also likely to have contributed to lower fluxes in 1999 via increased consumption of NO during denitrification. The pattern of rainfall following fertilisation influenced the magnitude and timing of peak emissions e.g. in 1998 initially enough rain (14 mm) fell to wash fertiliser to sites of microbial activity followed by < 0.5 mm permitting an increase in the aeration of the soil and NO emission. An increase in WFPS and a concomitant fall in soil mineral N influenced changes in the magnitude of NO fluxes during both years.

- There was no one factor or indeed one combination of factors which could explain the variation in the NO emissions over 1 or both years. Significant correlations between NO emission and soil nitrate and soil ammonium were shown in the first year when the largest fluxes were measured. Relationships between WFPS and NO emission were observed in both years, such that there was a marked shift in the magnitude of emissions above and below field capacity equivalent to a WFPS of 65%. Emissions when the WFPS was < 65% were up to 50 μg NO-N m^{-2} h^{-1}, whilst above a WFPS of 65% emissions were never more than 15 μg NO-N m^{-2} h^{-1}. The results suggest that at this study site both mineral N and WFPS may exert a control on the emission of NO.
- The available evidence from statistical analysis and NO:N_2O ratios suggests that both nitrification and denitrification contributed to the NO flux from the ploughed treatments, whereas the role of nitrification is liable to be minimal in the direct drilled treatments.
- An increase in the sampling frequency throughout the year and particularly following significant farming operations e.g. fertilisation or harvest will generate more data points and therefore improve the potential of detecting significant relationships between soil and environmental variables which may control the emission of NO at this site.
- A suitable abatement strategy to reduce NO emission from arable agricultural soil would seem to be a move to zero-tillage. However, particularly in the cooler,

moist climate of Scotland the reduction in yield or even total crop failure is likely to be an economic issue, whilst the substantial rise in the emission of the greenhouse gas N_2O is of environmental concern. A shift in agricultural policy to a less intensive system which encourages lower crop yields may promote the use of zero-tillage within the UK, although the accompanying frequent increase in the use of pesticides and herbicides will need to be resolved. The use of zero-tillage in rotation with conventional ploughing has been suggested previously in the literature in the hope that a more sustainable system of agriculture develops. Although the long term benefits of zero-tillage to the soil are well documented, the use of such a system in rotation with conventional ploughing would need to be thoroughly researched.

- The importance and environmental impact of the loss of NO from an agricultural system and potential abatement strategies should not be viewed in isolation, but should be examined in relation to a more complete budget of nitrogen losses from soil including N_2O, N_2, NH_3, NO_3^- and crop offtake.

Chapter 4. Organic and inorganic fertilisers and their effects on soil NO emissions

4.1 Introduction

4.1.1 Introduction

The application of both organic and inorganic N fertiliser is routinely used in agriculture when the anticipated supply of N is inadequate to meet the demand imposed by the crop (MAFF, 2000). Organic manures used as an agricultural fertiliser e.g. cattle slurry and sewage sludge (biosolids) can provide major plant nutrients (e.g. N, phosphorus, sulphur and magnesium) as well as adding beneficial organic matter to the soil, although if used incorrectly organic manures can pose a significant risk to the environment (MAFF, 2000).

4.1.2 Background to sewage sludge

Currently in the UK, the major routes for sewage sludge disposal are land application, landfill and incineration (Anon, 2001). Incineration and landfill of sewage sludge in the UK are expensive, whereas the recycling of sewage sludge to land is a cheap method of disposal and produces a low-cost fertiliser. Although the EU Sewage Sludge Directive 86/278/EEC (CEC., 1986) encourages the use of sewage sludge to obtain agricultural benefit, it also aims to avoid damage to human and animal health and the wider environment, especially soil, surface- and ground-water.

4.1.3 Sewage sludge production

It was estimated that in 1996/97 1.1 million tonnes of sewage sludge (dry solids) was produced annually in the UK (Gendebien *et al.* 1998). Approximately 47% of the sludge (515 000 tonnes of dry solids) was applied to agricultural land, over a total land area of 80,000 ha, although this only represents 0.5 % of the total agricultural land coverage (Gendebien *et al.* 1998).

As a result of the European Urban Wastewater Treatment Directive 91/71/EEC (CEC, 1991), there is now a requirement to treat sewage discharged directly into the sea through outfall pipes. The production of sewage sludge is, therefore predicted to increase from 1.1 million tonnes of dry solids in 1996/97 to 1.5 million tonnes by 2005/06 (Gendebien *et al.* 1998). Additional pressure will also be imposed on agricultural land (through the same directive), as a consequence of the prohibition of sewage sludge disposal at sea. Recycling of sludge for use in agriculture is expected to remain as the major disposal route (Bacon *et al.* 2001), consequently the amount applied to agricultural land is also expected to rise from 520,000 tonnes of dry solids in 1996/97 to 732,000 tonnes by 2005/06 (Gendebien *et al.* 1998).

4.1.4 Different forms of sewage sludge and merits of each

One of the most commonly applied forms of sewage sludge is digested liquid sewage sludge (mean dry matter of 4%) (MAFF, 2000), for which anaerobic digestion is the main technique used to stabilise the sludge (Gendebien *et al.* 1998). Recently, however, there has been a substantial rise in the production of thermally dried sludge (mean dry matter of 95%) (MAFF, 2000). Thermally dried sewage sludge pellets are N-rich, contain > 50% organic matter and remain intact when wet (Aitken, 1997) and act as a slow release fertiliser delivering nutrients throughout the crop growing season and in subsequent years.

4.1.5 Inorganic fertilisers

The use of chemical, nitrogenous fertilisers in agriculture is widespread. Fertilisers such as ammonium nitrate (NH_4NO_3) contain N in a form that is rapidly available for plant uptake. However, even if the N is applied close to the time of rapid crop growth and N uptake, due to its high availability, it is likely that there will be an immediate excess and, therefore, there is the potential to lose some N via leaching, denitrification, nitrification and volatilisation. The development of slow-release N fertilisers which improve the timing of mineral N availability with crop

As a result of the European Urban Wastewater Treatment Directive 91/71/EEC (CEC, 1991), there is now a requirement to treat sewage discharged directly into the sea through outfall pipes. The production of sewage sludge is, therefore predicted to increase from 1.1 million tonnes of dry solids in 1996/97 to 1.5 million tonnes by 2005/06 (Gendebien *et al.* 1998). Additional pressure will also be imposed on agricultural land (through the same directive), as a consequence of the prohibition of sewage sludge disposal at sea. Recycling of sludge for use in agriculture is expected to remain as the major disposal route (Bacon *et al.* 2001), consequently the amount applied to agricultural land is also expected to rise from 520,000 tonnes of dry solids in 1996/97 to 732,000 tonnes by 2005/06 (Gendebien *et al.* 1998).

4.1.4 Different forms of sewage sludge and merits of each

One of the most commonly applied forms of sewage sludge is digested liquid sewage sludge (mean dry matter of 4%) (MAFF, 2000), for which anaerobic digestion is the main technique used to stabilise the sludge (Gendebien *et al.* 1998). Recently, however, there has been a substantial rise in the production of thermally dried sludge (mean dry matter of 95%) (MAFF, 2000). Thermally dried sewage sludge pellets are N-rich, contain > 50% organic matter and remain intact when wet (Aitken, 1997) and act as a slow release fertiliser delivering nutrients throughout the crop growing season and in subsequent years.

4.1.5 Inorganic fertilisers

The use of chemical, nitrogenous fertilisers in agriculture is widespread. Fertilisers such as ammonium nitrate (NH_4NO_3) contain N in a form that is rapidly available for plant uptake. However, even if the N is applied close to the time of rapid crop growth and N uptake, due to its high availability, it is likely that there will be an immediate excess and, therefore, there is the potential to lose some N via leaching, denitrification, nitrification and volatilisation. The development of slow-release N fertilisers which improve the timing of mineral N availability with crop

requirement reduces the potential for such losses. Slow-release fertilisers are not widely used in conventional agriculture, however, due to the relatively large purchase cost at *ca.* 5 times the price of conventional inorganic N fertiliser (Pers. Comm., Dr. I. McTaggart, 2001).

4.1.6 Farm manures

Farm manures contain a significant N content, since up to 80% of consumed N is excreted by livestock (Dyson, 1992), although compared to inorganic N fertilisers, a much smaller percentage of the total N loading is in a readily plant-available form. In a typical cattle slurry only 50% of the total N will be present in the readily available NH_4^+-N form. The N content in farm manures is generally not simple to predict and is further complicated because the organic N in the manure is mineralised (i.e. released) slowly and becomes plant available over a period of months to years.

Sewage sludge, along with other organic manures can act as an effective, economic fertiliser for agricultural land, but may potentially be a source of gaseous emissions. There are, however, very few field or laboratory studies of NO emission following the application of either livestock manures or sewage sludge to agricultural soils. The aim of this study was, therefore, to compare NO emissions from agricultural land fertilised with typical rates of two forms of sewage sludge, cattle slurry and inorganic fertilisers.

4.2 Sites and treatments

The Cowpark (CP) experimental site (Plate 4.2) is located on the Bush Estate, *ca.* 15 km south of Edinburgh (NT238862), at an altitude of 190 m. The site had previously been managed between 1989 and 1994 in an arable rotation (winter barley, spring oil seed rape, potatoes, spring barley) for a long-term compaction experiment (Dickson & Ritchie, 1996a; Dickson & Ritchie, 1996b). The site was ploughed and re-seeded to Perennial Rye-grass (*Lolium perenne*) in August 1996. White clover (*Trifolium repens L.*), however, had established and during this trial was particularly noticeable in the control plots where N fertiliser was not applied. A 3 year field trial was initiated in 1998 by the Scottish Agricultural College (SAC) to examine N_2O and CO_2 emissions from ungrazed grassland following the application of organic wastes and mineral fertilisers and to ultimately enable balanced crop nutrient budgeting (McTaggart *et al.* 1999). My PhD research used six of the eleven treatments established for this organic wastes experiment.

The soil at this site is characterised as the Winton soil series. The soil and the prevailing climate were previously described in chapter 3. The site had been historically, artificially drained with small-bore (< 50 mm-internal diameter) clay pipes to a depth of *ca.* 1 m.

Table 4.1 Timings of field operations on ungrazed grassland plots at Cowpark field, 1998-1999

Experimental Year	Fertiliser Application		Sward Mown
	April	July	
1998	-	1st-20th July	26th August & 20th October
1999	26th-29th April	5th-9th July	23rd June, 1st September & 8th November

The timings of the field operations on the plots are displayed in Table 4.1. The site plan remained unchanged over the experiment and is illustrated in Figures

4.2 Sites and treatments

The Cowpark (CP) experimental site (Plate 4.2) is located on the Bush Estate, *ca.* 15 km south of Edinburgh (NT238862), at an altitude of 190 m. The site had previously been managed between 1989 and 1994 in an arable rotation (winter barley, spring oil seed rape, potatoes, spring barley) for a long-term compaction experiment (Dickson & Ritchie, 1996a; Dickson & Ritchie, 1996b). The site was ploughed and re-seeded to Perennial Rye-grass (*Lolium perenne*) in August 1996. White clover (*Trifolium repens L.*), however, had established and during this trial was particularly noticeable in the control plots where N fertiliser was not applied. A 3 year field trial was initiated in 1998 by the Scottish Agricultural College (SAC) to examine N_2O and CO_2 emissions from ungrazed grassland following the application of organic wastes and mineral fertilisers and to ultimately enable balanced crop nutrient budgeting (McTaggart *et al.* 1999). My PhD research used six of the eleven treatments established for this organic wastes experiment.

The soil at this site is characterised as the Winton soil series. The soil and the prevailing climate were previously described in chapter 3. The site had been historically, artificially drained with small-bore (< 50 mm-internal diameter) clay pipes to a depth of *ca.* 1 m.

Table 4.1 Timings of field operations on ungrazed grassland plots at Cowpark field, 1998-1999

Experimental Year	Fertiliser Application		Sward Mown
	April	July	
1998	-	1st-20th July	26th August & 20th October
1999	26th-29th April	5th-9th July	23rd June, 1st September & 8th November

The timings of the field operations on the plots are displayed in Table 4.1. The site plan remained unchanged over the experiment and is illustrated in Figures

4.1 and 4.2. The 12 m long and 6 m wide plots were either amended with ammonium nitrate mineral (21-8-11 NPK) fertiliser (NPK), Ficote 70® (14-14-14 NPK) *slow-release* mineral fertiliser (SR), anaerobically digested *sewage sludge liquid* provided by East of Scotland Water (SSL), thermally dried *sewage sludge pellets* provided by Wessex Water (SSP), dairy *cattle slurry* provided by the Scottish Agricultural College -SAC (CS) or received no N fertiliser and acted as a *control* (CON). For the majority of the storage period the cattle slurry accumulated in an open, above-ground tank, although it was necessary for the slurry to be emptied into an underground store prior to removal for application. This procedure was performed at various time intervals before slurry application and it is therefore likely that the composition of the slurry was affected.

The cattle slurry and liquid sewage sludge were both applied using an umbilical system and shallow injected to a depth of 70 mm. The vertical injection slots were *ca.* 20 mm in width and *ca.* 250 mm apart (Plate 4.3). In contrast, both of the mineral fertilisers and the sewage sludge pellets (Plate 4.4) were spread on the soil surface. The pellets were applied using a Vicon "vary spreader" and the mineral fertilisers were added with an easy to calibrate Bamlett tive pneumatic spreader.

The site received one fertiliser application (July) in the first experimental year and two applications (April & July) in the second year. Each fertiliser application was applied at a target rate of 120 kg available N ha^{-1}. In 1998, however, the SSL treatment was added at a much lower rate (*ca.* 20 kg available N ha^{-1}) due to an error by the providing water company.

At the three fertiliser application timings it was not possible to apply all of the treatments on the same day, particularly in 1998, due to fertiliser availability, weather and time. Consequently at every measurement occasion a varied amount of time had elapsed since application of each treatment. Tables 4.2 and 4.3 indicate the days after fertilisation for each treatment at each NO sampling event in 1998 and 1999 respectively.

The grass was mown to an approximate height of 75-100 mm, once in 1998 and twice in 1999 (Table 4.1). No pesticides or herbicides were applied to the site during the trial.

Table 4.2 The number of days after fertilisation elapsed at each measurement occasion in 1998, on a per treatment basis

Treatment		NPK	SR	SSL	SSP	CS
Fertiliser Application Date		17th July	17th July	3rd July	20th July	1st July
Sampling Date	15/07/98	-	-	12	-	14
	24/07/98	7	7	21	4	23
	06/08/98	20	20	34	17	36
	25/08/98	39	39	53	36	55
	03/10/98	78	78	92	75	94
	20/10/98	95	95	109	92	111

Table 4.3 The number of days after fertilisation elapsed at each measurement occasion in 1999, on a per treatment basis

Treatment		NPK	SR	SSL	SSP	CS
First Fertiliser Application Date		30th April	30th April	28th April	27th April	26th April
Sampling Date	26/04/99	-	-	-	-	0
	29/04/99	-	-	1	2	3
	07/05/99	7	7	9	10	11
	18/05/99	18	18	20	21	22
	15/06/99	46	46	48	49	50
Second Fertiliser Application Date		9th July	NA	7th July	6th July	5th July
Sampling date	06/07/99	-	67	-	-	1
	08/07/99	-	69	1	2	3
	15/07/99	6	76	8	9	10
	22/07/99	13	83	15	16	17
	02/09/99	55	125	57	58	59
	07/10/99	90	160	92	93	94

Nitric oxide measurements commenced following fertiliser application and continued at weekly to monthly intervals (dependent on weather, laboratory access for soil processing and analyser operating capability) until early to mid October when sampling was unfeasible due to trafficability problems with the transport of the analyser in the field.

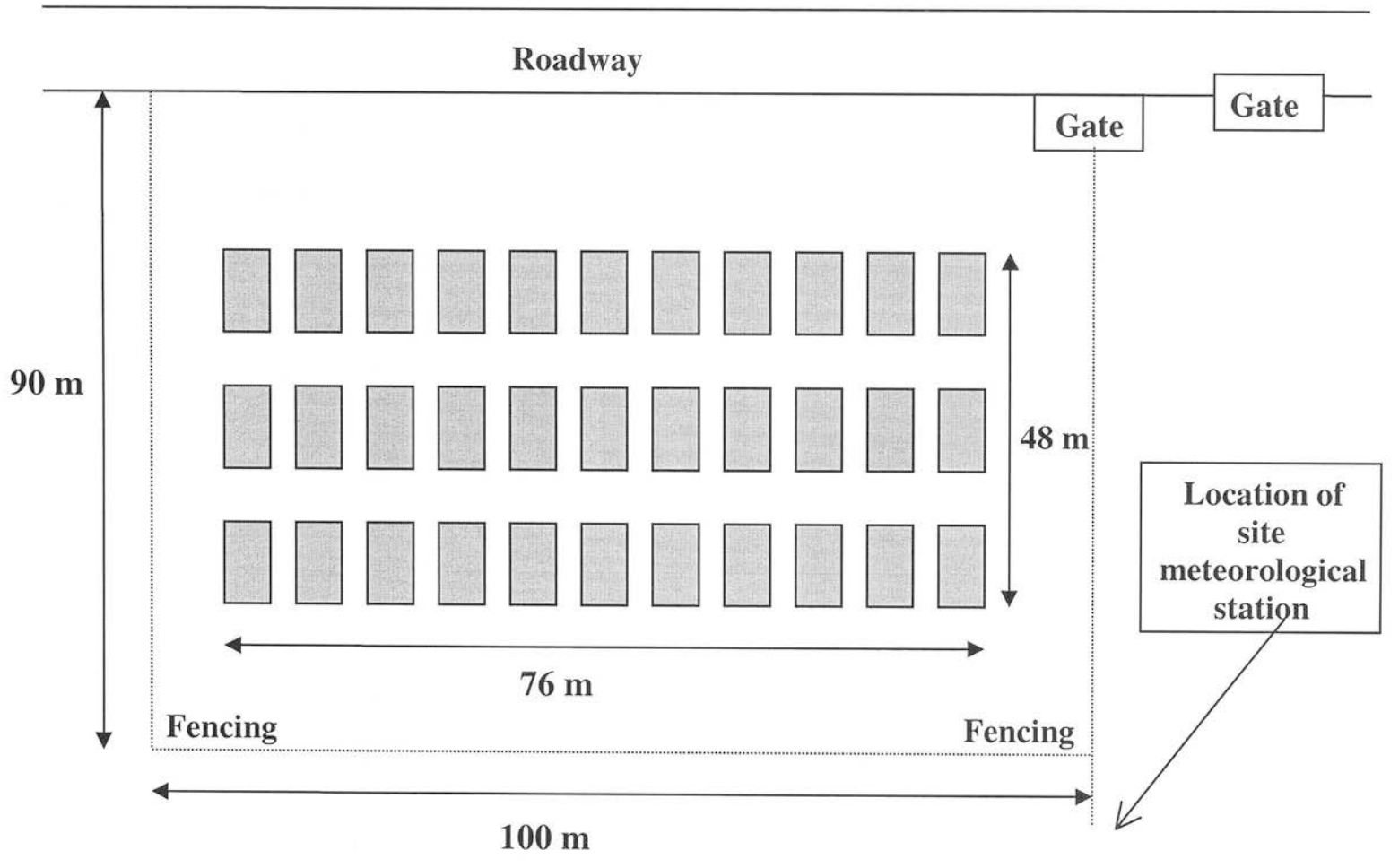

Figure 4.1 Cowpark Field Experiment (ungrazed grassland) Overview

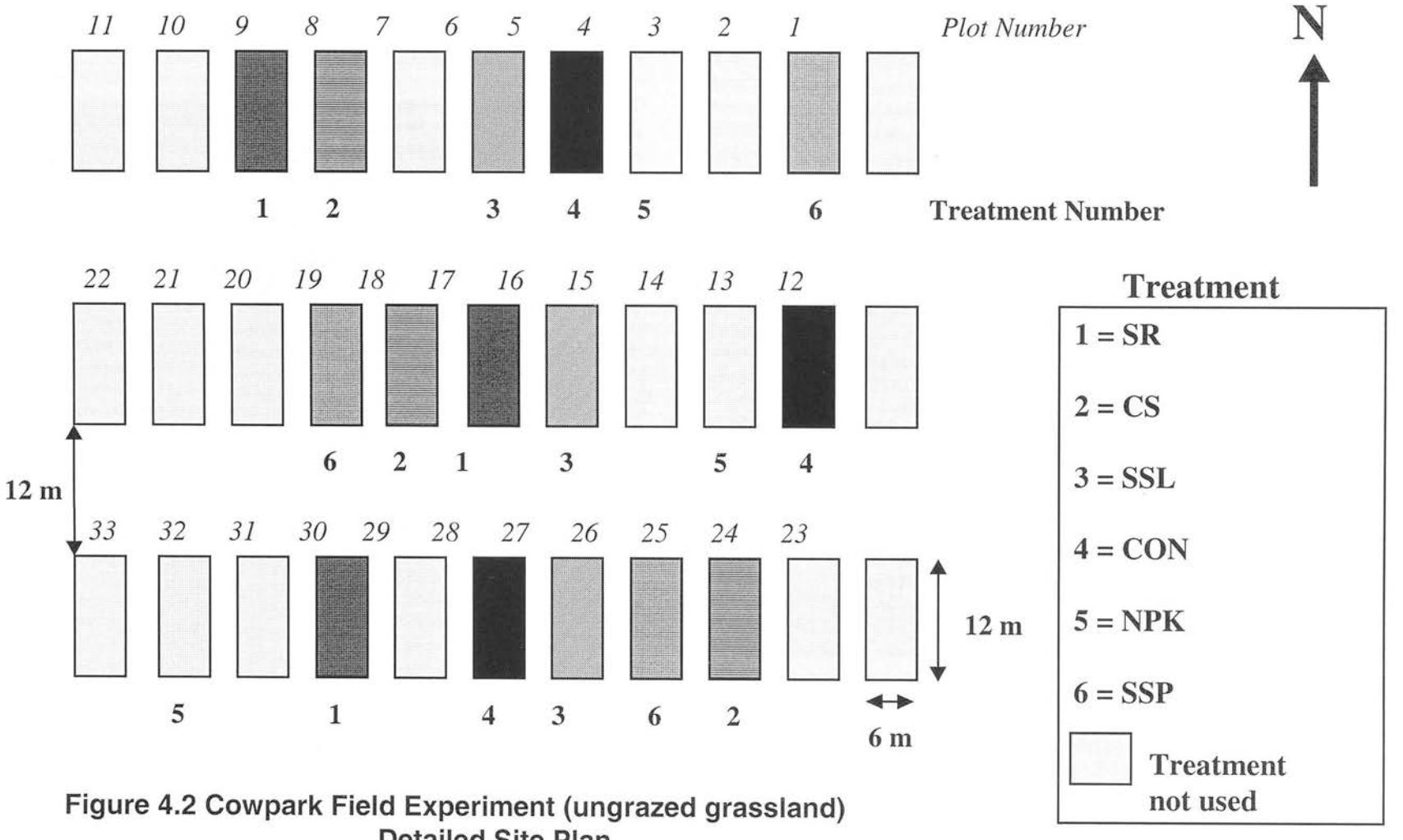

Figure 4.2 Cowpark Field Experiment (ungrazed grassland) Detailed Site Plan

Plate 4.1 Gas sampling system using the Scintrex analyser

Plate 4.2 Block 3, Cowpark field site showing the meteorological station

Plate 4.3 Chamber positioned over injection slots on the cattle slurry treatment

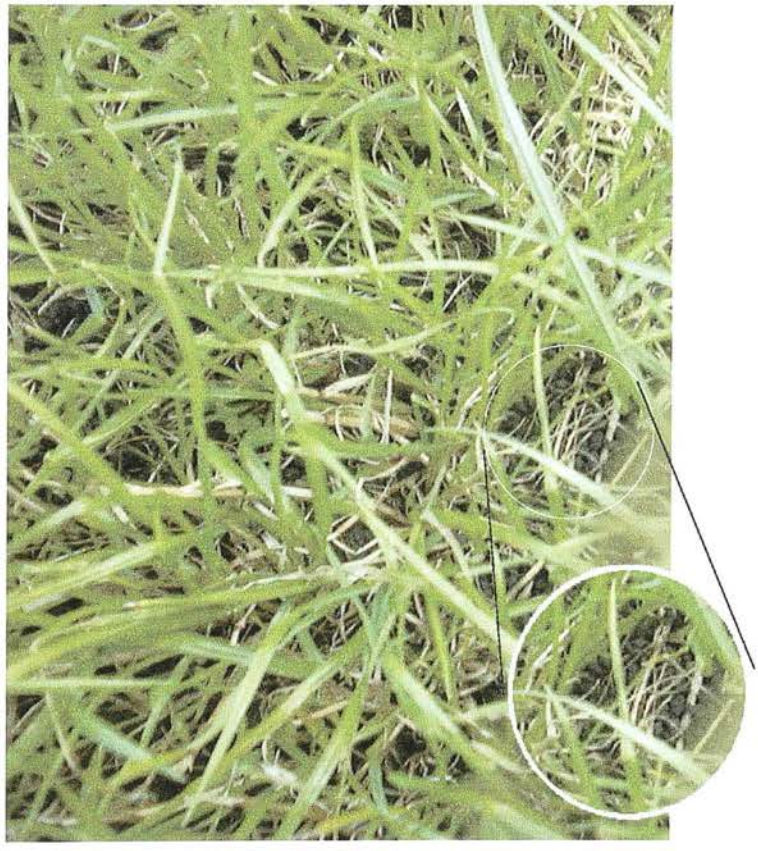

Plate 4.4. Dried sewage sludge pellets (SSP) following application

4.3 Materials and Methods

The same method was used as at the Beechgrove field site (section 3.3). Plate 4.1 shows the internally battery powered Scintrex which was transported between plots on a small sack-trolley.

4.4 Results

The experiment was designed as a fully randomised block design with three replicates per treatment (Figures 4.1 and 4.2). The NO emission data from the trial were analysed using a repeated measures analysis of variance (ANOVA) (Genstat release 4.2). In 1999 the emission following each fertiliser application was analysed separately, since the gas chambers were relocated between applications. The available soil NH_4^+-N, soil NO_3^--N and soil WFPS data were analysed using a analysis of variance (ANOVA) within a general linear model (Minitab release 10.2) with treatment and block as factors. Treatment combinations were analysed with the Tukey test ($P < 0.05$), since it has more protection against a Type 1 error than the LSD or Duncan's test.

Cumulative losses were calculated for each plot (replicate) by linearly interpolating the data points and integrating the underlying area over the sampling period i.e. a 111 d and 165 d period following fertilisation in 1998 and 1999, respectively. N loss as NO due to application of fertiliser was estimated via the cumulative NO emission emitted from the control treatments being subtracted from the cumulative NO emissions calculated for each of the fertilised treatments and the differences were related to the total N and the total ammoniacal N (TAN) applied.

4.4.1 Experimental year 1998

The daily patterns of precipitation and mean air temperature (April-October) recorded at the meteorological station located within Cowpark field approximately 10 m south-east of the field plots (Figure 4.1) are shown in Figure 4.3. The site received more than the typical rainfall with a total annual precipitation during 1998 of 1056 mm, which is 186 mm greater than the usually quoted average quantity. Over the 4 month (July to October) sampling period the site received 37 % of the 1998 annual rainfall at 389.0 mm of rain. The monthly rainfall associated with the fertiliser applications (July) amounted to 116.3 mm, with 17-52 mm falling in the 7 d prior to application and 2.5-44.2 mm falling in the 7 d period following application. The mean daily air temperature for the month of July ranged from 7.4 °C to 20.5 °C with an average of 13.5 °C, which was 1.3 °C colder than the typical value (14.8 °C).

The mean daily air temperature reported for the fertiliser application dates varied from 12.0 °C to 15.7 °C.

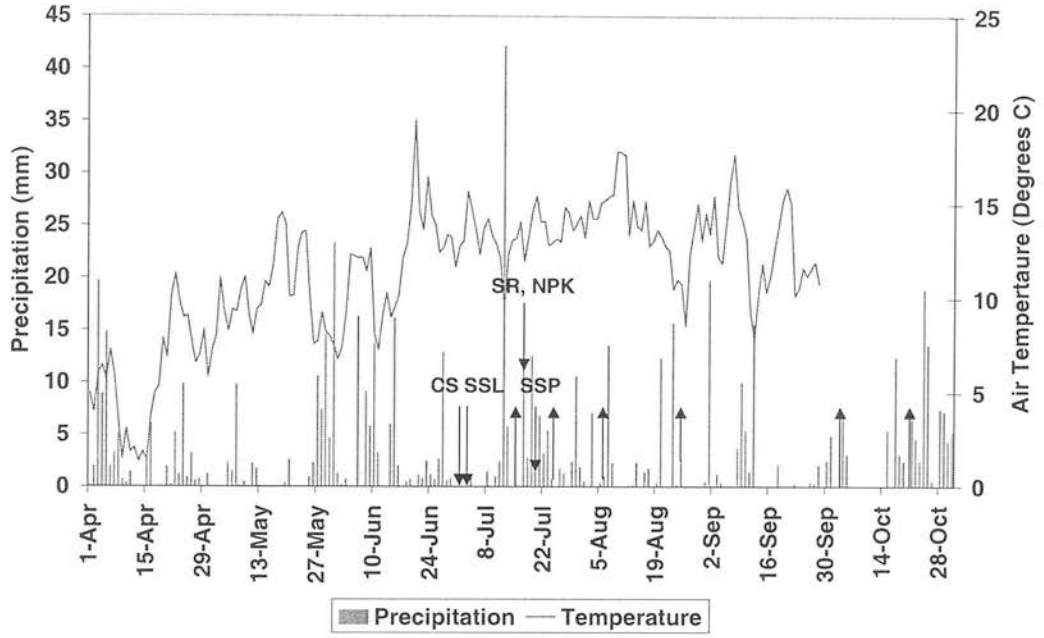

Figure 4.3 The precipitation and air temperature recorded during April to October 1998 (downward arrow and treatment code indicates application date of N fertiliser; upward solid arrow indicates NO measurement taken)

4.4.1.1 Fertiliser properties

Table 4.4 Composition of both organic wastes and inorganic fertilisers applied to grassland in July 1998

Treatment	Dry matter (%)	Total carbon (kg t^{-1})	Application rate (m^3 ha^{-1} or t ha^{-1})	Total N applied (kg ha^{-1})	TAN applied (kg ha^{-1})
SSL	0.4	NA	60	24	16
SSP	97.4	296	15	513	111
CS	5.9	NA	60	216	119
NPK	-	-	0.57	120	60
SR	-	-	0.86	120	60

NA = not available - samples were not analysed for the carbon content.

Table 4.4 summarises the composition and application rates of both the organic and inorganic fertilisers applied (Pers. Comm. Iain McTaggart, 2001). The total N applied in the SSP was more than twice that in the CS and *ca.* twenty-one times more than in the SSL. The N supplied by both the SSP and CS was approximately equal to the target application rate of 120 kg ha^{-1} of available N, since in these organic manures virtually all of the readily available N would be in the ammoniacal form rather than as NO_3^--N (Table 4.4). It should be noted though, that over the growing season available N would additionally have been made free through mineralisation of organic N present in the manure and consequently the target rate of 120 kg ha^{-1} of available N would probably have been exceeded.

Analysis of the SSL indicated that the material was substantially more dilute than was expected. This is mirrored in the dry matter content of only 0.4%, which is an order of magnitude smaller than the typical value quoted of 4% (MAFF, 2000). The total N content of the sludge liquid (0.4 kg m^{-3}) was $1/5^{th}$ of the typical value (2 kg m^{-3}) (MAFF, 2000) and consequently the actual application rate of available N was 16 kg ha^{-1} instead of the target rate of 120 kg ha^{-1} of available N. The relative ease of calculating N content in inorganic fertilisers is reflected in the accuracy of the application rate for both NPK and SR fertilisers, which were 120 kg ha^{-1} available N, the target application rate (Table 4.4).

4.4.1.2 Gaseous emissions

4.4.1.2.1 NO emissions

The low NO fluxes measured from the soil to atmosphere at this ungrazed, grassland site are displayed in Figure 4.4. and only varied from -0.16 to 4.03 μg NO-N m^{-2} h^{-1} for the fertilised treatments and from -0.36 to 0.63 μg NO-N m^{-2} h^{-1} for the unfertilised, CON treatment.

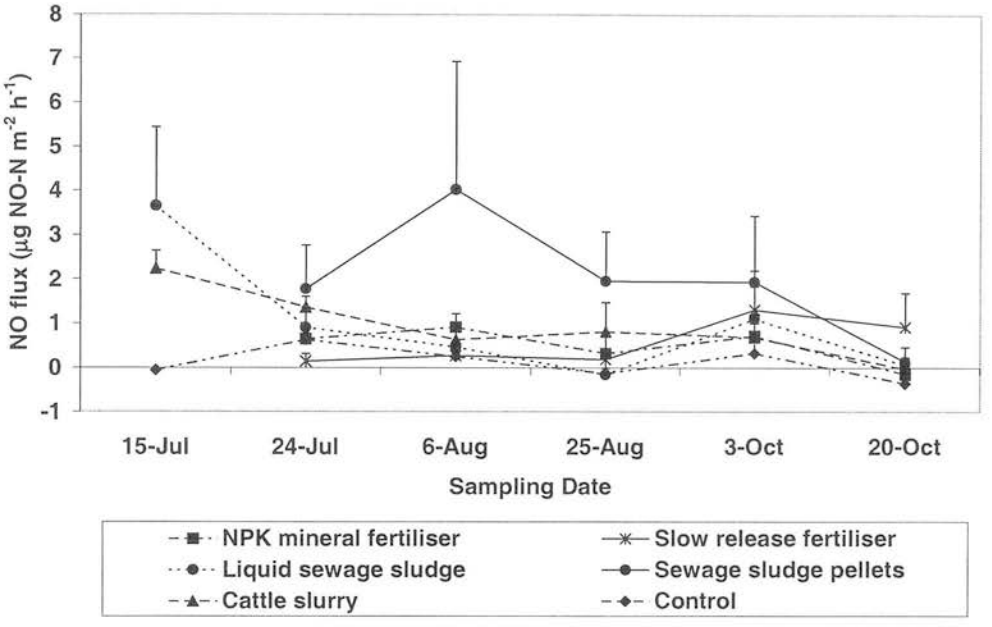

Figure 4.4 Mean NO flux measured in 1998 from ungrazed grassland amended with organic wastes and inorganic fertilisers. Error bars represent one standard error (N=3). Cattle slurry applied on 1st July, sewage sludge liquid applied on 3rd July, slow-release and NPK fertilisers applied on the 17th July and sewage sludge pellets applied on the 20th July.

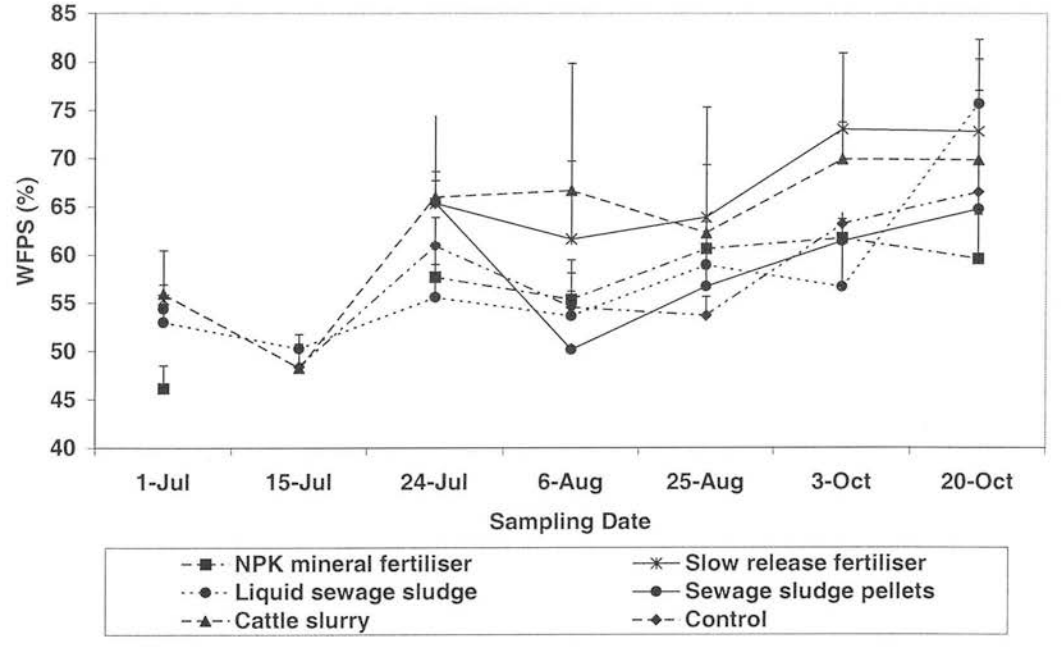

Figure 4.5 Mean WFPS measured in 1998 from ungrazed grassland amended with organic wastes and inorganic fertilisers. Error bars represent one standard error (N=3).

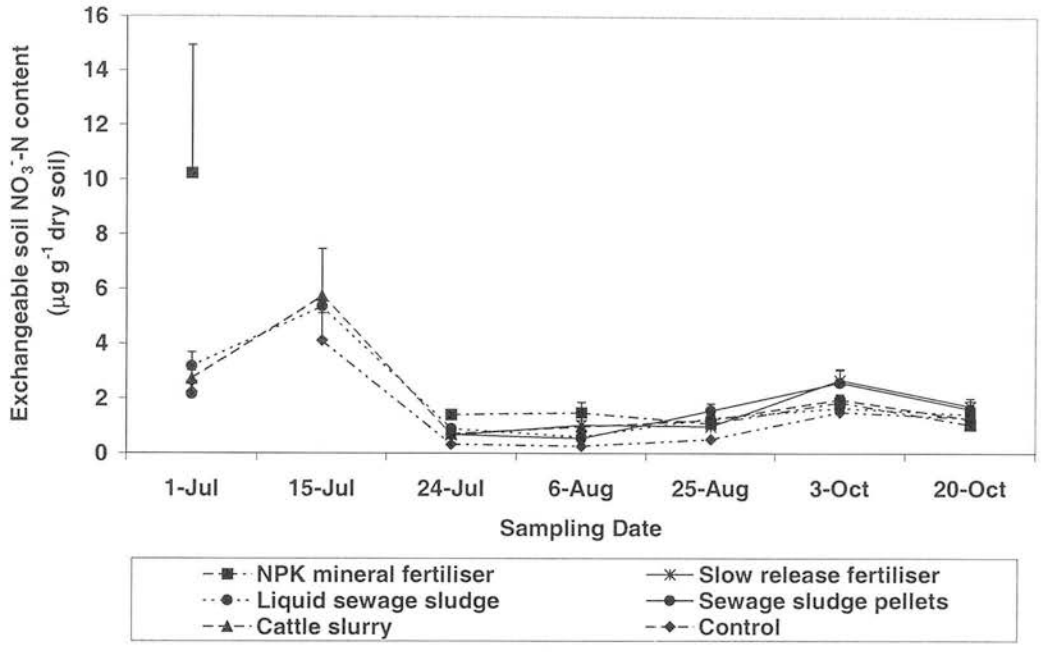

Figure 4.6 Mean soil NO_3^--N measured in 1998 from ungrazed grassland amended with organic wastes and inorganic fertilisers. Error bars represent one standard error (N=3).

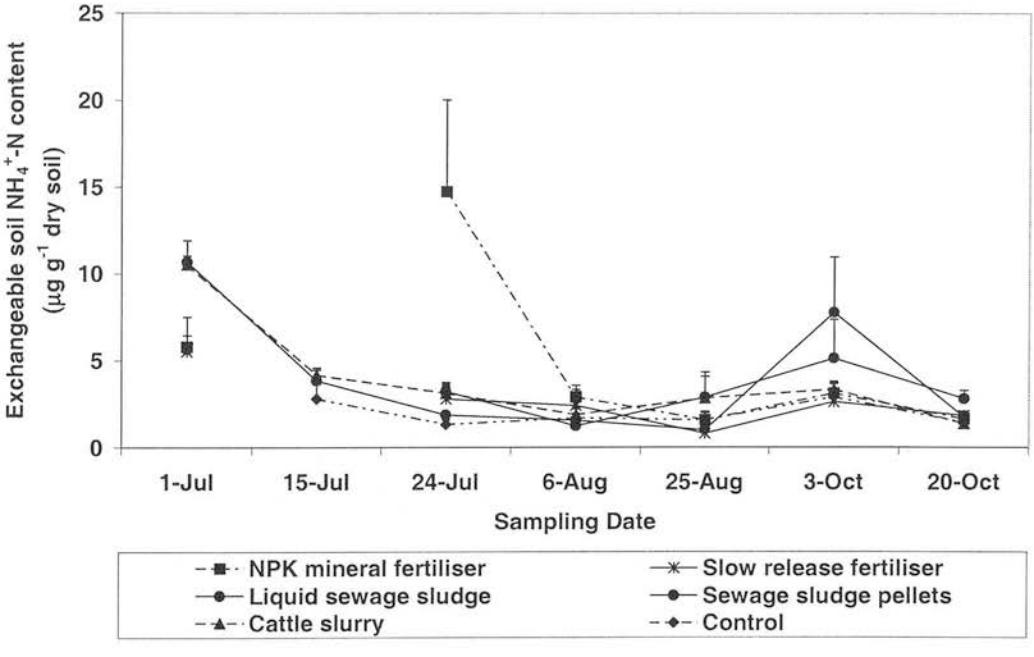

Figure 4.7 Mean soil NH_4^+-N measured in 1998 from ungrazed grassland amended with organic wastes and inorganic fertilisers. Error bars represent one standard error (N=3).

There was a significant effect ($P < 0.01$) of time on the emission of NO at this site (Table 4.5). The timing of the measured NO emission peaks varied between treatments, although this may have been an artefact of the 20 d period in which the fertiliser applications occurred. NO emission from both of the injected organic wastes (SSL & CS) peaked, 12 and 14 d after fertilisation respectively (Figure 4.4), which corresponded to the maximum soil NO_3^--N concentrations (Figure 4.6) and the lowest water filled pore spaces (Figure 4.5) recorded for each treatment. Furthermore, the peak NO emission from the CS treatment also coincided with the maximum measured soil NH_4^+- concentration (Figure 4.7) .

Table 4.5 Table of mean values (time) produced as a result of repeated measures ANOVA of NO flux (μg NO-N m^{-2} h^{-1}) in 1998.

Time - Sampling date						Degrees of freedom	Standard error of differences of means
15/07	24/07	06/08	25/08	03/10	20/10		
2.1	0.9	1.1	0.5	1.0	0.1	51	0.3

Peak emissions from two of the surface applied fertilisers (NPK & SSP) were measured 20 and 17 d after fertilisation respectively, whilst from the surface applied SR treatment the peak emission was not recorded until 78 d after fertilisation. The majority of treatments experienced a secondary smaller peak on the 3rd October, which appeared to coincide with a rise in both soil NO_3^--N and NH_4^+-N concentrations (Figures 4.6 and 4.7).

Emission minima were predominantly measured on the last sampling occurrence (20th October) with the exception of the SR and SSL treatments when the lowest fluxes were recorded 7 d and 53 d after fertilisation, respectively (Figure 4.4). Negative fluxes of NO were observed on several occasions, primarily from the CON treatment and generally corresponded to low soil available N concentrations (< 2.0 μg g^{-1} NH_4^+-N; < 1.5 μg g^{-1} NO_3^--N) (Figures 4.6 and 4.7).

Measured NO fluxes were used to calculate potential cumulative losses over a 111 d period following fertilisation (July to October) (Figure 4.8). Despite the

There was a significant effect ($P < 0.01$) of time on the emission of NO at this site (Table 4.5). The timing of the measured NO emission peaks varied between treatments, although this may have been an artefact of the 20 d period in which the fertiliser applications occurred. NO emission from both of the injected organic wastes (SSL & CS) peaked, 12 and 14 d after fertilisation respectively (Figure 4.4), which corresponded to the maximum soil NO_3^--N concentrations (Figure 4.6) and the lowest water filled pore spaces (Figure 4.5) recorded for each treatment. Furthermore, the peak NO emission from the CS treatment also coincided with the maximum measured soil NH_4^+- concentration (Figure 4.7) .

Table 4.5 Table of mean values (time) produced as a result of repeated measures ANOVA of NO flux (μg NO-N m^{-2} h^{-1}) in 1998.

Time - Sampling date						Degrees of freedom	Standard error of differences of means
15/07	24/07	06/08	25/08	03/10	20/10		
2.1	0.9	1.1	0.5	1.0	0.1	51	0.3

Peak emissions from two of the surface applied fertilisers (NPK & SSP) were measured 20 and 17 d after fertilisation respectively, whilst from the surface applied SR treatment the peak emission was not recorded until 78 d after fertilisation. The majority of treatments experienced a secondary smaller peak on the 3rd October, which appeared to coincide with a rise in both soil NO_3^--N and NH_4^+-N concentrations (Figures 4.6 and 4.7).

Emission minima were predominantly measured on the last sampling occurrence (20th October) with the exception of the SR and SSL treatments when the lowest fluxes were recorded 7 d and 53 d after fertilisation, respectively (Figure 4.4). Negative fluxes of NO were observed on several occasions, primarily from the CON treatment and generally corresponded to low soil available N concentrations (< 2.0 μg g^{-1} NH_4^+-N; < 1.5 μg g^{-1} NO_3^--N) (Figures 4.6 and 4.7).

Measured NO fluxes were used to calculate potential cumulative losses over a 111 d period following fertilisation (July to October) (Figure 4.8). Despite the

apparent disparity in cumulative NO emission between treatments, statistically significant differences were not detected, primarily due to the large variability between plots within an individual treatment (Figure 4.8).

Cumulative NO loss ranged from 15 – 200 μg NO-N m^{-2}, with the lowest emission measured from CON and the highest observed from SSP. The cumulative emissions from the inorganic fertiliser treated plots were *ca.* 4 times higher than CON plots. This difference in flux magnitude was enhanced further with the plots amended with organic fertilisers emitting *ca.* 6 - 13 times more NO than the CON plots (Figure 4.8).

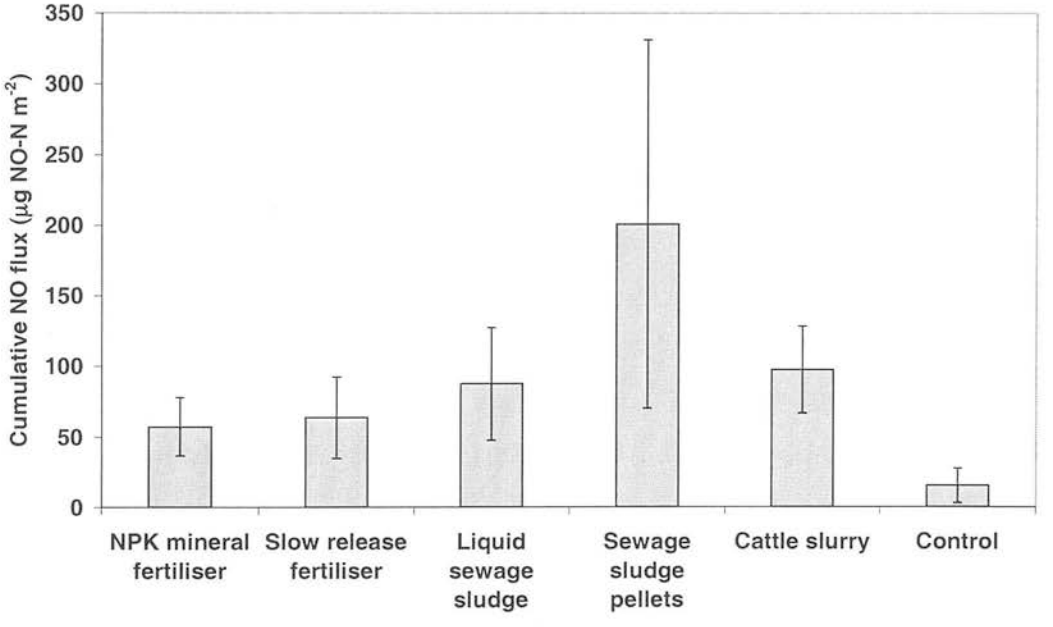

Figure 4.8 Cumulative NO emission over a 111 d period following fertilisation in 1998 from ungrazed grassland amended with organic wastes and inorganic fertilisers. Error bars represent ± one standard error (N=3).

The percentage of total N applied in fertiliser and subsequently lost as NO was very similar between treatments with the exception of that lost from SSL, which was an order of magnitude larger (Table 4.6). Despite this there was no significant difference ($P > 0.05$) between treatments of either the percentage of total N applied lost as NO or the percentage of TAN applied lost as NO.

Table 4.6 The percentage of fertiliser N lost as NO in 1998 over a 111 d period following fertilisation

Treatment	Total N applied lost as NO (%)	TAN applied lost as NO (%)
NPK	0.0003	0.0007
SR	0.0004	0.0008
SSL	0.0030	0.0050
SSP	0.0004	0.0020
CS	0.0004	0.0010

Comparison between fertiliser treatments

The measured NO emissions were very low (< 5 μg NO-N m^{-2} h^{-1}) and combined with the high variability (CV of 25-734%) accounted for the lack of a significant difference ($P > 0.05$) between treatments.

In spite of the addition of liquid with the SSL and CS treatments, the application of the various manures did not appear to influence the measured soil WFPS (Table 4.6). As expected, the application of the fertilisers affected the soil available mineral N contents, although not on every measurement occasion. Significant differences in soil NO_3^--N and soil NH_4^+-N between the different treatments were observed on two of the measurement occasions (Table 4.7).

On the 24th July and the 25th August, significantly higher soil NO_3^--N concentrations were reported in the plots amended with NPK and SSP respectively, as compared to the CON treatment (Tukey test, $P < 0.05$).

Following the first soil sampling event (1st July) there was a significant difference ($P < 0.01$) between treatments (excluding CON, which was not measured) such that the soil NH_4^+-N concentration was larger from CS (Tukey test, $P < 0.05$). Surprisingly though, since sewage sludge application had not yet occurred, soil NH_4^+-N was also significantly higher from the SSL plots (Tukey test, $P < 0.05$), which demonstrates the natural variability of soil variables (Table 4.7).

Table 4.7 Summary of ANOVA results (*P* values and associated level of significance) for soil NO_3^-, NH_4^+ and WFPS at each sampling date in 1998

Data	Sampling Date						
	01/07	15/07	24/07	06/08	25/08	03/10	20/10
Soil available NO_3^-	NS	NS	0.049 *	NS	0.027 *	NS	NS
Soil available NH_4^+	0.004 **	NS	0.011 *	NS	NS	NS	NS
WFPS	NS	NS	NS	NS	NS	NS	NS

*** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$; NS = not significant;
NA = NO emission not measured.

On the 24th July, a significant difference in soil NH_4^+-N was measured between treatments. The Tukey test ($P < 0.05$) determined that the soil NH_4^+-N concentration was much greater in all of the fertiliser amended treatments (except SSL) compared to the CON plots. Furthermore, the soil sampled from the CS and SSP treatments contained more NH_4^+-N than that from the SSL, and the soil NH_4^+-N measured from the NPK treatment was significantly higher than from all other treatments.

There was no evidence of a significant ($P < 0.01$) relationship between NO emissions from all treatments and either soil WFPS, available NH_4^+-N, available NO_3^--N, surface temperature or temperature at a depth of 5 cm. Nevertheless, significant relationships ($P < 0.05$ to $P < 0.01$) were observed between NO emissions and the various soil parameters for individual treatments.

Figure 4.9 demonstrates the three significant relationship obtained between NO emission and soil WFPS from the SSP ($P < 0.01$) treatment. The relationship was negative and able to explain 73% of the variation in the data. Moreover a significant inverse relationship was additionally observed between NO emission and

cumulative precipitation over the 3 days prior to sampling from the NPK treatment ($r^2 = 0.90$, $P < 0.05$).

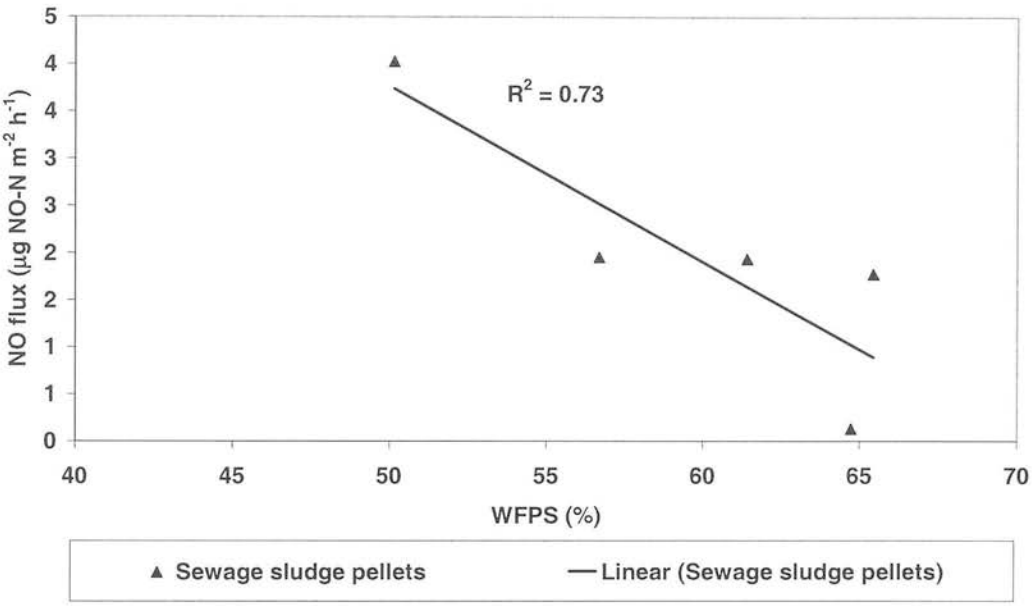

Figure 4.9 The relationship between NO emission and soil WFPS measured in 1998 from the SSP SR treatment

Fluxes of NO from the SR treatment appeared to be influenced by the soil NO_3^--N concentration, such that 96% of the variation in the NO data could be explained (Figure 4.10). An increase in soil NO_3^--N was significantly ($P < 0.01$) correlated with a rise in the NO emission. One significant ($P < 0.05$; $r^2 = 0.77$) positive relationship between NO emission and soil NH_4^+-N of was observed from the CS treatment (Figure 4.10).

A combination of soil parameters was, however, able to explain more of the variation in the NO data from 3 of the treatments than individual factors alone. Soil NO_3^--N and WFPS together were able to explain 99 and 92% of the variation in the data from the SR ($P < 0.01$) and SSL ($P < 0.05$) treatments respectively, whilst WFPS and soil NH_4^+-N yielded a significant ($P < 0.05$) relationship that could account for 91% of the variation in the NO data from the CS treatment.

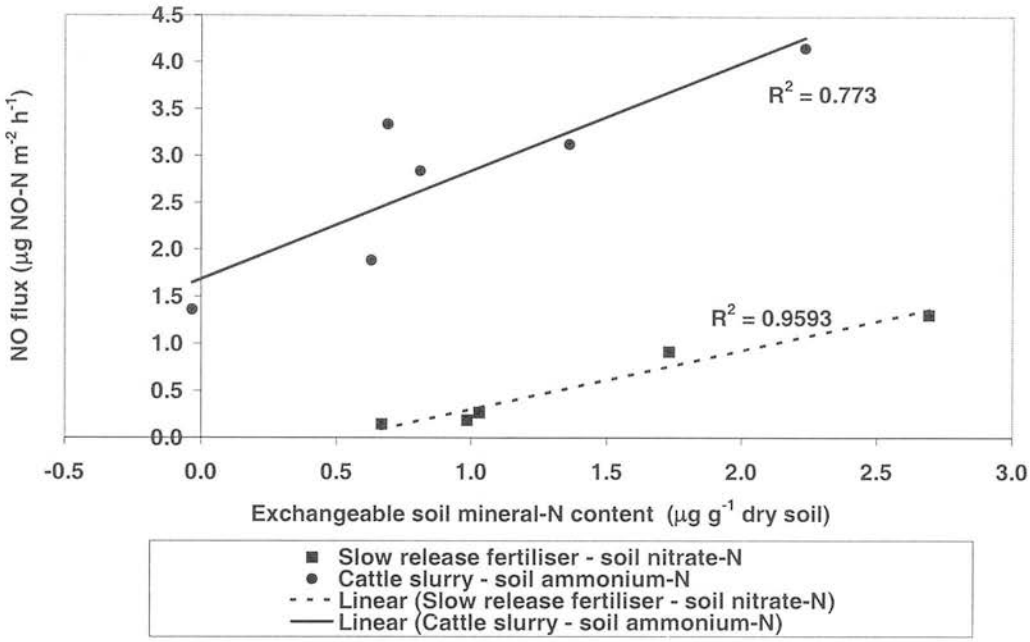

Figure 4.10 The relationship between mean NO emission and exchangeable soil NO_3^--N from the slow release fertiliser and soil NH_4^+-N concentration from the cattle slurry measured in 1998 (N=3).

Temperature at both the soil surface and at a depth of 5 cm emerged as an important parameter affecting NO emission from the NPK treatment. A marginally better correlation and a higher degree of significance, however, was observed with the temperature at depth (r^2 =0.98, P = 0.012) rather than at the soil surface (r^2 = 0.96, P = 0.020). Loss of NO from plots amended with SSP also appeared to be linked to soil surface temperature with a significant positive (P = 0.05) relationship of r^2 = 0.90.

Only on the 25th August (5th sampling occasion) could a single factor or combined factors account for the daily variation in NO fluxes when soil NH_4^+-N was able to account for 67% of the variation.

4.4.1.2.2 N_2O emissions

Measurements of N_2O were taken as part of the parent experiment and these results are shown by kind permission of Dr. Iain McTaggart (SAC, Edinburgh).

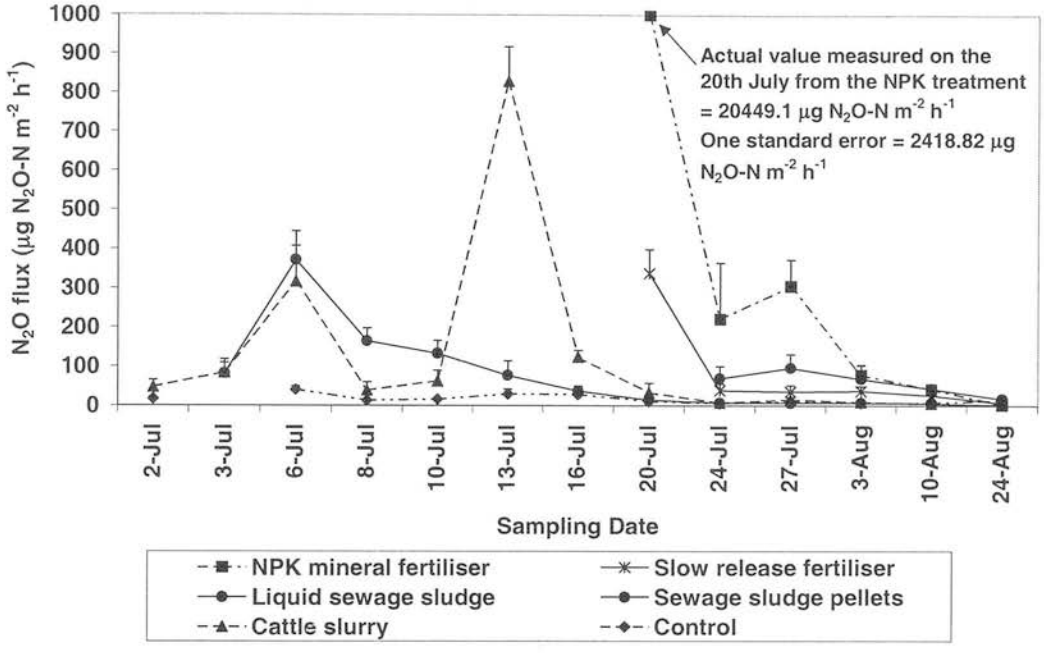

Figure 4.11 N_2O flux measured in 1998 (2nd July - 24th August) from ungrazed grassland amended with organic wastes and inorganic fertilisers. Error bars represent the standard error (N=3). Cattle slurry applied on 1st July, sewage sludge liquid applied on 3rd July, slow-release and NPK fertilisers applied on the 17th July and sewage sludge pellets applied on the 20th July.

The N_2O fluxes measured from the 2nd July to the 24th August are shown in Figure 4.11. Fluxes of N_2O over the whole sampling period (2nd July to the 19th October) varied from 0.3 to 20449 µg N_2O-N m^{-2} h^{-1} for fertilised plots and up to 40.3 µg N_2O-N m^{-2} h^{-1} for unfertilised plots.

4.4.2 Experimental year 1999

The daily mean air temperature and precipitation patterns (April-October) recorded at the meteorological station previously described are illustrated in Figure 4.12. The meteorological data collected at the site in 1999 indicated a drier than average year with a total annual precipitation of 816 mm, 54 mm less than the typical value. During the April to October sampling period the site received *ca.* half of the yearly total rainfall at 406.5 mm. Over the sampling period comparable to that used in 1998 (i.e. July to October) the amount of rain which fell in 1999 was just less than

half of that collected in 1998. Furthermore, in 1999 the site received less than half of the 1998 monthly rainfall associated with the July fertiliser applications. Only 0.5-5.4 mm of precipitation fell in the 7 d prior to the 1999 April fertiliser applications and 0.0-5.1 mm fell in the 7 d period following these applications. In contrast, more precipitation was associated with the July applications, with 18.0-22.2 mm falling in the 7 d prior to application and 3.6-6.9 mm falling in the 7 d period following application.

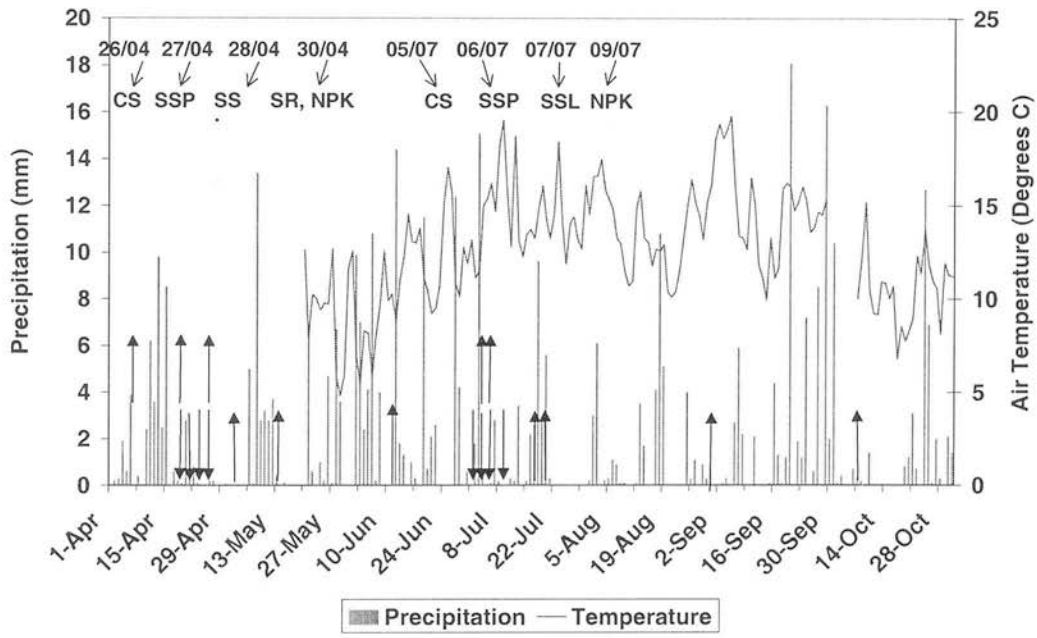

Figure 4.12 The precipitation and air temperature recorded during April to October 1999 (downward arrow and treatment code indicates application date of N fertiliser; upward solid arrow indicates NO measurement taken)

The mean daily air temperature for the month of July of 14.5 °C was similar to the typical value (14.8 °C) and ranged from 9.9 °C to 19.5 °C, but was 1 °C colder than the value recorded in 1998. The mean daily air temperature reported for the first fertiliser application dates in April varied from 7.7 °C to 12.0 °C and from 15.4 °C to 19.5 °C for the second application dates in July. Due to a malfunction of the site meteorological station, the April air temperatures were obtained from the Met Office, Edinburgh, Turnhouse station (3159E 6739N, 35 m above sea level).

4.4.2.1. Fertiliser properties

Tables 4.8 and 4.9 outline the composition and application rates of the organic and inorganic fertilisers added in both April and July. All three application rates of the organic wastes were increased from the 1998 rates largely due to a change in composition of the materials, although the SSP used at both of the 1999 applications were from the same batch as those applied in 1998. All of the five fertiliser treatments received two applications (April and July), except for the SR plots. These were given only one double application (240 kg ha^{-1} available N) in April in order to supply the crop throughout the growing season.

Table 4.8 Composition of both organic wastes and inorganic fertilisers applied to grassland in April 1999

Treatment	Dry matter (%)	Total carbon (kg t^{-1})	Application rate (m^3 ha^{-1} or t ha^{-1})	Total N applied (kg ha^{-1})	TAN applied (kg ha^{-1})
SSL	3.4	8	120	132	116
SSP	97.4	296	17.4	595	129
CS	10.0	39	100	430	154
NPK	-	-	0.57	120	60
SR	-	-	1.72	240	120

Table 4.9 Composition of both organic wastes and inorganic fertilisers applied to grassland in July 1999

Treatment	Dry matter (%)	Total carbon (kg t^{-1})	Application rate (m^3 ha^{-1} or t ha^{-1})	Total N applied (kg ha^{-1})	TAN applied (kg ha^{-1})
SSL	2.6	9	120	108	103
SSP	97.4	296	17.4	595	129
CS	3.7	11	100	190	68
NPK	-	-	0.57	120	60
SR	-	-	NA	NA	NA

NA = not available - only one fertiliser application required in April 1999.

As with the previous organic waste applications in 1998 at both applications in 1999, the total N and carbon contents were substantially larger in the SSP than in the CS or SSL (Table 4.9). The total and ammoniacal N were lower in the July application than at the April application, especially with respect to the CS, which contained less than half of the previously recorded values.

4.4.2.2. Gaseous emissions

4.4.2.2.1. NO emissions

The NO fluxes measured in 1999 after fertiliser application in both April and July are shown in Figure 4.13 and ranged from -2.6 to 28.4 μg NO-N m^{-2} h^{-1}. Fluxes from all fertilised treatments (except SR) were noticeably higher than those measured in 1998 (Figure 4.4), such that peak emissions were 6-15 times larger.

There was a highly significant ($P < 0.001$) effect of time on the NO emissions following both the April and July fertiliser applications (Table 4.10). Following each fertiliser application the peak NO emissions from the majority of the treatments (excluding CON and SR) were observed on the same measurement occasions (7th May and 15th July), which corresponded to the period of 6 to 11 days after fertilisation (Figure 4.13). Accompanying the gradual increase in emission magnitude from the initial measurements to the peak losses was a fall in soil water-filled pore space (WFPS) (Figure 4.14). This was particularly noticeable following the July application when there was an approximate 10% fall in WFPS from that measured at the sampling occasion prior to the peak emission (8th July) to that recorded on the same day as the peak emission (15th July).

Throughout the growing season, fluxes of NO were consistently very low from both the SR and CON treatments, consequently negative NO fluxes were frequently measured. Fluxes varied from only -2.6 to 0.86 μg NO-N m^{-2} h^{-1}, except for the peak emissions of 3.4 μg NO-N m^{-2} h^{-1} (SR) and 2.8 μg NO-N m^{-2} h^{-1} (CON), which both occurred on the 2nd September and coincided with a low WFPS (< 50%), a relatively high soil surface temperature of *ca.* 22 °C and a rise in the soil NO_3^--N concentration.

Table 4.10 Table of mean values (treatment and time) produced as a result of repeated measures ANOVA of NO flux (μg NO-N m^{-2} h^{-1}) in 1999 for both the April and July fertiliser applications.

Treatment (April)						Degrees of freedom	Standard error of differences of means
SR	CS	SSL	CON	NPK	SSP		
-2.3	7.5	8.4	-0.7	3.9	11.9	10	2.8

Time (April) - Days after fertilisation					Degrees of freedom	Standard error of differences of means
26/04	29/04	07/05	18/05	15/07		
2.8	5.2	13.2	3.3	-0.5	21	1.1

Treatment (July)						Degrees of freedom	Standard error of differences of means
SR	CS	SSL	CON	NPK	SSP		
-0.5	5.6	3.8	-0.6	3.6	6.4	9	1.6

Time (July) - Days after fertilisation						Degrees of freedom	Standard error of differences of means
06/07	08/07	15/07	22/07	02/09	07/10		
2.0	5.2	5.8	2.1	4.2	-1.1	29	0.9

The peak emissions generated as a result of the April fertiliser application corresponded with the maximum recorded soil NH_4^+-N (Figure 4.16). Furthermore, peak emissions from NPK, SSL and CON coincided with the largest soil NO_3^--N concentrations (Figure 4.15), and those from NPK, SSP and SR with the lowest soil WFPS (Figure 4.14). Fluxes from all but the SSP either remained at, or declined to, background levels (< 0.5 μg NO-N m^{-2} h^{-1}) by the last sampling occasion before the 2nd application - 46-50 days after fertilisation (Figure 4.13). The flux from the NPK treatment was, however, no different to that of the control 18 days after fertilisation.

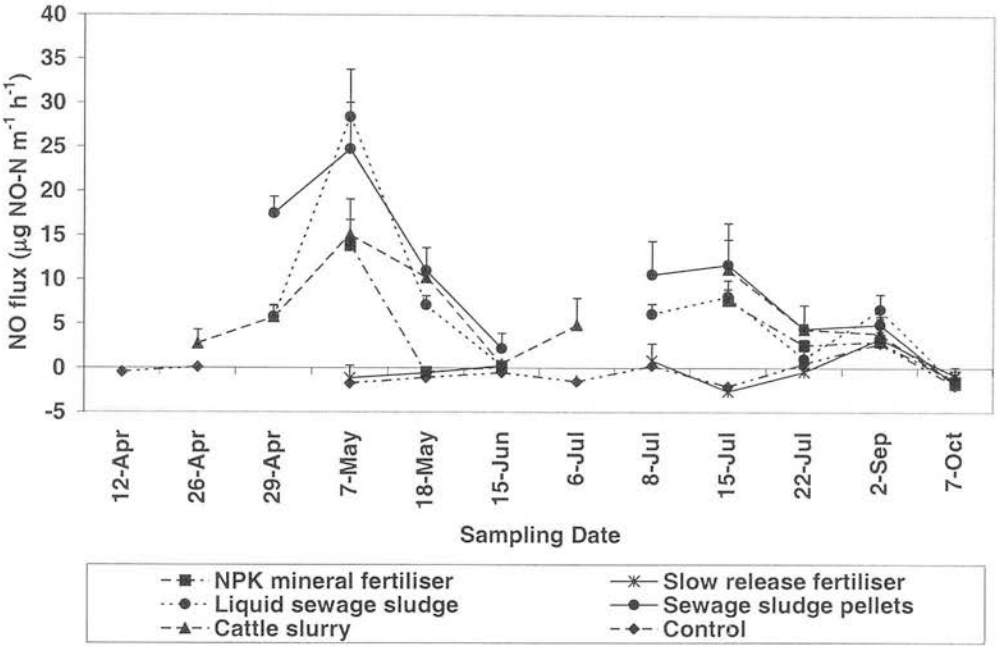

Figure 4.13 NO flux measured in 1999 from ungrazed grassland amended with organic wastes and inorganic fertilisers in April and July. Error bars represent one standard error (N=3). Note the different scale to that in 1998 (Figure 4.4). Cattle slurry applied on 26th April and 5th July, sewage sludge liquid applied on 28th April and 7th July, slow-release applied on 30th April, NPK fertiliser applied on the 30th April and 9th July and sewage sludge pellets applied on the 27th April and 5th July.

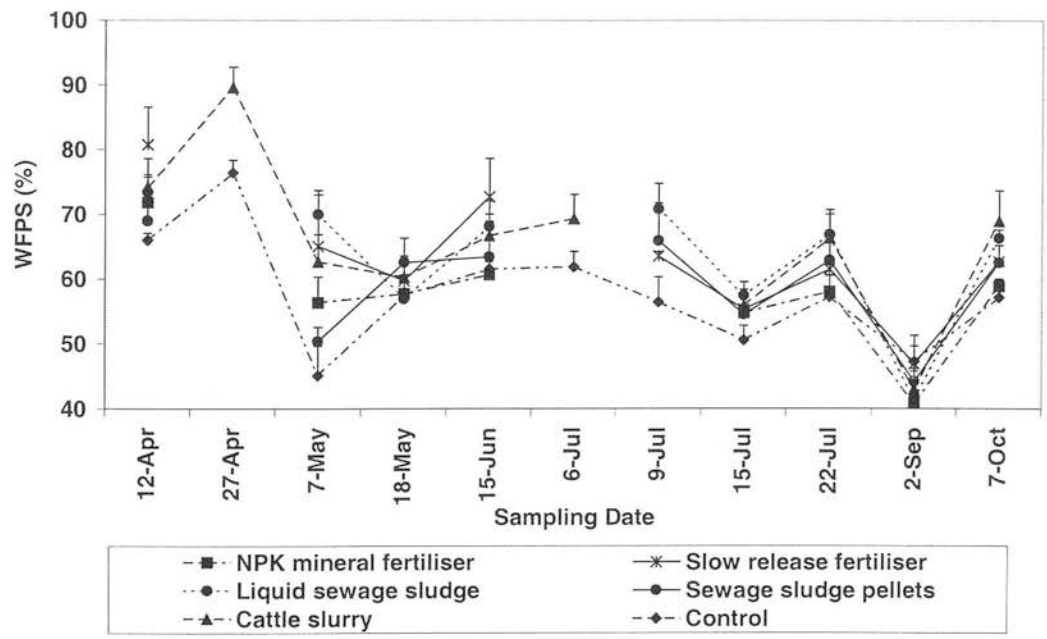

Figure 4.14 Mean WFPS measured in 1999 from ungrazed grassland amended with organic wastes and inorganic fertilisers. Error bars represent one standard error (N=3).

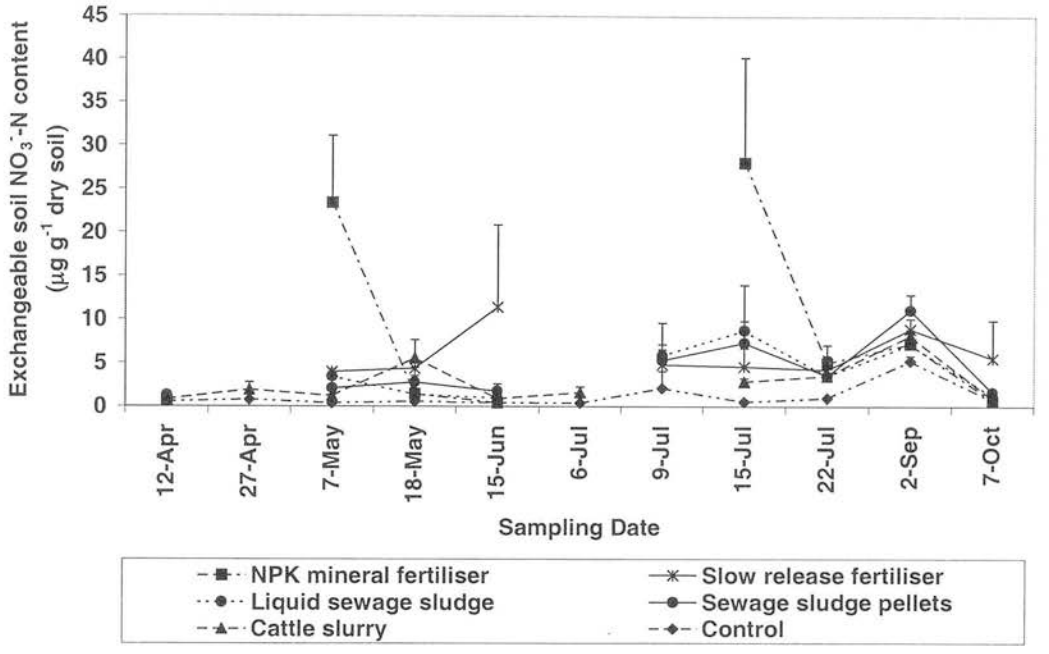

Figure 4.15 Mean soil NO_3^--N measured in 1999 from ungrazed grassland amended with organic wastes and inorganic fertilisers. Error bars represent one standard error (N=3).

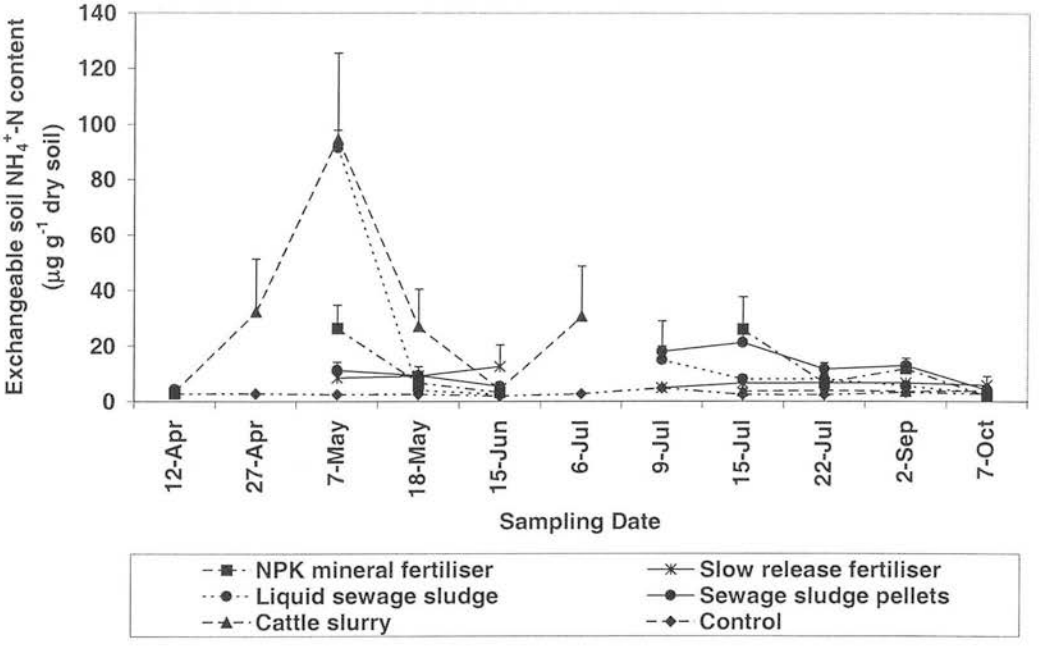

Figure 4.16 Mean soil NH_4^+-N measured in 1999 from ungrazed grassland amended with organic wastes and inorganic fertilisers. Error bars represent one standard error (N=3).

After the July application, the majority of the peak NO emissions did not only correspond to a reduction in WFPS, but those from the NPK, SSL and CON treatments also coincided with the largest soil NO_3^--N concentrations (Figure 4.15), and that from the SSP treatment with the highest soil NH_4^+-N concentrations (Figure 4.16). Similarly to the SR and CON treatments, the other treatments (excluding CS) experienced an increase in NO emission from the 22nd July to the 2nd September concurrent with a sharp decrease in the WFPS of between 17 and 25% (Figure 4.14), a rise in soil surface temperature from about 17 to 22 °C and an increase in soil NO_3^--N concentration to > 7 μg g dry soil^{-1} (Figure 4.15). Furthermore, the soil NH_4^+-N concentration remained constant or increased, although to a lesser extent than the soil NO_3^--N levels, in the NPK, SR, SSP and CON treatments.

Emission minima following the July application were measured on the last measurement occasion (7th October) from all but the CON and SR treatments, however all treatments showed negative NO fluxes. These negative fluxes occurred when the soil surface temperature was *ca.* 11 °C and the soil available mineral N concentrations were generally low (< 4 μg g^{-1} NH_4^+-N; < 2 μg g^{-1} NO_3^--N).

Measured NO fluxes were used to calculate potential cumulative losses over a 165 d period (April to October) following fertiliser application in April and July (Figure 4.17). Cumulative NO loss ranged from 24 – 1000 μg NO-N m^{-2}, with the lowest emission measured from CON and the highest observed from SSP. The cumulative emission from SR was only 4 times larger than the CON and that from NPK differed by a factor of 15. However, the magnitude of the disparity increased yet further with the organic fertilisers, as the cumulative loss from both SSL and CS was *ca.* 30 times the amount from the CON treatment, whilst that of SSP was 42 times greater.

There was a significant effect of N fertilisation ($P < 0.001$, d.f = 10) on NO emission from the soil surface (Figure 4.17). The Tukey multiple comparison test demonstrated that the organic wastes did not differ significantly from each other, nor did the inorganic fertilisers, or the SR and CON treatments. The organic wastes did, however, emit more NO than the inorganic wastes and the CON treatment, and the NPK also lost more than the CON (Tukey test, $P < 0.05$).

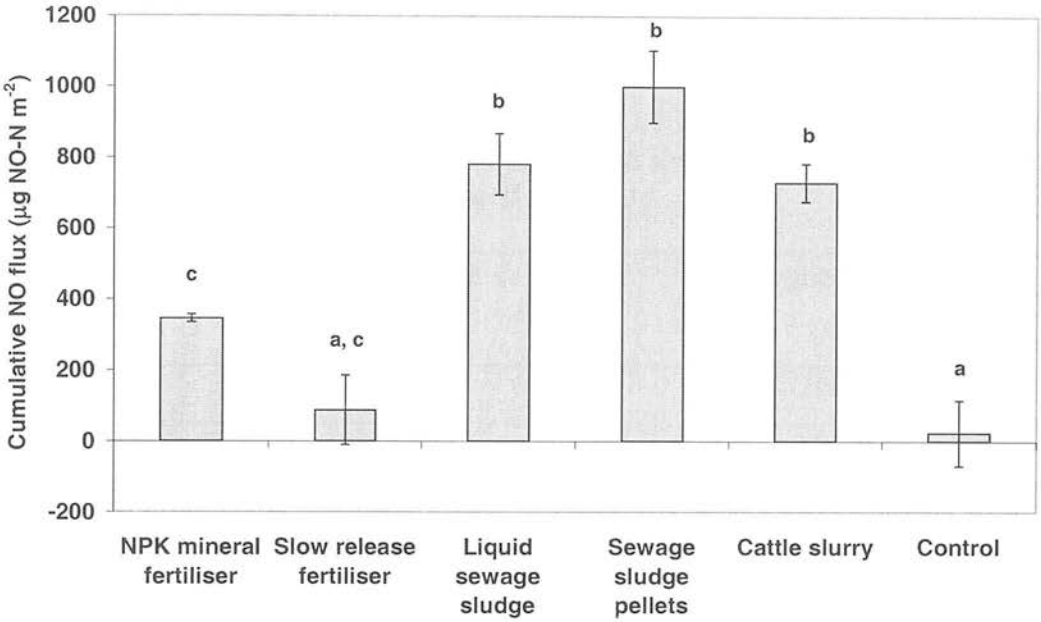

Figure 4.17 Cumulative NO emission over a 165 d period following fertilisation in 1999 from ungrazed grassland amended with organic wastes and inorganic fertilisers. Error bars represent ± one standard error (N=3). Means with the same letter code do not differ significantly (Tukey test $P > 0.05$).

There was a significant difference ($P < 0.001$; sed = 0.0002; df = 8) between treatments of the percentage of total N lost from the applied fertiliser as NO, although it was extremely small (<< 1%) and varied between treatments in the order of SSL > NPK ≈ CS > SSP > SR (Table 4.11). There was no significant difference ($P > 0.05$) between organic fertilisers of the percentage of applied TAN lost as NO. In 1999 the amount of TAN applied lost as NO from the SSP and CS treatments was *ca.* twice that in 1998 and was concurrent with an approximate doubling in the amount of TAN applied (Tables 4.6 and 4.11). Conversely the percentage of TAN lost as NO from the SSL treatment was *ca.* 1.5 times higher in 1998 despite a 14 fold increase in the quantity of TAN added in 1999 (Tables 4.6 and 4.11).

Table 4.12 summarises the cumulative NO emissions calculated after each fertiliser application. The SR treatment was not included, since only one application was made at the start of the growing season. Despite the apparent variation in total NO emission between the April and July application dates, with higher emissions recorded from the 3 organic waste treatments in April and lower fluxes from NPK

and CON, the variability masked any statistically significant differences ($P < 0.05$) (Table 4.12).

Table 4.11 The percentage of fertiliser N lost as NO in 1999 over a 165 d period following fertilisation in April and July

Treatment	Total N applied lost as NO (%)	TAN applied lost as NO (%)
NPK	0.0010	0.0030
SR	0.0003	0.0005
SSL	0.0030	0.0030
SSP	0.0008	0.0040
CS	0.0010	0.0030

Table 4.12 Cumulative NO losses following the application of fertiliser (excluding SR) in April and July 1999 and the subsequent percentage of fertiliser N lost as NO. Values in parentheses represent 1 standard error of the mean.

Treatment	Cumulative NO (μg NO-N m^2)		Total N applied lost as NO (%)		TAN applied lost as NO (%)	
	April	July	April	July	April	July
NPK	137.8 (20.9)	207.8 (21.3)	0.0020	0.0010	0.0030	0.0020
SSL	452.6 (53.6)	328.4 (52.9)	0.0040	0.0020	0.0040	0.0030
SSP	607.7 (101.1)	393.2 (20.6)	0.0010	0.0005	0.0050	0.0030
CS	398.3 (48.5)	331.4 (60.3)	0.0010	0.0010	0.0030	0.0040
CON	-43.6 (13.1)	67.2 (80.4)	-	-	-	-

There was a significant difference ($P < 0.001$; sed = 0.0007; df = 18) between the treatments in the percentage of total N added lost as NO following both application dates, again the amount was very low (<< 1%) and resulted in a similar pattern as to the overall cumulative losses: SSL > NPK > CS ≈ SSP in April, and SSL > NPK > CS > SSP in July (Table 4.12). Due to the large quantity of total N added in the SSP, the NO loss was an order of magnitude lower than from the other treatments. There was no significant difference ($P > 0.05$) between the organic fertilisers in the percentage NO loss from applied TAN. Furthermore, there was neither a significant difference ($P > 0.05$) in the percentage of total N or TAN lost as NO from the April and July applications.

Cumulative losses per treatment were substantially larger in 1999 than in 1998, although the measurements in 1998 included only one fertiliser application and were carried out over a shorter time period, 111 d as opposed to 165 d. Nevertheless, despite the slightly shorter calculation period (*ca.* 21 d less) the cumulative NO emission from each treatment (except SR) following the July application in 1999 was still 2 to 4.4 times larger than that calculated after the July fertiliser application in 1998 (Figures 4.8 and 4.17 and Tables 4.5 and 4.11).

Comparison between fertiliser treatments

Repeated measures ANOVA showed that there was a significant difference ($P < 0.01$) in the emission of NO from the treatments following both the April and July fertiliser application (Table 3.2). The results suggested that NO emissions following the April fertiliser application were largest from the SSP treatment and lowest from the SR and CON treatments. After the July application there appeared to be little difference between all of the treatments, except CON and SR, where NO emissions were much lower (Table 3.2). There were also significant interactions ($P < 0.01$) between time and treatments, such that the effect of time was different for the various treatments.

Table 4.13 Summary of ANOVA results (*P* values and associated level of significance) for soil NO_3^-, NH_4^+ and WFPS at each sampling date in 1999

Sampling date	Data		
	Soil available NO_3^-	Soil available NH_4^+	WFPS
12/04/99	NS	NS	NS
26/04/99	NS	NS	NS
29/04/99	NA	NA	NA
07/05/99	0.003 **	0.002 **	0.031 *
18/05/99	0.037 *	NS	NS
15/06/99	NS	NS	NS
06/07/99	NS	NS	NS
08/07/99	NS	NS	NS
15/07/99	NS	NS	NS
22/07/99	NS	0.018 *	NS
02/09/99	0.042 *	0.001 **	NS
07/10/99	NS	NS	NS

*** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$; NS = not significant

NA = not available - Sample not taken or only CON measured.

A significant effect of fertilisation application on the soil WFPS was observed once 9 days after the April application (7th May) when WFPS from the SSL treatment was significantly wetter ($P < 0.05$) than either the CON or SSP treatments (Table 4.12).

As expected, statistically significant effects of fertiliser application on the soil available mineral N were periodically recorded, particularly on the sampling occasions when peak emissions were measured (Table 4.13). On the 7th May, soil NO_3^--N was significantly ($P < 0.01$) larger from NPK than from the other treatments and soil NH_4^+-N was significantly ($P < 0.01$) greater from SSL and CS compared to the concentration in CON, SR and SSP. However, at the next sampling occasion on the 18th May, only a significantly ($P < 0.05$) larger soil NO_3^--N concentration was measured from the CS treatment compared to that in the CON treatment.

As a result of the July fertiliser application soil NH_4^+-N was significantly ($P < 0.05$) larger in the SSP treatment than that in the CON and CS treatments on the 22nd July. On the 2nd September, the soil NH_4^+-N concentration in the SSP treatment, along with that in the NPK, were also significantly greater than the concentration in the other treatments. Furthermore, soil NO_3^--N measured on the same day was significantly ($P < 0.05$) higher in the SSP treatment than in the CON.

A highly significant ($P < 0.001$) positive relationship between NO emissions from all treatments and soil available NH_4^+-N ($r^2 = 0.45$) was observed. The variation in the NO data could be explained further ($R^2 = 0.50$, $P < 0.001$) by a combination of soil WFPS and soil NH_4^+-N. None of the other soil parameters measured appeared to be able to explain the magnitude of NO fluxes gathered across the treatments, although individual treatments are likely to be influenced by different factors.

The data suggest that soil mineral N strongly influenced the variation in NO emission measured from the NPK treatment. Both soil NH_4^+-N and NO_3^--N yielded statistically significant, positive relationships with NO: $r^2 = 0.87$ ($P < 0.01$) and $r^2 = 0.79$ ($P < 0.01$), respectively (Figures 4.18 and 4.19). Neither a single factor or a combination of factors could explain the size of the NO fluxes from either the SSP treatment, CS treatment, SSL treatment or from the SR treatment.

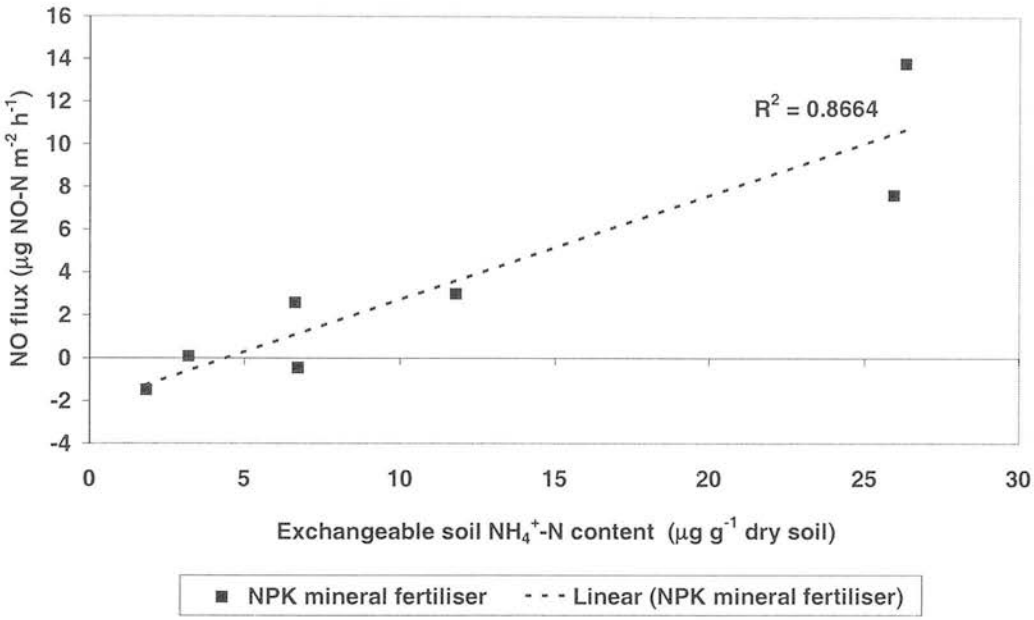

Figure 4.18 The significant relationship between NO emission and soil NH_4^+-N measured in 1999 from the NPK treatment

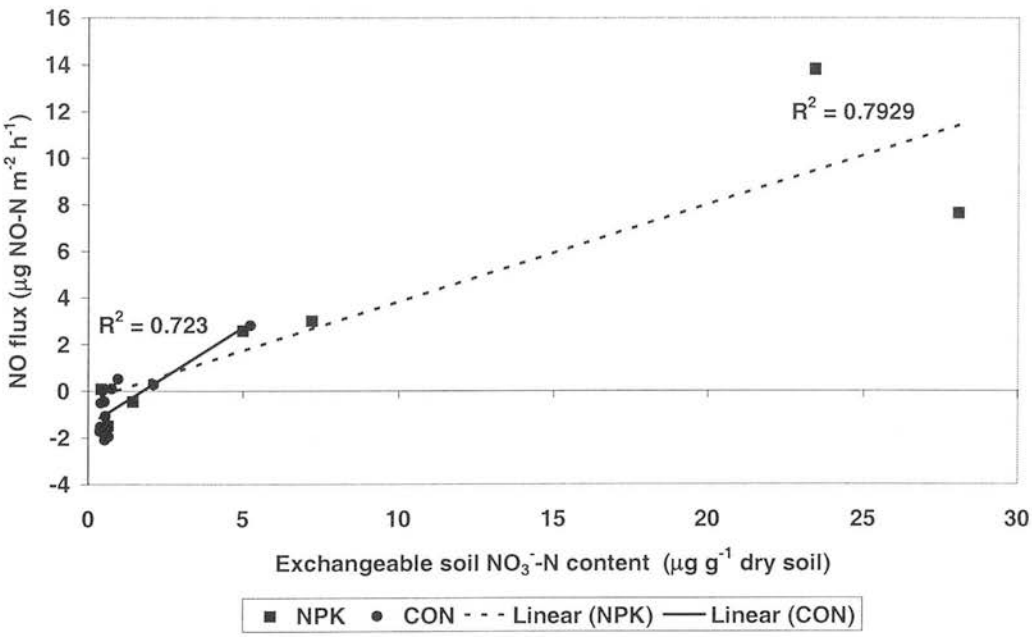

Figure 4.19 The significant relationship between NO emission and soil NO_3^--N measured in 1999 from the NPK and CON treatments

Soil NO_3^--N was estimated to account for 72% of the variation ($P < 0.001$) in the NO emission data obtained from the CON treatment (Figure 4.19), however, 84% could be accounted for with a combination of both soil NO_3^--N and WFPS ($P < 0.001$). On only 1 measurement day did any of the examined factors account for a proportion of the variation observed in the daily NO emission across all treatments. Soil NH_4^+-N alone was able to explain 96% of the variation on the 8th July ($P < 0.05$).

4.4.2.2.2 N_2O emissions

Measurements of N_2O were taken as part of the parent experiment and these results are shown by kind permission of Dr. Iain McTaggart (SAC, Edinburgh).

Table 4.14 Cumulative N_2O flux (μg N_2O-N m^{-2}) measured following all 3 fertiliser applications from ungrazed grassland amended with organic wastes and inorganic fertilisers

Treatment	Application date		
	July 1998	April 1999	July 1999
NPK	15744	1701	5850
SR	1070	401	1053
SSL	869	1825	4574
SSP	793	1869	3226
CS	1660	3144	4458
CON	367	91	240

Fluxes of N_2O over the whole sampling period (27th April to the 19th October 1999) varied from -1 to 3620 μg N_2O-N m^{-2} h^{-1} for fertilised plots and from 3 to 32 μg N_2O-N m^{-2} h^{-1} for unfertilised plots. Table 4.14 displays the cumulative N_2O emissions following each fertiliser application. The largest losses were observed from the SSL, SSP and CS treatments after the July 1999 application and from the NPK, SR and CON treatments following the July 1998 application.

4.4.3 Agronomic properties

The dry matter yields and N uptake data shown were obtained via the parent project and use of such data is gratefully acknowledged.

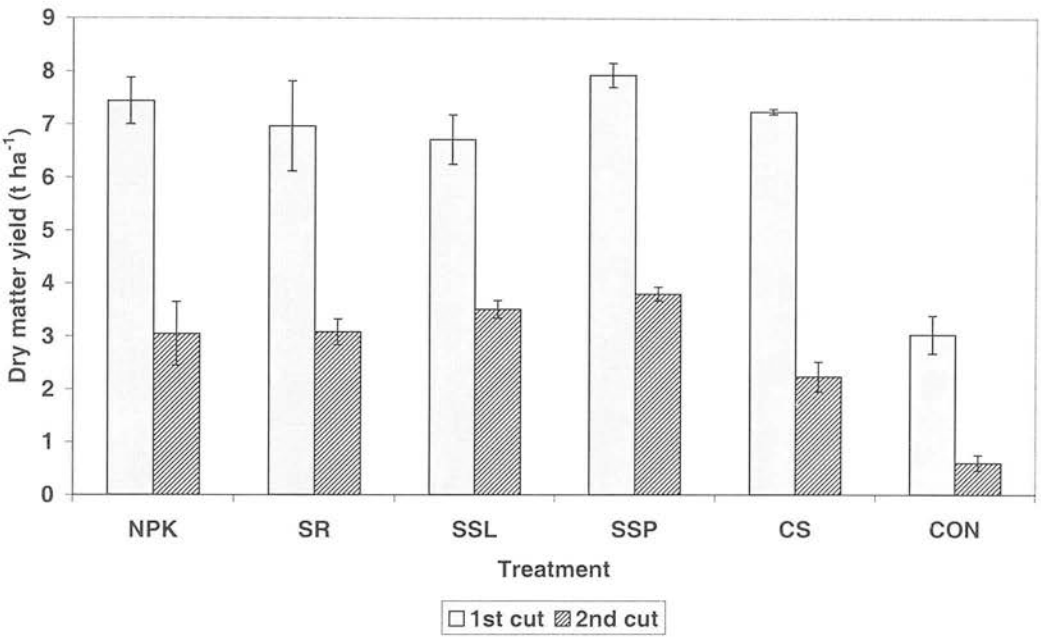

Figure 4.20 Grass yield from all treatments measured following the first and second harvests in 1999. Error bars represent ± one standard error of the mean (N=3).

No analysis of the harvested grass was carried out in 1998, but grass yields and total N uptake were calculated in 1999 following harvest on the 23rd June and the 1st September. Each plot was divided into two and the material was collected from the specifically designated half. Due to a shorter growth period the yields from the 2nd cut of grass were less than half that obtained from the 1st cut (Figure 4.20). Data from both cuts demonstrated the stimulating effect of fertilisation on the growth of the grass, with all treatments producing a statistically larger ($P < 0.05$) yield than that collected from the CON treatment. Furthermore, the yields obtained following the 2nd cut from the NPK, SR, SSL and SSP treatments were significantly greater ($P < 0.05$) than that from the CS treatment. Despite the small variability between treatments, no other statistical differences were recorded.

The pattern of N uptake between the treatments closely mirrored that of the dry matter yields, particularly with the 1st cut (Figure 4.21). There was, however, slightly more variation between treatments in the 2nd cut. Consequently, all of the fertilised treatments, except CS, demonstrated a significantly ($P < 0.05$) higher N uptake than the CON treatment, and all fertilised treatments, except NPK, displayed a significantly ($P < 0.05$) larger N uptake than CS. No other treatments were statistically different from each other.

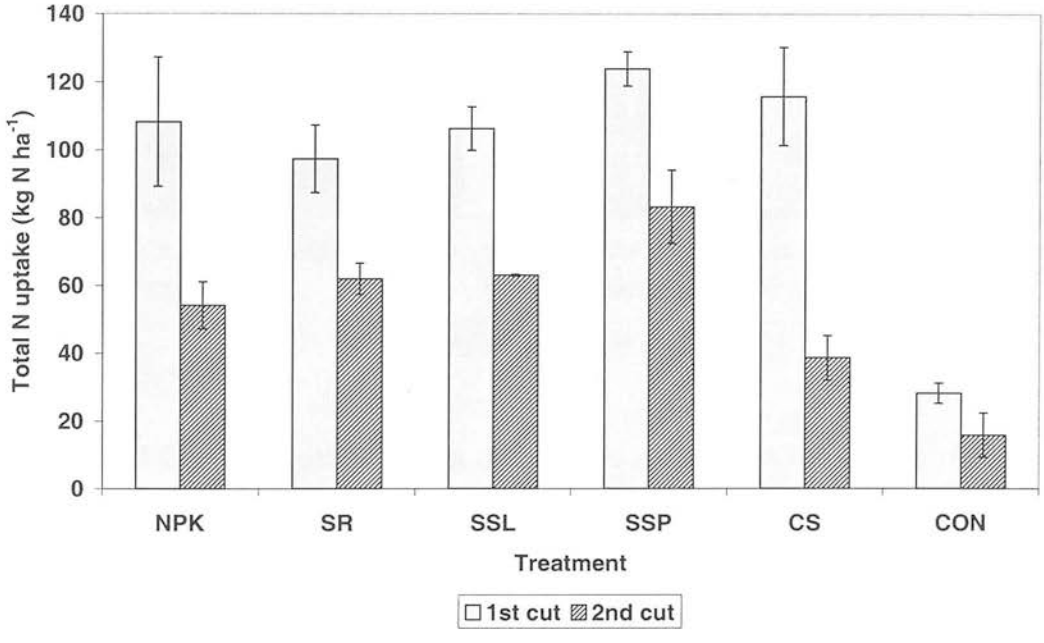

Figure 4.21 Total N uptake from all treatments measured following the first and second harvests in 1999. Error bars represent ± one standard error of the mean (N=3).

The crop height for each plot was averaged from a set of five measurements taken from inside the gas chamber. The crop was mown and removed twice during the sampling period, which is reflected in the reduction in measured crop height (Figure 4.22). Additionally, on the 10th June the crop was manually trimmed within each chamber and the grass removed. This was necessary to allow free gas exchange between the soil and the atmosphere within the chamber. At the taller crop heights it was noticeable that the grass growing in the CON plots was generally shorter than

that from the fertilised treatments; this was confirmed through the values obtained for the grass yields.

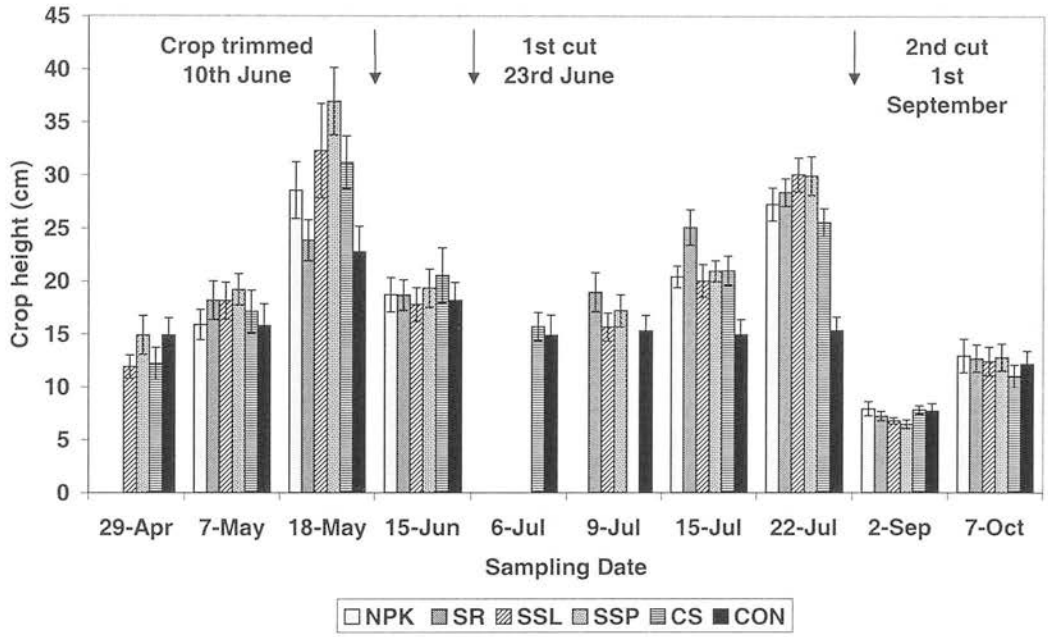

Figure 4.22 Crop height of grass measured in 1999 from all treatments

4.5 Discussion

4.5.1 Effect of fertiliser treatment on NO emission

The field trials suggest that the application of both organic and inorganic fertilisers to ungrazed grassland enhanced NO emissions through the stimulation of NO production processes, principally as a consequence of the addition of microbial substrates.

In 1998, although there were no statistically significant differences cumulative NO emissions from the different treatments followed the same emission pattern as 1999, with larger NO fluxes emitted from the organic treatments compared to the inorganic and unfertilised plots (Figures 4.8 and 4.17). Frequently in conjunction with the enhanced emissions, mineral N concentrations were also recorded at significantly higher concentrations in the fertilised plots. The influence of both the application of organic and inorganic fertilisers on NO emission and soil available mineral N did, however, generally diminish during the growing season. Actively growing grass possesses a high capacity to take up N (Whitehead, 1995) and therefore, reduce that remaining in the soil which could potentially be lost via NO emission.

Measured NO emissions from the fertilised soils in this experiment (target of 120 kg ha^{-1} available N) were comparable (Figures 4.4 and 4.13) to the median NO fluxes (1.5-15.7 μg NO-N m^{-2} h^{-1}) measured following similar fertiliser application (150 kg ha^{-1} NH_4NO_3-N) to a ryegrass sward located close to this field site and of the same soil series. Furthermore, the NO emissions from the CON treatment were also similar to those measured by Skiba *et al.* (1992) from unfertilised ryegrass (< 1 μg NO-N m^{-2} h^{-1}) in the same field and to the mean emission (0.7 ± 1.8 μg NO-N m^{-2} h^{-1}) from an unfertilised vegetated sandy loam in Mainz, Germany (Slemr & Seiler, 1991). The NO emissions recorded from the various treatments at this site, however, were considerably smaller than the peak NO emissions (> 1000 g NO-N m^{-2} h^{-1}) reported from a grassland soil amended with both cattle excreta and urine (Watanabe *et al.* 1997). The combined total N application rate was, however, at 601 kg N ha^{-1} about 5 times higher than in my experiment and therefore, since the majority of this N would be readily available (MAFF, 2000) higher fluxes are not unexpected.

Veldkamp & Keller (1997) estimate that *ca.* 0.5 % of applied inorganic N fertiliser is lost as NO during the crop growing season. This is a comparable figure to that suggested by Watanabe *et al.* (1997) in a study to measure NO emission from grassland soils after the application of excreta from cattle (0.48% of the total N added) and swine (0.45% of the total N added). In a laboratory based study, Paul *et al.* (1993) showed that cumulative NO emission calculated over a much shorter time period (only 6 days) than used in this work, averaged 0.26% of the added total N in liquid dairy cattle manure and fresh solid beef manure. The results from these two experiments are 2-3 orders of magnitude greater than that measured (0.0004-0.003 % of the total N applied) from this grassland site over the 111 d and 165 d period following fertilisation in both 1998 and 1999 respectively (Tables 4.5 and 4.11).

However, Chadwick & Harrison (2001) reported a fertiliser loss rate from grassland soils in the South of England of only one order of magnitude greater than in this experiment, albeit calculated over only the first 14 days following fertiliser application. In March, dairy slurry either surface spread or shallow injected into a sandy loam soil lost < 0.01% of the added total N as NO, as did June surface applied beef cattle slurry to a clay loam soil. Nonetheless, Chadwick and Harrison (2001) calculated that the percentage of applied TAN lost as NO was generally greater, at < 0.01 to 0.06%, than measured in this study over the whole experimental period (0.001-0.005% of TAN added) or over a comparable 14 day period following fertilisation (0.001-0.002% of added TAN) (Tables 4.5 and 4.11).

The discrepancy in the percentage loss of added N calculated in this work and that of other values reported in the literature may be due to the cool, moist climate of Scotland. Such conditions are likely to encourage denitrification and hence N_2O and N_2 production rather than large NO emissions. In particular, the soil and manures used in the laboratory study of Paul *et al.* (1993) were incubated at a temperature of 25 °C and the soil temperature (at a depth of 10 cm) reached > 20 °C in the field experiment carried out by Watanabe *et al.* (1997). Similarly, the average soil temperature at a depth of 5 cm in the UK clay loam soil was > 17 °C (Chadwick & Harrison, 2001). These temperatures were much higher than was generally measured in this project where an average soil temperature (at a depth of 5 cm) of 17 °C was only exceeded (19-23°C) on 3 measurement occasions and consequently, the

microbial processes producing NO would be expected to proceed at a far greater rate (Davidson & Schimel, 1995).

The texture of the soil used may also have enhanced the emissions of NO reported in the literature. The use of a sandy loam soil (Chadwick & Harrison, 2001) would encourage gaseous diffusion and Paul *et al.* (1993) themselves commented that their experimental set-up with only 55 g of soil in a 250 ml bottle may have promoted greater gaseous diffusion than would occur naturally in the field. Consequently, the loss of NO to consumption during denitrification would be reduced and the generation of NO from nitrification would rise.

Furthermore, the results of the study by Watanabe *et al.* (1997) were based on only one replicate so must be treated with caution as the variability in my experiment was large with CVs of 25-734%. The discrepancy between my results and those of Paul *et al.* (1993) may have also been influenced by the manure application method. Paul *et al.* (1993) intimately mixed the manure with the whole of the soil sample thereby exposing a larger population of micro-organisms to mineral N substrate than would have occurred with the surface application or injection of manure in my experiment.

The percentage of added (i.e. fertiliser) total N and TAN lost as NO is useful when comparing emissions from treatments where a variable quantity of particularly manure N has been applied. In calculating the percentage of TAN lost, it is assumed that emitted NO is derived from the manure NH_4^+-N, since commonly the quantity of NO_3^--N in digested sludges (Parker & Sommers, 1983) and animal waste (MAFF, 2000) is negligible. Nonetheless, the percentage may be overestimated, as over the growing season some mineralisation of organic N will occur, which will release bio-available NH_4^+-N into the soil. The amount of mineralisation will vary, however, with environmental factors e.g. temperature and with the composition of the manure.

The percentage of TAN lost as NO from ammonium nitrate fertiliser, however, is a less reliable calculation, because the NO_3^--N as well as the NH_4^+-N present in the compound may act as a substrate for biological NO production. It is generally acknowledged, however, that the majority of NO released to the atmosphere from soil is produced during nitrification (Davidson & Schimel, 1995;

Williams *et al.* 1998) and hence it is likely that NH_4^+-N will be the dominant microbial substrate.

In terms of season and the individual applications of 1999, the treatment with the apparently highest measured cumulative emissions (SSP) typically showed the largest percentage NO lost from TAN, although between the organic fertilisers there was no significant difference (Figure 4.17 and Table 4.12). The surface placement of the SSP would have resulted in a steady supply of NH_4^+-N into the relatively more aerated top few cm of the soil profile. The potential for NO production by nitrification would therefore rise, especially due to the relatively shorter diffusion pathway to the atmosphere and the subsequent reduced risk of NO loss via consumption. Nevertheless in 1998, the largest percentage of TAN as well as total N lost as NO was calculated from the SSL treatment, although again this was not statistically significant.

The composition and the far smaller N application rate of the SSL treatment in 1998 would have contributed to the relatively high NO loss observed compared to that in 1999. The very dilute nature (DM of 0.4%) and the high proportion (67%) of ammoniacal N in the SSL would have resulted in a rapid infiltration of a large concentration of microbial substrate into the soil matrix and hence to sites of nitrification. It is quite possible that a large NO emission peak may have been missed in the 11 d prior to the first measurement occasion, especially since prior to application rainfall was low and the WFPS was less than 60%, the value that is generally thought to mark the point between the dominance of nitrification or denitrification as the major NO production mechanism (Davidson, 1993).

In 1998 there was no significant difference in the percentage of NO lost as TAN and total N from the 2 injected liquid organic manures CS and SSL. Following the 1999 application of fertilisers, there was also no significant difference in the percentage of NO lost as TAN from the three organic fertiliser treatments.

The smallest measured fluxes from fertilised plots were consistently observed from the two inorganic fertiliser treatments and correspondingly, so were the lowest percentage of TAN lost as NO. The low emissions of NO measured in both years from the SR treatment were not unexpected, since the release of a large concentration of mineral N into the soil was prevented. The flow of mineral N out of the fertiliser

granule is synchronised with the crop demand and consequently, there is little excess N available for microbial denitrification or nitrification.

Despite the fact that the SSP and NPK fertilisers were both applied to the soil surface, the fluxes measured and the subsequent percentage of TAN lost as NO were different, although not statistically so in 1998 (Figures 4.4 and 4.13). The larger emissions from the SSP treatment may be associated with the organic nature of the fertiliser and differences in solubility. Unlike the SSP fertiliser, the NPK is purely inorganic and therefore does not have a slow and gradual release of mineral N over time for use by the microbial population. Additionally, the NPK fertiliser is much more soluble than the SSP and consequently mineral N will be washed further into the soil matrix, such that the subsequent diffusion pathway for any produced NO is increased and therefore so is the chance of NO consumption.

The difference between the two fertilisers may also be partly due to the different application dates and the consequent variation in rainfall following application. Particularly, in the first field season, during the period between fertiliser addition and the first sampling point, 2 ½ times as much rain fell on the NPK plots than on the SSP plots. The highly soluble nature of the mineral fertiliser will have facilitated the washing in of the surface applied substrates deeper into the soil and therefore, increasing the diffusion path length for NO escape. The wet soil conditions and the reduced potential for NO loss from the soil would have retarded production by nitrification, but stimulated denitrification, particularly with the presence of added fertiliser NO_3^--N. Indeed, the strong capability for denitrification is reflected in the extremely high N_2O (20449.10 μg N_2O-N m^{-2} h^{-1}) flux measured 3 d after the application of NPK (Figure 4.11).

4.5.2 Variation in NO emission over the sampling period

The pattern of NO emission in both years (Figures 4.4 and 4.13) of the experiment was similar to that of numerous studies, which have all demonstrated the stimulation of production following the application of organic and inorganic nitrogenous fertilisers (Anderson & Levine, 1987; Hutchinson & Brams, 1992; Skiba *et al.* 1992; Thornton *et al.* 1996; Watanabe *et al.* 1997). Subsequent to the fertiliser-induced peak emissions, NO losses declined, primarily as a result of the decrease in

plant-available soil mineral N due to uptake by the growing grass sward. It has been estimated that actively growing grass can take up most of the fertiliser-added NH_4^+-N and NO_3^--N in the 3-4 weeks after application (Whitehead, 1995).

In the 1998 field season, the measured peak NO emission from both of the injected treatments (SSL and CS) occurred 12 and 14 days after fertilisation, although these were the first measurement occasions and large fluxes may have been missed (Figure 4.4). It is possible that a contribution to the peak emission from the injected treatments was made from the actual action of the injecting implement, carving slots through the soil. In a manner similar to the action of ploughing, soil aggregates would have become disrupted and organic N exposed to microorganisms. Subsequently, bio-available N would have been made available for use in nitrification and denitrification (Dowdell & Cannell, 1975; Paustian *et al.* 1997).

The maximum NO losses measured from the surface-applied fertiliser treatments of NPK and SSP occurred around the same number of days after fertiliser application as the maximum losses from the SSL and CS treatments, at 20 and 17 days respectively (Figure 4.4). These peak emissions were recorded at the 2nd measurement occasion rather than the first, 7 and 4 days after fertilisation. This suggests that the peak NO measured by the SSL and CS treatments was a 'true' peak. However, there was a substantial difference in the pattern of precipitation around the first two sampling events, which was likely to influence the magnitude of the NO losses. In the 4 days prior to the 1st sampling occasion 30.2 mm of rain fell at the site, whereas in the 4 days before the 2nd measurement event only 9.1 mm of precipitation was recorded (Figure 4.3). The soil conditions at the 1st sampling would therefore be wetter and indeed this was reflected in the soil WFPS, particularly in the SSP treatment where there was a decrease in WFPS of *ca.* 15% (Figure 4.5). As the WFPS of a soil increases so does the degree of anaerobicity, which is known to inhibit NO release through reduced gaseous diffusion and increased NO consumption during denitrification (Firestone & Davidson, 1989). In contrast the lower rainfall before the measured peak emissions would yield drier soils and the accompanying improved aeration would tend to promote nitrification.

Despite the nature of the SR fertiliser, which releases more mineral N as the temperature increases throughout the growing season, the demand by the crop

seemed to be appropriately matched, so that virtually no surplus mineral N was available for use by the microbial biomass. The measured NO loss was consequently not significantly different to the background CON treatment (Figure 4.4). The emission of NO from the unamended CON treatment did not demonstrate any marked pattern and was consistently < 0.7 μg NO-N m^{-2}.

The majority of the treatments (including the control) showed a secondary smaller peak emission on the 3rd October 1998 (Figure 4.4), which coincided with a rise in mineral N, particularly soil NH_4^+-N (Figure 4.7). In the autumn, mineralisation of soil organic N coupled with the absence of an actively growing grass crop is likely to increase the availability of N for use as a substrate by microorganisms. Additionally, the soil surface temperature (data not shown) was recorded at *ca.* 16°C, which would have stimulated the microbial NO production processes yet further.

Not unexpectedly NO emission minima generally occurred on the last sampling date (20th October), when the stimulating effect of fertilisation had ceased and the soil surface temperature was *ca.* 6°C (data not shown). Consequently, the microbial NO generation processes of denitrification and nitrification rates were liable to be significantly reduced (Williams & Fehsenfeld, 1991; Otter *et al.* 1999).

In the 1999 field season, the maximum NO flux measured after each of the two fertiliser applications (i.e. April and July) occurred 6-11 d after fertilisation, although not from either the CON or SR treatments (Figure 4.13). Peak fluxes corresponded to an approximate 10% fall in WFPS to generally < 60% (Figure 4.14). As the WFPS drops below 60%, the dominance of denitrification recedes and the rate of nitrification rises, consequently the NO:N_2O ratio increases as more NO is produced from nitrification and less is consumed during denitrification (Davidson, 1991).

Following the April application of fertiliser, the largest measured NO emissions from the NPK, SSP and SR treatments corresponded to the lowest recorded WFPS (≤ 56%) (Figures 4.13 and 4.14). Moreover, peak emissions from all treatments (including the control) coincided with the peak soil NH_4^+-N concentrations (Figure 4.16) and with the maximum soil NO_3^--N levels (Figure 4.15) in the NPK, SSL and CON treatments. The stimulation of NO fluxes from the added

N, however, rapidly declined to background levels within 18 d after fertilisation from the NPK treatment and 46-50 d from the CS and SSL treatments, whereas NO release continued from the SSP treatment. This variation in length of influence of the applied manure is likely to reflect the level of potential mineralisation of added organic N. Since the inorganic NPK fertiliser did not contain any organic N and was applied at the lowest rate of NH_4^+-N, it was not unexpected that emissions declined more quickly. In contrast, emissions were sustained for a longer period from the organic fertilisers, as ammoniacal N was slowly released during mineralisation. The SSP treatment, known for its slow release properties, was able to maintain mineralisation and hence NO production yet further, due to its extremely high organic N content (3.4%).

Similarly to the April application, peak emissions as a result of the July fertilisation were governed by the soil mineral N content. The maximum fluxes from the NPK, SSL and CON treatments corresponded to peak soil NO_3^--N concentrations and the highest flux measured from the SSP treatment coincided with the highest soil NH_4^+-N levels.

A secondary emission peak was detected 7 weeks (2nd September) after the maximum (15th July) fluxes were recorded from the NPK, SSL, SSP and CS treatments, and occurred at the same time as the peak NO fluxes were measured for the SR and CON treatments (Figure 4.13). The elevated emissions may have been related to a number of factors. This sampling event took place the day after the second yield cut and even though the gas chambers were reinserted immediately following harvest, the soil may have been disturbed enough to promote microbial activity, which continued through to the next day. Similarly, Anderson & Levine (1987) attributed enhanced NO emissions to the accidental removal and subsequent reinstallation of a chamber collar a week before analysis.

The resultant shortened grass length (Figure 4.23) combined with a maximum air temperature of > 26°C will have contributed to the *ca.* 5°C increase in soil surface temperature, which would have stimulated microbial NO production. Furthermore, the relatively high soil temperature may have increased the availability of mineral N released from the slow release fertiliser, which may have contributed to the peak emission observed.

Notably, this burst of NO corresponded to a sharp decline in soil WFPS of 17-25% (Figure 4.14) and a rise in soil NO_3^--N of 4-8 μg g^{-1} (Figure 4.15) from the previously measured values. The elevated NO_3^--N may be a consequence of the grass harvest, since frequent defoliation of grass throughout the growing season has been shown to temporarily, dramatically reduce NO_3^--N uptake (Jarvis and Macduff, 1989). An increase in soil NO_3^--N was not, however, noticeable in June when the grass within the chamber was manually trimmed 5 d prior to sampling. It is possible that the higher NO_3^--N levels were the result of reduced denitrification in the relatively dry weather prior to the sampling point and/or the product of an increase in the rate of nitrification ensuing from the low WFPS of < 50%. Certainly, the substrate for nitrification, soil NH_4^+-N, had increased in samples taken from the NPK, SSP and CON plots and remained constant in the SR treatment. The elevated NH_4^+-N concentrations were probably the effect of increased mineralisation of both soil and applied organic N in the drier, warmer conditions. Mineralisation is known to proceed more rapidly in moist, but well aerated soils (i.e. *ca.* 60% WFPS) (Brady & Weil, 1999) and with increasing temperatures (Terry *et al.* 1981).

The minimum NO flux from all but the SR and CON treatments was monitored on the last sampling day, the 7th October 1999 (Figure 4.13). Significantly, mineral N levels were generally at background levels and the soil surface temperature was *ca.* 11°C and hence the microbial processes of both nitrification and denitrification would have slowed down appreciably. Negative NO fluxes were indicated on this measurement occasion, although it was unlikely that consumption of NO via anaerobic denitrification occurred as the majority of treatments recorded a WFPS of *ca.* 60% and a very low soil NO_3^--N concentration (< 2 μg g dry $soil^{-1}$). Nevertheless, with minimal production of NO by nitrification (NH_4^+-N levels of *ca.* < 4 μg g dry $soil^{-1}$), anaerobic denitrification may have continued in microsites and used NO instead of NO_3^- as a terminal electron acceptor.

4.5.3 Variation in NO emission in 1999 between the April and July fertiliser applications

The variation in NO emission between that measured following the April fertiliser application and that recorded after the July addition can be clearly seen in Figure 4.13. The maximum rate of grass growth occurs shortly prior to ear emergence, which at this site was early June (Whitehead, 1995). It may be expected that with more vigorous sward growth, less mineral N would be available for microbial use and therefore emissions may be smaller following the April fertiliser application. Peak emissions following the July application were however, substantially smaller and consequently higher cumulative emissions were estimated from the three organic fertiliser treatments after the April application, although the difference was not statistically significant. Cumulative NO loss from the NPK and CON treatments, in contrast, was smaller after the April fertiliser addition and may well be related to the number and timing of the gas measurements, as well as the length over which the cumulative fluxes were calculated (Table 4.12). The low number of sampling occasions after each application ($n \leq 7$ and $n \leq 5$ after the 1st and 2nd application respectively) meant that each measured emission was strongly relied upon in the calculation of the cumulative flux and therefore, heavily affected the interpolation between points. In addition, the cumulative emissions were calculated over twice as long a period in July than in April with fewer actual measurements.

The difference in NO emission between fertiliser application timings is unlikely to be entirely linked to the quantity of total N and TAN applied. Although less TAN was added to the CS and SSL treatments following the July application, the same amount was applied to the SSP and NPK plots (Tables 4.8 and 4.9). Alternatively, the disparity may be explained by the distribution of precipitation around the fertiliser application dates. In the 7 d prior to the addition of fertiliser in April and July, 0.5-5.4 mm and 18.0-22.2 mm of rain fell and in the 7 d following the fertiliser amendment 0.0-5.1 mm and 3.6-6.9 mm were recorded respectively. The wetter soil conditions at the time of the July fertiliser application would have reduced aeration and consequently inhibited nitrification and the production of NO. The cumulative N_2O emissions measured at the site support this idea, with larger total

losses reported following the July application compared to losses calculated after the April application (Table 4.14).

4.5.4 Variation in NO emission between field seasons

There was a marked difference between the cumulative NO emissions determined following the July fertiliser applications in 1998 and 1999 (Figures 4.8 and 4.17). Despite the shorter calculation period (21 d less) in 1999, NO losses were 2 to 4.4 times larger than in 1998. More total N and TAN were added in 1999 from the SSP and SSL treatments, although less was applied on the CS treatment and there was no difference in the applied rate of NPK (Tables 4.4, 4.8 and 4.9). It should be noted though, that the percentage of fertiliser total N and TAN lost as NO from the SSL treatment was larger in 1998, than 1999, whereas the reverse was true for all other treatments. The small, but very dilute addition of slurry is the probable explanation for the greater percentage loss observed in 1998.

The most plausible reason for the distinct difference between NO emissions observed in the two experimental years is the pattern of precipitation at fertilisation. Throughout the sampling period of July to October approximately twice as much rain fell in 1998 than in 1999 and significantly in the month of fertiliser application (July), the monthly precipitation in 1998 was more than double that received in 1999. In 1998, 44.2 mm and 20.7 mm fell in the 7 days after application of the surface applied NPK and SSP respectively, whereas only 6.3 and 7.0 mm was recorded in 1999. The larger rainfall in 1998 would result in wetter soil, which is therefore likely to have increased the number of anaerobic microsites and subsequently reduced the NO fluxes. This explanation, however, is not generally supported by the measurements of WFPS, which are similar in both years. It is possible that calculating WFPS from soil cores to a depth of 10 cm missed anaerobic microsites in the top few cm of soil.

Further evidence, however, for precipitation influencing the difference in NO emissions between the 2 years is provided by the N_2O flux. The cumulative N_2O emission from the NPK treatment in 1998 was more than 2.5 times that in 1999 (Figure 4.13). The application of NPK was the only treatment which involved the immediate addition of substantial amounts of the denitrification substrate of NO_3^--N

(Figures 4.6 and 4.15). In contrast, denitrification and subsequent N_2O production in the organic treatments largely depends on the production of NO_3^--N following nitrification of the applied NH_4^+-N. If increased precipitation in 1998 reduced nitrification the generation of NO_3^--N would be similarly curtailed and N_2O emissions would be smaller than in 1999. Indeed N_2O emissions from the three organic treatments were *ca.* 3-25 times larger in 1999 than in 1998. In a study to investigate rates of denitrification following the application of slurry to grassland, Thompson & Pain (1989) reported little denitrification in a poorly drained soil. This was attributed to a lack of NO_3^--N substrate, as a consequence of reduced aeration preventing nitrification of the slurry NH_4^+-N.

It is possible that a contribution to the increased emissions in 1999 was due to release of mineral N from mineralisation of material added in the previous season and/or April application, although this could not account for the increase in emissions from the inorganic treatments. Decomposition and mineralisation of manures has been shown to persist in subsequent years, such that 3-10% of the organic N applied in one season is taken up by the crop in the next (Whitehead, 1995).

4.5.5 Factors controlling NO emission

In 1998 no individual soil parameters were shown to affect the NO emission. Soil NH_4^+-N, however, could account for 45% of the variation in the relatively high NO fluxes measured in 1999 and a combination of soil NH_4^+-N and WFPS could explain 50% of the emission data. Nonetheless, in each year individual treatments appeared to be influenced to varying degrees by different soil variables, which were further affected by the year of study.

In 1998 the magnitude in the emission of NO from the CS treatment increased with the level of NH_4^+-N. For the SSP treatment in 1998 the impact of soil moisture on NO loss was apparent. The emission of NO was shown to rise with a lower WFPS. For the NPK treatment it is clear from the data collected that the emission of NO was influenced by different soil variables in the 2 years. In 1999, the loss of NO appeared to be most strongly affected by the soil NH_4^+-N and NO_3^--N levels, such that emissions were stimulated as the mineral N concentration rose.

However, in the low NO emission year of 1998, the importance of mineral N was not detected, but an increase in the cumulative rainfall during the 3 d before each measurement occasion resulted in lower NO emissions.

For the SR treatment a correlation between soil NO_3^--N and NO emission was demonstrated in 1998, such that the larger NO emissions were associated with the highest soil NO_3^--N concentrations. An influence of temperature on the generation of NO may have been expected, because the release of mineral N from slow release fertilisers are principally triggered by warmth, however no such relationship was found. In 1998 losses of NO from the surface applied SSP and NPK treatments were, however, enhanced as the temperature of the soil surface increased.

For the control, the only measured variable that seemed to have an effect on the emission of NO was the soil NO_3^--N concentration in 1999. The importance of WFPS on the emission of NO was demonstrated, since in a combination with soil NO_3^--N, 84% of the variation in the NO data from the control could be accounted for.

During 1998 when NO emissions were low (≤ 4 μg NO-N m^{-2} h^{-1}) compared to 1999, the effect of temperature on NO emission was shown on two of the surface placed fertilisers, NPK and SSP. Furthermore, WFPS and cumulative precipitation before sampling were able to account for some of the variation in the data from these treatments. The strong control of mineral N over the magnitude of NO emission when both soil mineral N and NO emissions are high may mask the role of variables such as temperature e.g. in 1999 when both the soil mineral N concentration and NO emissions from the NPK treatment were high (peak of *ca.* 30 μg N g^{-1} dry soil and 15 μg NO-N m^{-2} h^{-1} respectively) there was a strong relationship between NO emission and soil mineral N, but in 1998 with lower fluxes and lower mineral N concentration (peak of *ca.* 15 μg N g^{-1} dry soil) such an association was not evident.

The effect that different measured soil parameters had on the emission of NO from each treatment, particularly immediately following fertilisation, is illustrated in the fact that in 1998 a common influence across all treatments was not evident until 39-55 days after fertilisation when fluxes were < 2 μg NO-N m^{-2}. On one separate measurement occasion soil NH_4^+-N was able to explain 67% of the variation in NO emission data. The importance of soil NH_4^+-N was, however, demonstrated on a sampling occasion immediately after fertilisation when 96% of the variation in the

NO data could be explained, although emissions were not monitored from each treatment.

The results from both years demonstrated that no one soil or environmental variable was strongly influential on NO emission from all treatments and in both years. Several relationships between NO emission and soil variables were shown, but were strongly influenced by 1 or 2 points and were therefore discarded. It is possible that over a larger and more comprehensive data range significant relationships may be shown. It is also likely that other environmental parameters and interactions not measured must be significant in influencing the magnitude of emissions e.g. soil carbon, plant cover, mineralisation rate etc.

4.5.6 Source of NO emission

At this grassland site with a mean soil pH of 6.7, it is unlikely that conditions in the bulk soil were acidic enough to support a large population of heterotrophic nitrifiers or to stimulate chemodenitrification at a significant rate (Killham, 1986; Skiba *et al.* 1997). The emission of NO, therefore, is likely to arise predominantly from microbial autotrophic nitrification and/or anaerobic denitrification.

In this study, without the use of labelled fertiliser components or use of nitrification inhibitors, it was not feasible to differentiate between the effect of the individual components of the fertiliser (i.e. NH_4^+ and NO_3^-) on NO emission and therefore to indicate whether the emission source was from nitrification or denitrification. Nevertheless, numerous researchers have interpreted statistically significant correlations between soil variables and NO emissions to suggest a possible source process (Anderson & Levine, 1987; Skiba *et al.* 1992).

Larger N_2O emissions from organic treatments compared to inorganic treatments have been used in several studies to imply that N_2O production was primarily from denitrification (Christensen 1983; Maag, 1989; Ellis *et al.* 1998). The argument suggests that a factor present in the organic manures, which is not in synthetic fertilisers is responsible for the higher fluxes. The presence of carbonaceous material in the manures is the often-cited parameter, as it is known to stimulate denitrification directly and also indirectly by enhancing anaerobic conditions. It would follow therefore, that the higher NO emissions recorded from

the organic waste treatments at this site are produced from denitrification. However, there is little evidence to substantiate this theory and furthermore the nature of organic wastes can be used to indicate NO production from nitrification. The larger NO emissions measured from the organic treatments compared to those from the inorganic treatments may potentially be the result of NH_4^+-N produced as the result of mineralisation of the added organic N. Synthetic fertilisers do not contain organic N and, therefore, any substrate for nitrification must be provided by the readily available mineral N.

The significant positive relationship established between NO emission and soil NH_4^+-N in the injected CS treatment suggests the use of NH_4^+-N as a substrate in microbial nitrification. Additionally in 1998, the soil NH_4^+-N concentrations recorded from both of the injected organic fertilisers of CS and SSL, approximately halved from the 1st July to the 15th July and over the same period the soil NO_3^--N concentrations roughly doubled. This simultaneous decrease of NH_4^+-N and increase of NO_3^--N suggests that the nitrification process was proceeding and therefore NO production from this source was likely.

Despite the lack of any statistically significant correlations between mineral N and NO emission from the SSP treatment, the inverse relationships between WFPS and NO emission suggests that nitrification may have been the source of NO. Although both nitrification and denitrification rates are stimulated with an increase in temperature, the positive relationship between soil surface temperature and NO loss may reflect a potential for increased evaporation of moisture from the soil surface. This loss in soil moisture and concurrent improvement in the aeration status of the soil would tend to favour nitrification rather than denitrification.

The relationships observed between NO emissions from the inorganic NPK treatment and with the soil variables also suggests that the production of NO was from nitrification. Losses of NO increased with soil surface temperature as well as with lower cumulative rainfall values. Even though the higher measured NO emissions were associated with larger concentrations of both soil NH_4^+-N and NO_3^--N, it is doubtful that the relationship with NO_3^--N signified NO generation from denitrification. An equal quantity of NH_4^+-N and NO_3^--N was added to the soil with the application of NPK and therefore, an initially large NO emission measured at the

same time as a high NO_3^--N concentration may purely be a coincidence and as suggested the high NO losses were a consequence of nitrification of the added NH_4^+-N. If NO was primarily evolved from denitrification, then one would expect to see the largest measured emissions from the treatment with the highest concentration of NO_3^--N i.e. from the NPK treatment, which did not occur at this site.

At this ungrazed grassland site the data suggests that nitrification may have been the source process for the production of NO released to the atmosphere. Similarly, Skiba *et al.* (1992) indicated that a negative response of NO emission to increased precipitation from the same soil series at a nearby site (NT 239629), was because nitrification was the primary production process. Nonetheless, over the range of water contents commonly present in agricultural soils, both nitrification and denitrification may proceed simultaneously (Linn & Doran, 1984). The relative significance of each process is principally determined by the O_2 concentration in the soil, which in turn is heavily affected by the soil water content. Hence the percentage of WFPS may be a valuable gauge of the potential for aerobic and anaerobic microbial activity in the soil (Linn & Doran, 1984).

Across both field seasons, the WFPS ranged from *ca.* 40-90% and thus traversed the estimated transition point of 60 % between processes which predominantly operate anaerobically and aerobically (Davidson, 1993). A WFPS of $\leq$ 60 % is generally thought to predominantly release NO from autotrophic nitrification (Schuster & Conrad, 1992; Hutchinson *et al.* 1993b; Williams *et al.* 1992), whereas the emission of NO from a WFPS of > 60% is primarily expected to be produced from the denitrification process (Schuster & Conrad, 1992; Hutchinson *et al.* 1993b; Williams *et al.* 1992). This information would, therefore, suggest that the production of NO may have been from both nitrification and denitrification, although limited nitrification material still operate at a WFPS > 60% (Davidson, 1993). However, there have been several reports of nitrification proceeding at much higher moisture contents than 60% WFPS. For example, Ellis *et al.* (1998) indicated that vigorous nitrification had occurred at the soil-slurry interface in a silty clay loam, grassland soil, where the WFPS ranged from 70-81%. In a laboratory study, Clemens & Huschka, (2001) reported that nitrification was not limited by soil

moisture in a loamy sand, such that nitrification was demonstrated at a WFPS of 71%.

The relative emission of NO-N and N_2O-N may be used to differentiate between nitrification and denitrification as the source of gaseous production (Williams *et al.* 1998). The concept relies upon the assumption that NO is essentially produced during the autotrophic nitrification process and in substantially larger quantities than N_2O (Davidson & Schimel, 1995; Williams *et al.* 1998). In contrast, the generation of N_2O is principally associated with denitrification (Williams *et al.* 1998), whereas NO is typically not regarded as a key product of denitrification (Firestone & Davidson, 1989).

In 1998 and 1999 there were only one and 5 measurement occasions when both NO and N_2O samples were taken on the same day, albeit at different times of the day and from different gas chambers, but of the same dimensions (Tables 4.15 and 4.16). The NO:N_2O ratios should, therefore, be considered with care.

Table 4.15 NO:N_2O ratios for losses measured from ungrazed grassland in 1998. The sampling date in parentheses represents the date when N_2O was measured.

Treatment	Sampling Date					
	15/07 (16/07)	24/07 (24/07)	06/08 (10/08)	25/08 (24/08)	03/10 (05/10)	20/10 (19/10)
NPK	-	0.003	0.022	0.114	0.065	-0.112
SR	-	0.004	0.010	0.029	0.034	0.276
SSL	0.100	0.125	0.078	-0.125	0.035	0.049
SSP	-	0.026	0.094	0.114	0.092	0.391
CS	0.018	0.187	0.115	0.624	0.025	-0.084
CON	-0.002	0.120	0.034	-0.010	0.018	-0.213

In both years, the NO:N_2O ratios were consistently < 1. A dominant production process of nitrification has been estimated to yield an NO:N_2O ratio > 1 (Anderson & Levine, 1986) and, therefore, these results suggest that nitrification was not the primary production process. In contrast, denitrification has been implied as

the dominant production process if the NO:N_2O ratio is *ca.* 0.01 (Anderson & Levine, 1987; Bouwman, 1990; Williams *et al.* 1988). Although some NO:N_2O ratios were equal to or less than that reported for denitrification, the majority of ratios derived from the emissions measured at this site lie within the range 0.01-1.00, neither large enough to be principally from nitrification nor small enough to be predominantly from denitrification. It is suggested, therefore, that both nitrification and denitrification are operating simultaneously and in particular the production of N_2O is largely from denitrification, whilst nitrification may contribute to the production of NO. Certainly McTaggart *et al.* (1999) infer that the generation of N_2O used in the above NO:N_2O ratios was generated from the denitrification process. However, in order to be able to conclusively determine the source process for the production of NO and N_2O, studies would need to be carried out using inhibitors e.g. C_2H_2, nitrapyrin etc.

Table 4.16 NO:N_2O ratios for losses measured from ungrazed grassland in 1999. The sampling date in parentheses represents the date when N_2O was measured.

Sampling Date	Treatment					
	NPK	SR	SSL	SSP	CS	CON
26/04 (27/04)	-	-	-	-	0.25	0.03
29/04 (29/04)	-	-	0.22	0.30	0.39	-
07/05 (07/05)	0.18	-0.02	0.05	0.07	0.01	-0.13
18/05 (25/05)	-0.03	-0.07	0.66	0.98	0.02	-0.15
15/06 (08/06)	0.02	0.01	0.05	0.01	0.01	-0.12
06/07 (06/07)	-	-	-	-	0.06	-0.18
08/07 (08/07)	-	0.001	0.05	0.01	-	0.01
15/07 (16/07)	0.17	-0.11	0.04	0.27	0.03	-0.50
22/07 (22/07)	0.003	-0.02	0.002	0.02	0.02	0.04
2/09 (24/08)	0.27	0.50	0.83	0.58	0.24	0.60
07/10 (05/10)	-0.37	-0.07	-0.30	-0.37	-0.23	-0.84

4.6 Conclusion

- In the first year of this study we have discovered that there was no statistically significant difference in the NO emission from the fertilised and unfertilised treatments. Cumulative NO emissions from the inorganic fertilisers were 4 times larger than from the control and fluxes from the organic fertilisers were 6-13 times larger than from the control with the largest loss from the sewage sludge pellets.
- In the second year of the study we have shown that the application of slow release fertiliser significantly reduces NO emission over NPK mineral fertiliser by 75% and over the organic fertilisers by 90%. Emissions of NO from slow release fertilisers were not significantly different to those from the unfertilised treatment. There were no significant differences in the NO emitted from the 3 organic treatments, although the largest NO fluxes were emitted from the plots amended with thermally dried sewage sludge pellets. Cumulative NO fluxes were 1.3 - 42.3 times larger from these plots than from all other treatments.
- In the second year of the study we have reported that the application of NPK fertiliser significantly reduces NO emission over sewage sludge pellets by 65%. This difference in NO emissions shown between the 2 surface placed fertilisers reflects their composition. Sewage sludge pellets slowly release mineral N into the more aerated soil surface, whereas following rain the highly soluble NPK fertiliser will be quickly washed further into the soil matrix increasing the diffusive path length for and increasing the risk of NO consumption before emission.
- In the first year of the study the low dry matter content (0.4%) and low application rate (16 kg N ha^{-1}) of the liquid sewage sludge affected the percentage of total ammoniacal N and total N applied lost as NO, although there was no significant difference between this and other treatments.
- In the 2^{nd} year of the study there was no difference in the percentage of total ammoniacal N and total N applied lost as NO between treatments and from the April and July application dates.

- In both 1998 and 1999 the amount of total applied N lost as NO over the 111 d and 165 d period, respectively, following fertilisation was at, 0.0004-0.03 %, 1-2 orders of magnitude smaller than previously reported. The cool, wet climate and clay loam soil at this site is likely to have reduced losses by increasing denitrification and the consumption of NO.
- The different patterns of NO emission between the two field seasons and throughout the growing season appear to be influenced by the nature of the fertiliser, the timing of rainfall (particularly around fertiliser application), the soil WFPS and the concentration of soil mineral N. Precipitation and an increase in WFPS have been suggested as factors responsible for reductions in NO emissions, due to an increase in anaerobicity of the soil and stimulation of denitrification.
- In the first year of study when fluxes were less than 5 μg NO-N m^{-2} h^{-1} there was no one variable which was shown to give a significant relationship with NO emissions across all treatments. For individual treatments significant correlations were shown between NO emission and soil ammonium or soil nitrate or soil surface temperature or soil WFPS or soil moisture in the form of cumulative precipitation over 3 days prior to sampling.
- In the second year of the study when the peak NO fluxes were 6-15 times larger, only soil mineral N was demonstrated to show a significant relationship with NO emission. The importance of other variables on the magnitude of NO emissions appears to be masked by the more dominant factors of mineral N.
- The evidence suggests that NO emissions were produced during nitrification, although denitrification is also likely to have contributed, however in order to fully determine the production source, studies using inhibitors would need to be undertaken.
- A suitable abatement strategy to reduce NO emission from grassland soil would seem to favour the use of inorganic fertilisers, rather than organic wastes. The use of synthetic slow release fertilisers, in particular does not significantly increase emissions above background levels and with no apparent loss in grass yield, although economically their use is undesirable. With a wish for ecologically sound management of sewage sludge and the potential rise in the amount applied

to land, combined with a shift in UK agricultural policy to a more organic, sustainable regime, losses of NO may increase, particularly as nitrogen applied in organic wastes is likely to be less efficiently utilised than inorganic fertilisers due to the difficulties in synchronising crop demand to fertiliser application.

- The significance and environmental impact of the loss of NO from a grassland system amended with organic and inorganic fertilisers and potential abatement strategies should not be considered in isolation, but should be investigated in relation to N losses from soil including N_2O, N_2, NH_3, NO_3^- and crop offtake.

Chapter 5 – Laboratory experiments

5.1 Introduction

Soil moisture and nitrogen are major factors that strongly affect the production and release of both NO and N_2O gas from soil. Although the emissions of both trace gases have been routinely and successfully measured in field situations (Anderson & Levine, 1987; Hutchinson & Brams, 1992; Skiba *et al.* 1992; Yamulki *et al.* 1995; Thornton & Valente, 1996), such studies provide only limited knowledge on the influence of these two individual variables on emissions, because they can often be masked by the effects of others e.g. temperature.

Laboratory studies provide the ideal controlled environment to isolate and examine the effect of individual parameters, whilst keeping potential interfering variables constant. Many laboratory studies have been carried out using a known quantity of sieved, homogenous soil (e.g. Chae & Tabatabai, 1986; Klemedtsson *et al.* 1988a; Tortoso & Hutchinson, 1990; Maag & Vinther, 1996; Gödde & Conrad, 1998; Gödde & Conrad, 1999), however the results generated by this method may not necessarily relate to the structured bulk soil found in the field. The use of undisturbed soil cores of various dimensions (e.g. Parkin, 1987; Papen *et al.* 1993; Sierra, 1997; Williams *et al.* 1998) extracted directly from the field helps to retain soil conditions as close as possible to those in the field. Although intact soil cores reduces disturbance and therefore perturbation to the microbial community, they also introduce a large degree of variability in both the physical and biological factors involved in NO and N_2O trace gas production and surface emission.

The use of repacked soil cores using coarsely sieved soil permit the establishment of relatively similar conditions within each core and to a certain degree remove the inherent variability found in field conditions, which may hamper the ability to adequately explain variation in the measured emission data.

The aim of these experiments was to examine the individual effects of water filled pore space (WFPS) and nitrogen fertilisation on emissions of NO and N_2O from repacked soil cores under controlled conditions which may be related to field conditions.

5.2 Materials and Methods

5.2.1 Soil collection and soil core production

For each experiment, following collection, the soil was air dried on trays, passed through a sieve (5 mm mesh) and then stored at 4 °C in air tight, polythene bags until use. A mesh size of 5 mm was used in order to obtain a fairly homogenous soil sample, yet to avoid the large scale destruction of soil aggregates and the subsequent alteration of soil physical, chemical and biological characteristics.

Prior to core manufacture, a standard weight of air dry soil was wetted to the required final moisture content by the following method similar to that used by Tortoso & Hutchinson (1990). In order to minimise the risk of further aggregate damage, the soil was misted with deionised water from a hand-operated pump spray bottle while thoroughly mixing the soil and water on a balance covered with a plastic bag. Each repacked core was then formed from the addition of three approximately equal increments of the water-adjusted soil in a plastic cylinder (diameter of 5.5 cm) to achieve a target height of 10 cm and a dry bulk density of 1.2 g cm^{-3}. The bottom of the core was sealed with a plastic lid fitted with an airtight rubber seal.

Large pulses of both NO and N_2O have been measured following drying and the subsequent rewetting of soils (Davidson, 1992), therefore the soil cores were pre-incubated for 1 week before the start of the experiment. This time period was assumed to be sufficiently long enough to avoid the pulse of NO and N_2O emissions, since in previous laboratory experiments a period of 5 to 7 days was found to be adequate (Gödde & Conrad, 1998; Gödde & Conrad, 1999; Clemens & Huschka, 2001).

The experimental set up that was used was described in chapter 2 and is shown in Plate 5.1 and Figure 5.1. Each experiment was conducted within a controlled temperature room. All cores were positioned on the work bench in rows and columns as part of a fully randomised block design to take out any possible effects of bias as a result of water loss from the cores due to the fan unit. Each treatment was replicated three times. The column caps used during gas sampling were placed loosely over the top of the columns so as to allow gas exchange, but to minimise moisture loss.

During each experiment, the water content of the soil cores was controlled gravimetrically. On a daily basis (following gaseous measurements on a sampling day) additional water was added to the soils if necessary and any seedlings were gently removed with tweezers.

Temperature was logged (Campbell Scientific 21x) at 10 sec intervals from duplicate soil cores using thermocouples inserted through specially designed plastic columns at 3 depths, 0 cm (i.e. the soil surface), 2 cm and 5 cm.

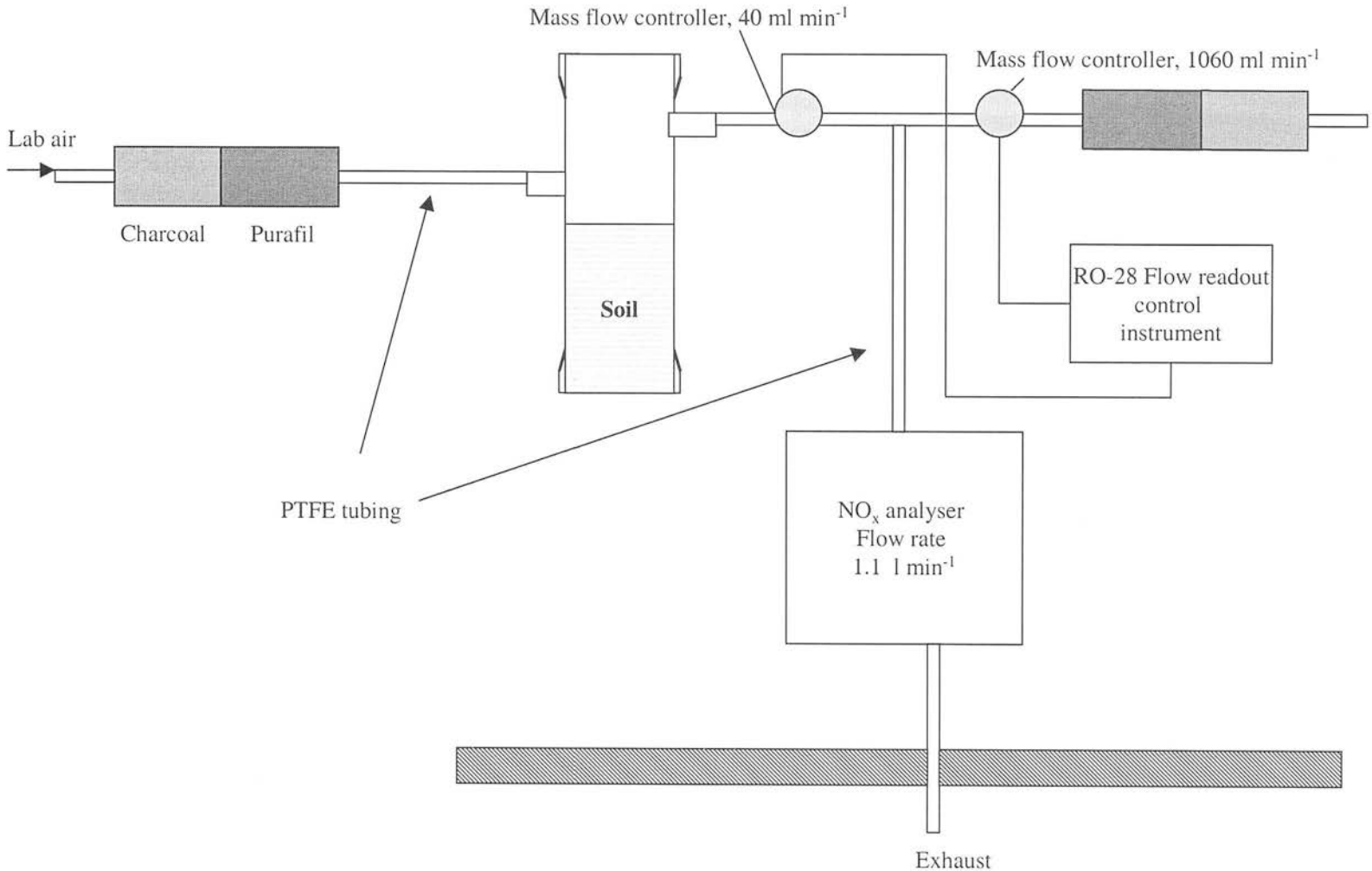

Figure 5.1 Flow through system used to measure NO flux from repacked soil cores

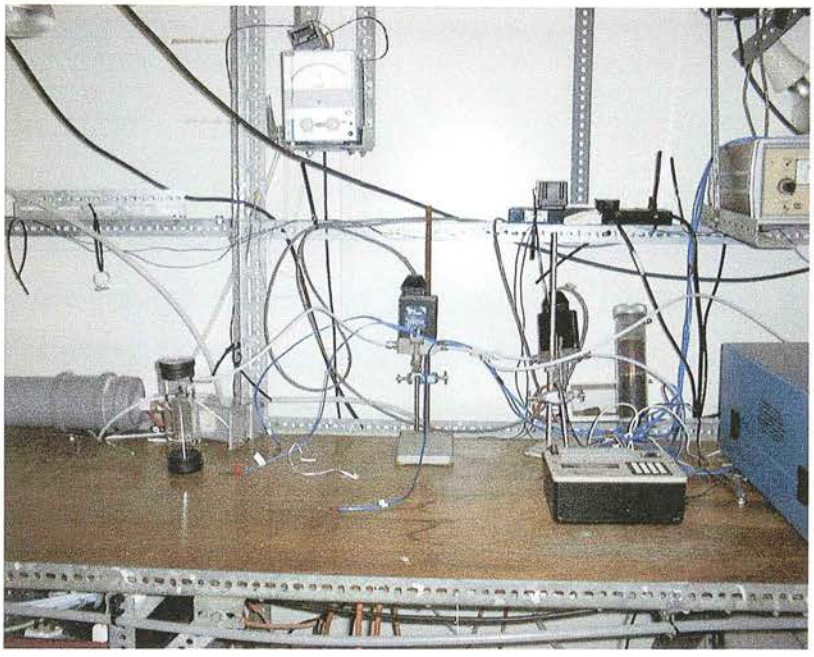

Plate 5.1 Laboratory experimental set up showing the flow-through system

5.3 Organic and inorganic fertilisers experiment

5.3.1 Introduction

The results of the field experiment at Cowpark to examine the loss of NO from ungrazed pasture amended with organic or inorganic fertilisers demonstrated that the source process of NO was probably from nitrification. Relationships established between NO emission and soil mineral N levels formed the basis of the evidence. However in the field other environmental variables e.g. temperature and WFPS, can strongly affect the flux magnitude with respect to their spatial and temporal variability. The aim of this experiment was, therefore, to provide an environment that was able to focus on the effect of mineral N addition, essentially by removing the variables of soil WFPS, precipitation, temperature and plant activity. It was hoped that with a more intense sampling regime and the virtually simultaneous measurement of N_2O emission together with NO, that the source process of NO at the Cowpark field site would be clarified. Additionally, it was anticipated that the isolation of mineral N as a controlling factor would alter the NO emission pattern to that seen in the field and would therefore highlight the significance of other variables in determining the magnitude of the flux.

5.3.2 Sites and treatments

The soil used in this study was collected from the top 10 cm beneath the vegetation of an ungrazed grassland located at the Cowpark (CP) experimental site, Bush Estate, *ca.* 15 km south of Edinburgh (NT238862). For a more detailed history of the field see chapter 4.

All of the soil cores were adjusted to a WFPS of 60% and incubated at a temperature of 17° ± 0.5°C, which corresponded to the mean WFPS and mean soil surface temperature calculated from Cowpark during the 2 field seasons of 1998 and 1999 (Chapter 4).

The cores were either amended with ammonium nitrate mineral (21-8-11 NPK) fertiliser (NPK), Ficote 70® (14-14-14 NPK) slow-release mineral fertiliser (SR), liquid anaerobically digested sewage sludge (SSL), thermally dried sewage sludge pellets (SSP), dairy cattle slurry (CS) all sourced as in Chapter 3 or received

no nitrogen fertiliser (CON). The cattle slurry and liquid sewage sludge were both added to the core in a pre-made slit with comparable dimensions to the injection slot used in the Cowpark field trial i.e. 70 mm deep and 20 mm wide. In contrast, both of the mineral fertilisers and the sewage sludge pellets were spread on the soil core surface.

The cores received one fertiliser application at a target rate of 120 kg available N ha^{-1} (the same as that used at the Cowpark site) one hour before the first gas sampling occasion. Immediately following fertiliser addition, all soil cores received deionised water by pipette equal to 2 mm of precipitation, the mean rainfall measured in the April and July field fertiliser application months of 1999.

Emissions of both NO and N_2O were measured on day -3, 0, 2, 4, 7, 10, 14 and 31. Available soil NH_4^+-N, NO_3^--N and moisture content were determined on 1 soil core per treatment, which were removed for destructive sampling on day 0, 2, 7, 14 and 31. After the final gas measurements on day 31 the cores themselves were used for the destructive soil analysis determinations. Consequently, the results were calculated from 3 cores per treatment. The layout of the soil cores is shown by treatment below in Figure 5.2

A	SSL	CS	NPK	SR	CON	SSP
C	CON	SSL	NPK	CS	SR	SSP
3	SSP	NPK	SR	CS	SSL	CON
2	NPK	CS	SSL	CON	SSP	SR
B	CON	NPK	CS	SSL	SSP	SR
1	SSP	CS	NPK	SSL	SR	CON
4	CON	SSP	SSL	CS	SR	NPK

Figure 5.2 A plan view of the soil core layout as positioned in the controlled temperature room. Rows A, B and C represent the 3 treatment blocks used for gas sampling and rows 1, 2, 3 and 4 represent the cores periodically removed for destructive soil analysis.

5.3.3 Results

5.3.3.1. Fertiliser properties

The organic fertiliser application rates were calculated prior to the manure composition analyses and were based on standard available nitrogen figures (Dyson, 1992; Aitken, 1997).

Table 5.1 Composition of both organic wastes and inorganic fertilisers applied to repacked soil columns

Treatment	Dry matter (%)	Total carbon (kg t^{-1})	pH	Total N applied (kg ha^{-1})	TAN applied (kg ha^{-1})
SSL	4.1	14.0	7.06	196	42
SSP	97.4	296.0	7.08	513	111
CS	4.2	18.8	7.05	192	90
NPK	-	-	-	120	60
SR	-	-	-	120	60

Table 5.1 summarises the composition and actual application rates of both the organic and inorganic fertilisers applied to the soil cores. The total nitrogen applied in the SSP treatment was more than twice that applied in the other two organic manures. The low quantity of TAN added in the SSL was much less than the target application rate of 120 kg ha^{-1} of available nitrogen. The TAN content of the SSP and CS was slightly lower than the target available N rate, although over a growing season in a field this may have been achieved through mineralisation of some of the organic nitrogen added. The contribution to the available N content would have been negligible from NO_3^--N in these organic slurries.

5.3.3.2 Gaseous Emissions

The measured NO fluxes emitted from the fertilised soil cores are shown in Figure 5.3 and varied from 2.0 to 93 μg NO-N m^{-2} h^{-1} for the fertilised treatments and from 1.1 to 18.5 μg NO-N m^{-2} h^{-1} for the unfertilised, CON treatment.

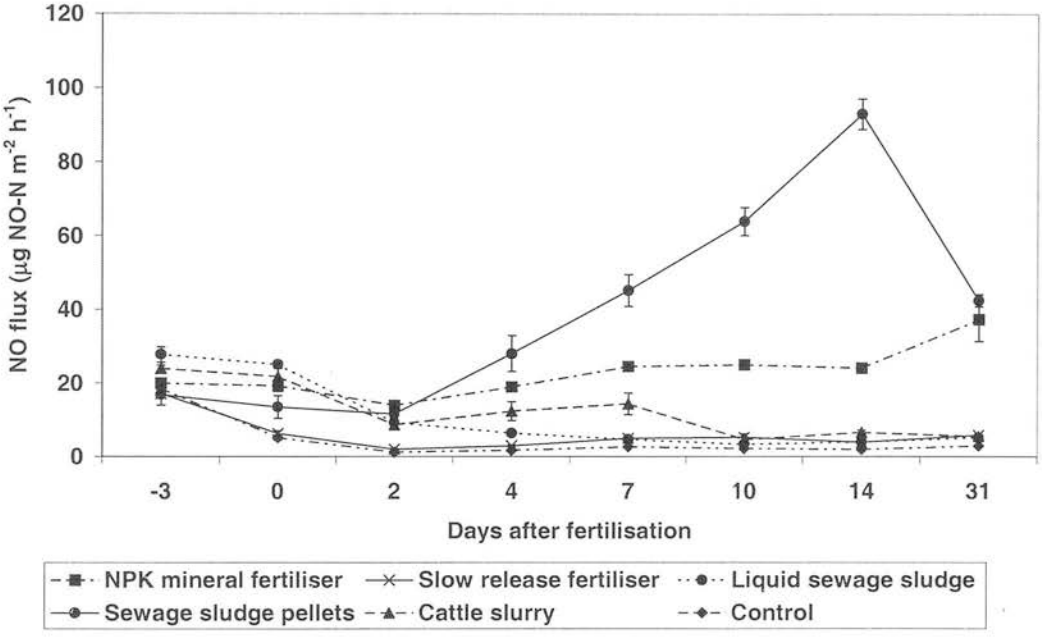

Figure 5.3 Mean NO flux measured from repacked soil cores amended with organic wastes and inorganic fertilisers. Error bars represent ± one standard error of the mean (N=3).

There was a significant ($P < 0.001$) effect of time on the emission of NO from the soil cores, but a significant interaction ($P < 0.001$) between time and treatment meant that the effect of time was different for the various treatments (Table 5.2). Following fertilisation the largest NO emissions measured were observed 1 hour later from 4 of the 6 treatments. The surface-placed SSP and NPK treatments showed maximum losses 14 and 31 or more days after fertilisation respectively. Emissions from the NPK treatment were relatively consistent throughout the study period and never fell below 13 μg NO-N m^{-2} h^{-1}.

Similar to 3 other treatments (NPK, SR and CON), the SSP demonstrated emission minima on the second measurement occasion only 2 days after fertilisation. Subsequently emissions steadily increased from the SSP treatment until peaking at

day 14 (14-31), whereas NO losses from SR and CON marginally rose and then fluctuated below 6 μg NO-N m^{-2} h^{-1}. Both of the liquid organic wastes declined from their initial peak until an emission minima was measured 10 days after fertilisation, although fluxes subsequently remained below 7 μg NO-N m^{-2} h^{-1}.

Table 5.2 Table of mean values (treatment and time) produced as a result of repeated measures ANOVA of NO flux (μg NO-N m^{-2} h^{-1}) and N_2O flux (μg N_2O-N m^{-2} h^{-1}) from repacked soil cores.

Flux type	Treatment						Degrees of freedom	Standard error of differences of means
	SR	CS	SSL	CON	NPK	SSP		
NO	6.0	12.2	10.7	4.5	22.8	39.3	10	3.3
ln N_2O	3.9	4.7	4.9	4.4	4.2	5.1	10	0.23

Flux type	Time - Days after fertilisation								Degrees of freedom	Standard error of differences of means
	-3	0	2	4	7	10	14	31		
NO	20.6	15.1	7.7	11.7	16.0	17.4	22.3	16.5	47	1.8
ln N_2O	5.1	5.1	5.1	5.2	4.5	4.0	3.8	3.3	83	0.2

The levels of NO measured 3 days before fertilisation indicated elevated emissions compared to those from the CON treatment measured following fertiliser application. Emissions of NO from the SR and CON treatments were larger before fertilisation than measured 1 hour afterwards, whereas N_2O emissions from the SR and SSP treatments were larger before fertilisation than measured 1 hour afterwards (Figure 5.4).

The N_2O fluxes ranged from 11 to 850 μg N_2O-N m^{-2} h^{-1} for the fertilised treatments and from 4 to 480 μg N_2O-N m^{-2} h^{-1} for the unfertilised, CON treatment.

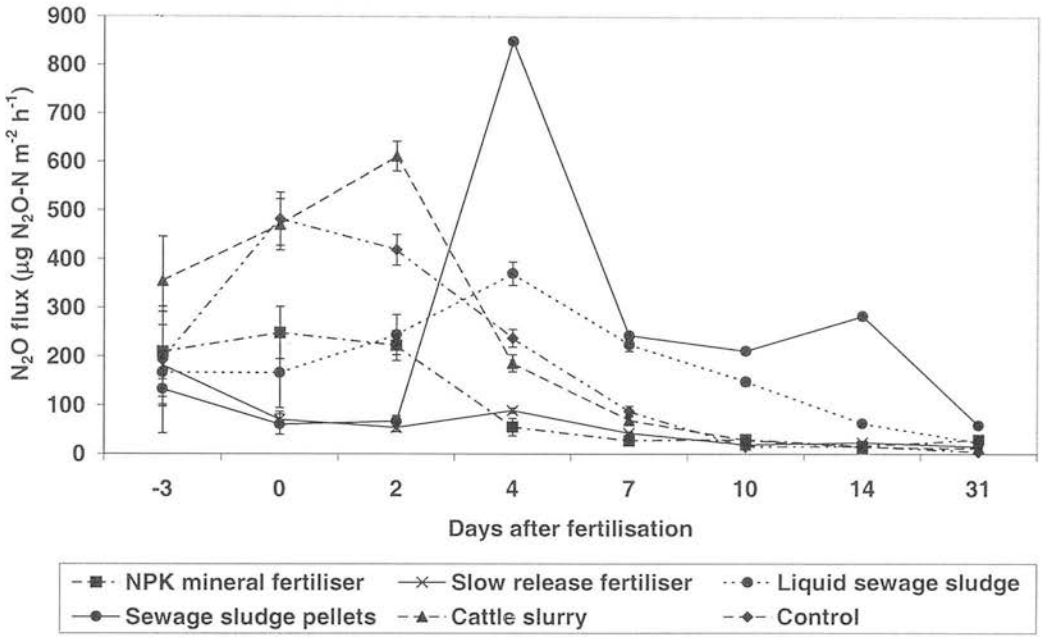

Figure 5.4 Mean N_2O flux measured from repacked soil cores amended with organic wastes and inorganic fertilisers. Error bars represent ± one standard error of the mean (N=3).

There was a significant ($P < 0.001$) effect of time on the emission of N_2O from the soil cores, but a significant interaction ($P < 0.01$) between time and treatment meant that the effect of time was different for the various treatments (Table 5.2). All but the NPK treatment showed the lowest fluxes on the last measuring date 31 days after fertilisation. Together with the CON treatment, the emissions from the NPK treatment peaked 1 hour following fertilisation and then gradually declined to the emission minima. Maximum losses of N_2O from both of the sewage sludge treatments and SR were observed 4 days after fertilisation. Similarly to the CS treatment, where the N_2O losses reached a maximum 2 days after fertilisation, the emissions from SR progressively decreased until 31 days after fertilisation.

Measured NO and N_2O emissions were used to calculate potential cumulative losses over the 32 d period following fertilisation (Figure 5.5 and Table 5.4). Cumulative NO loss ranged from 107 – 1850 µg NO-N m^{-2}, with the lowest emission measured from the CON treatment and the highest observed from the SSP treatment (Figure 5.5). The cumulative emission from the fertilised treatments were 2 to 17 times the value from the CON treatment. There was a highly significant effect of

treatment on the emission of NO from the soil cores ($P < 0.001$, d.f = 10). Losses of NO were significantly higher from the SSP and NPK treatments (Tukey test, $P < 0.05$), and cumulative emissions from the SSP treatment were significantly larger than from the NPK treatment (Tukey test, $P < 0.05$). Despite higher mean values all other treatments did not statistically differ from the CON treatment (Tukey test, $P > 0.05$).

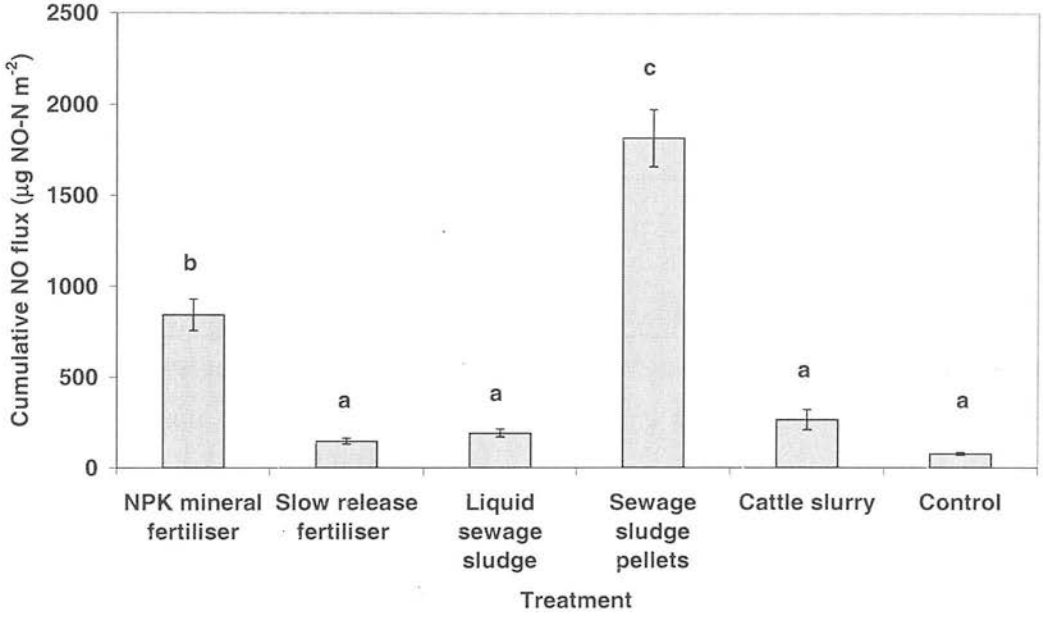

Figure 5.5 Cumulative NO emission over a 32 d period following fertilisation of repacked soil cores with and without added nitrogen fertiliser. Error bars represent one standard error of the mean. Means with the same letter code do not differ significantly (Tukey test $P > 0.05$).

There was a highly significant difference in both the percentage of total N ($P < 0.001$; sed = 0.0006; df = 8) and TAN ($P < 0.001$; sed = 0.0015; df = 8) lost as NO between the treatments. There was a significantly higher percentage of total N lost as NO from the NPK and SSP treatments (Table 5.3). This pattern was mimicked in losses of TAN, with comparable losses from the SSL, CS and SR treatments, but about an order of magnitude less than that lost from the SSP and NPK treatments (Table 5.3).

Table 5.3 The percentage of fertiliser nitrogen (total and TAN) lost as NO from the soil cores over the 32 d sampling period

Treatment	Total N applied lost as NO (%)	TAN applied lost as NO (%)
NPK	0.006	0.013
SR	0.001	0.001
SSL	0.001	0.003
SSP	0.003	0.016
CS	0.001	0.002
CON	-	-

Cumulative N_2O loss varied from 1030 – 7400 μg N_2O-N m^{-2}, with the highest emission measured from the SSP treatment and the smallest observed from the SR treatment (Table 5.4). Unusually, the CON treatment did not display the lowest cumulative emission, indeed, not only were the emissions from the SR treatment lower, but also from the NPK treatment. A significant effect of treatment on the emission of N_2O was observed ($P < 0.05$, d.f = 10). Losses of N_2O from the SSP treatment was significantly higher (Tukey test, $P < 0.05$) than from the SR and NPK treatments, although all other treatments did not statistically differ from each other (Tukey test, $P > 0.05$).

As a result of the very high N_2O emissions from the CON treatment, it was not possible to calculate the percentage of fertiliser nitrogen lost as N_2O from the SR and NPK treatments. Moreover, losses from the organic fertilisers were liable to be underestimated. The percentage of total nitrogen lost as N_2O from the organic manures varied between treatments in the order of SSP > SSL > CS, although there was no significant difference ($P > 0.05$) between the treatments (Table 5.4). Despite the larger amount of TAN lost as N_2O from the SSP and SSL treatments compared to the CS treatment, again there was no significant difference.

Table 5.4 Cumulative N_2O losses following the application of organic and inorganic fertilisers and the subsequent percentage of fertiliser nitrogen (total and TAN) lost as N_2O from the soil cores over the 32 d sampling period. Values in parentheses represent 1 standard error of the mean (N=3).

Treatment	Cumulative N_2O emission ($\mu g\ N_2O\text{-}N\ m^{-2}$)		Total N applied lost as N_2O (%)	TAN applied lost as N_2O (%)
	Total	Net		
NPK	2121.6 (489.4)	†	†	†
SR	1340.0 (312.6)	†	†	†
SSL	4904.3 (885.5)	1465.1	0.007	0.035
SSP	7615.4 (1610.8)	4176.1	0.008	0.038
CS	3938.1 (794.2)	498.9	0.005	0.006
CON	3439.2 (1110.9)	-	-	-

† Net treatment emission is negative, as total treatment emission is less than the CON total emission

The results from the analysis of the soil cores for WFPS, soil NO_3^--N and soil NH_4^+-N (except from the last sample occasion) are only from 1 core and, therefore, caution should be taken as the values shown may not be truly representative.

Figure 5.6 shows the variability of the WFPS between the soil cores, which were initially adjusted to a target WFPS of 60%.

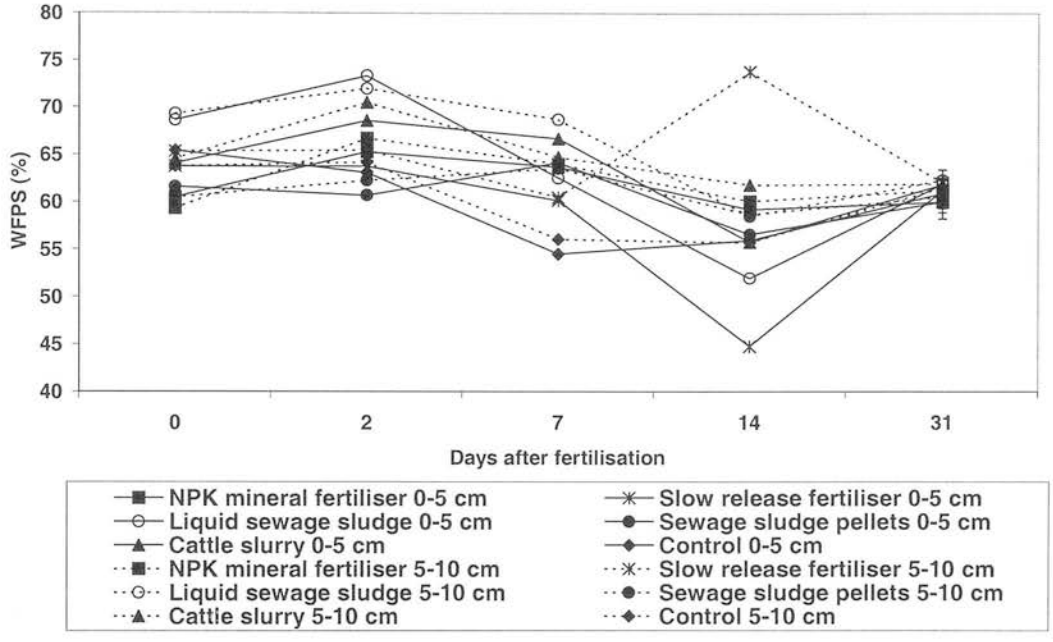

Figure 5.6 Mean WFPS measured at 2 depths (0-5 cm and 5-10 cm), in repacked soil cores amended with organic and inorganic fertilisers. Error bars 31 days after fertilisation represent ± 1 standard error of the mean (N=3).

For the non-liquid treatments, the WFPS values measured typically lay within the range of 55-65% WFPS. Following fertilisation the WFPS values obtained from the CS and SSL treatments increased up to 73%, as a result of the addition of extra liquid contained in the manures. In general, the difference in WFPS between the upper and lower 5 cm layers of the soil cores was less than the variability between core. The soil core from the SR treatment analysed 14 days after fertilisation indicated unusual behaviour, in that the WFPS of the upper layer was only *ca.* 45%, but that of the lower layer was *ca.* 74%. It is likely that within the soil core or indeed between the core and plastic column a route for the preferential flow of water from the upper to the lower soil layers was established.

Figure 5.7 demonstrates that throughout the experiment the soil NH_4^+-N concentration in the CON and SR treatments were consistently measured below 3.5 μg g dry $soil^{-1}$ and 4.5 μg g dry $soil^{-1}$ respectively. The addition of CS to the soil cores initially increased the NH_4^+-N concentration to *ca.* 40 μg g dry $soil^{-1}$ in both the top and bottom 5 cm of soil, although the NH_4^+-N levels diminished to

background levels 14 days after fertilisation. The NH_4^+-N concentration in the cores amended with SSL followed a similar pattern to the CS treatment, although, due to the smaller quantity of TAN added, levels in the top 5 cm were never more than 20 μg g dry soil^{-1}.

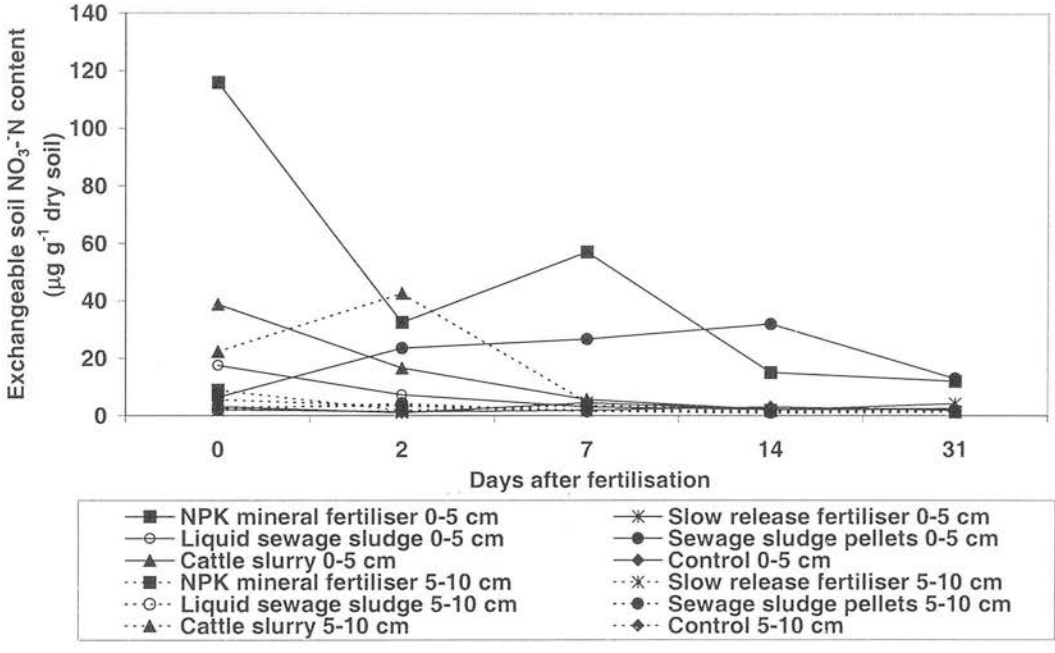

Figure 5.7 Soil NH_4^+-N measured at 2 depths (0-5 cm and 5-10 cm), in repacked soil cores amended with organic and inorganic fertilisers. Error bars 31 days after fertilisation represent ± 1 standard error of the mean (N=3).

The addition of NPK fertiliser resulted in elevated NH_4^+-N concentrations in the top 5 cm of soil to > 100 μg g dry soil^{-1} and although the levels gradually declined, values remained greatly higher than from the CON treatment even at 31 days after fertilisation. Despite the large rise in NH_4^+-N levels in the upper half of the soil cores, concentrations were always < 10 μg g dry soil^{-1} in the lower half of the soil cores. The surface application of SSP to the soil cores resulted in only a small (*ca.* 6 μg g dry soil^{-1}) rise in NH_4^+-N concentration 1 hour after fertilisation. A rapid increase in concentration subsequently followed to a peak of 32 μg g dry soil^{-1}, 14 days after fertilisation, which remained elevated even at 31 days after fertilisation. During the experimental period, the NH_4^+-N concentration in the bottom half of the SSP amended cores was comparable to levels in the CON treatment.

Throughout the experiment soil NO_3^--N concentrations from all treatments increased (Figure 5.8) with concentrations measured in the CON, SR, SSL, CS and SSP treatments rising to > 70 μg g dry soil^{-1}, > 90 μg g dry soil^{-1}, *ca.* 100 μg g dry soil^{-1}, > 120 μg g dry soil^{-1} and *ca.* 130 μg g dry soil^{-1} respectively, 31 days after fertilisation. Not only did soil NO_3^--N levels increase in the top soil layer, but they also increased in the lower layers, although not to such an extent. Soil NO_3^--N concentrations in the bottom 5 cm of the SSP treatment did not increase as much as the levels in the CS and SSL treatments.

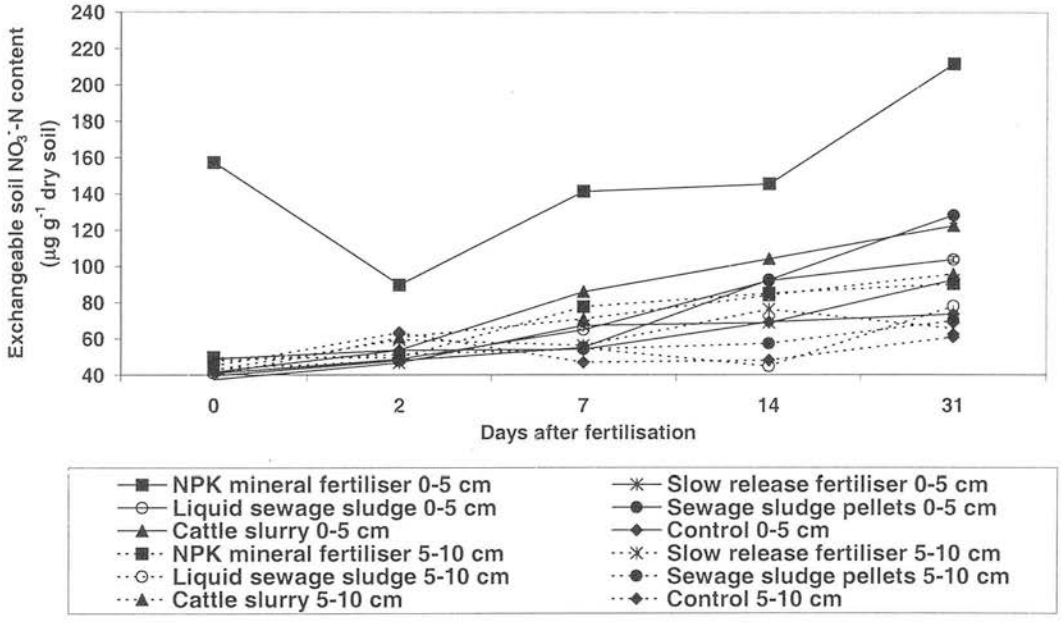

Figure 5.8 Soil NO_3^--N measured at 2 depths (0-5 cm and 5-10 cm), in repacked soil cores amended with organic and inorganic fertilisers. Error bars 31 days after fertilisation represent ± 1 standard error of the mean (N=3).

Concentrations of soil NO_3^--N rapidly increased in the upper half of the soil cores amended with NPK fertiliser to values > 150 μg g dry soil^{-1} only 1 hour after fertilisation. Concentrations were still highly elevated 31 days after fertilisation at > 200 μg g dry soil^{-1}. In contrast, the levels of soil NO_3^--N in the lower half of the core were comparable to those monitored in the CON treatment until 7 days after fertilisation when an increase was measured.

Comparison between fertiliser treatments

Repeated measures analysis of variance showed that there was a highly statistically significant effect of fertilisation treatment on the emission of NO ($P < 0.001$), with statistically higher emissions from the NPK and SSP treatments and no difference between emissions from the CON and SR treatments (Table 5.2). There was also a statistically significant effect of fertilisation treatment on the N_2O emission ($P < 0.01$) (Table 5.2). Losses of N_2O from the 2 sewage sludge treatments were significantly larger than from the inorganic fertilisers and the control despite the initially very high production of N_2O from the CON treatment. Emissions from the SSP treatment were significantly larger than those from the SSL treatment.

Table 5.5 Summary of ANOVA results (*P* values and associated level of significance) for soil NO_3^-, soil NH_4^+ and WFPS at 31 days after fertilisation

Data	Treatment	*P*-value	Significance level
Soil available NO_3^-	Fertiliser type	<0.001	***
	Depth	<0.001	***
	Fertiliser type x Depth	<0.001	***
Soil available NH_4^+	Fertiliser type	<0.001	***
	Depth	<0.001	***
	Fertiliser type x Depth	<0.001	***
WFPS	Fertiliser type	NS	-
	Depth	NS	-
	Fertiliser type x Depth	NS	-

*** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$; NS = not significant

The examination of replicate cores for soil NH_4^+-N and NO_3^--N concentration and WFPS on the last measurement occasion demonstrated a highly statistically significant ($P < 0.001$) influence of both the fertiliser treatment and soil depth on the mineral N concentrations (Table 5.5). As expected, since all cores were re-packed to a similar moisture content, there was no significant effect of either soil depth or

fertiliser treatment on the WFPS. Additionally, a significant interaction was observed between fertiliser treatment and soil depth, such that with the NPK and SSP treatments there was a greater impact of depth on both the soil NO_3^--N and NH_4^+-N concentration ($P < 0.001$) (Table 5.5). It was not possible to ascertain though, which individual treatments were statistically different from each other owing to the unavailability of appropriate statistical tables necessary for the Tukey multiple comparison test.

None of the soil parameters measured appeared to be able to account for any of the variation in the NO data across all treatments. Nevertheless, statistically significant relationships were established between NO emission and specific soil variables for some individual treatments. None of the variation in the NO data produced from either the SR, CON, CS or SSP treatments was explained by the measured variables of WFPS, soil NH_4^+-N or NO_3^--N. However, a significantly ($P < 0.05$) positive relationship between NO emission from the NPK treatment and soil NO_3^--N from the top 5 cm of the soil core was able to explain 84% of the variation in the data. A stepwise regression combining WFPS, soil NH_4^+-N and NO_3^--N was able to explain 99.9% of the variation in the NO data ($P < 0.01$) (NO = -92.5 + 1.28 WFPS - 0.0982 NH_4^-N + 0.291 NO_3^-N). The soil NH_4^+-N concentration in both the upper and lower layer of the soil core was able to account for virtually all the variation in the NO data from the SSL treatment at 99% ($P < 0.001$) and 94% ($P < 0.01$) respectively (Figure 5.9).

In contrast to the NO data, the measured soil variables were partially able to account for the pattern of N_2O emissions from all the treatments. A significant negative and positive relationship between ln (natural log) transformed N_2O emissions from all treatments and soil available NO_3^--N ($r^2 = 0.15$, $P < 0.05$) and WFPS ($r^2 = 0.24$, $P < 0.01$) from the top 5 cm of soil was observed, respectively. A combination of WFPS and soil NH_4^+-N from the bottom half of the soil cores was able to explain 51% of the variation in the ln transformed N_2O data ($P < 0.001$). Furthermore, the soil NO_3^--N concentration in the lower 5 cm of soil was able to explain a larger percentage of the data variation (36% [$P < 0.001$]) than the concentration in the top 5 cm.

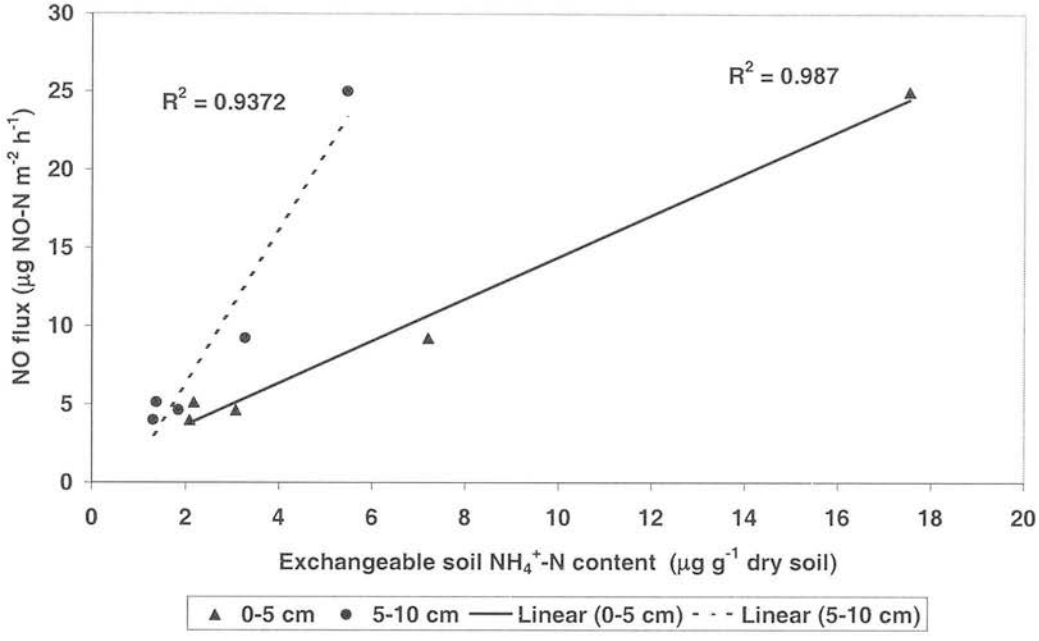

Figure 5.9 The relationship between mean NO emission and mean soil NH_4^+-N concentration measured from repacked soil cores amended with SSL fertiliser (N=3).

Soil NO_3^--N concentration from the top 5 cm of the soil cores was able to account for more of the variation in the ln transformed N_2O data from individual treatments than it could for the pattern of NO emission. The ln transformed N_2O emission from SR, SSL, CS and CON treatments all established a significantly ($P < 0.01$ to $P < 0.05$) inverse relationship with the soil NO_3^--N concentration in the upper soil core layer ($r^2 = 0.80$ to $r^2 = 0.96$). Moreover, this relationship was repeated in the lower soil core layer from the CS treatment.

The effect of the soil NH_4^+-N concentration in the bottom 5 cm of the soil core was demonstrated in the CS and CON treatments. The ln transformed N_2O emission data from the 2 treatments formed significant positive relationships with NH_4^+-N ($P < 0.05$) and accounted for 79% and 65% respectively. Apparently, none of the measured soil variables in the upper soil core layer could account for the variation in the ln transformed N_2O data from the NPK treatment, although 92% of the variation could be accounted for in a significantly ($P < 0.05$) negative relationship with the soil NO_3^--N concentration in the lower soil core layer.

Four days after fertilisation fungi was observed on the surface of the soil cores amended with sewage sludge pellets (Plate 5.2). The surface of the cores became more prolific with fungi over the next 10 days, but were observed to have no fungi or minimal colonisation 31 days after fertilisation. The fungi was identified by Mr. Kevin Ingleby (CEH, Edinburgh) as belonging to the Zygomycetes class, which include many common soil saprophytes (Hudson, 1972). Many of the species are important in the breakdown of organic material including animal manure, particularly if it is moist (Hudson, 1972). The fungi are able to grow very quickly and produce a copious mycelium of coarse, sparsely branched, grey or white hyphae, which allows rapid colonisation of organic material (Hudson, 1972).

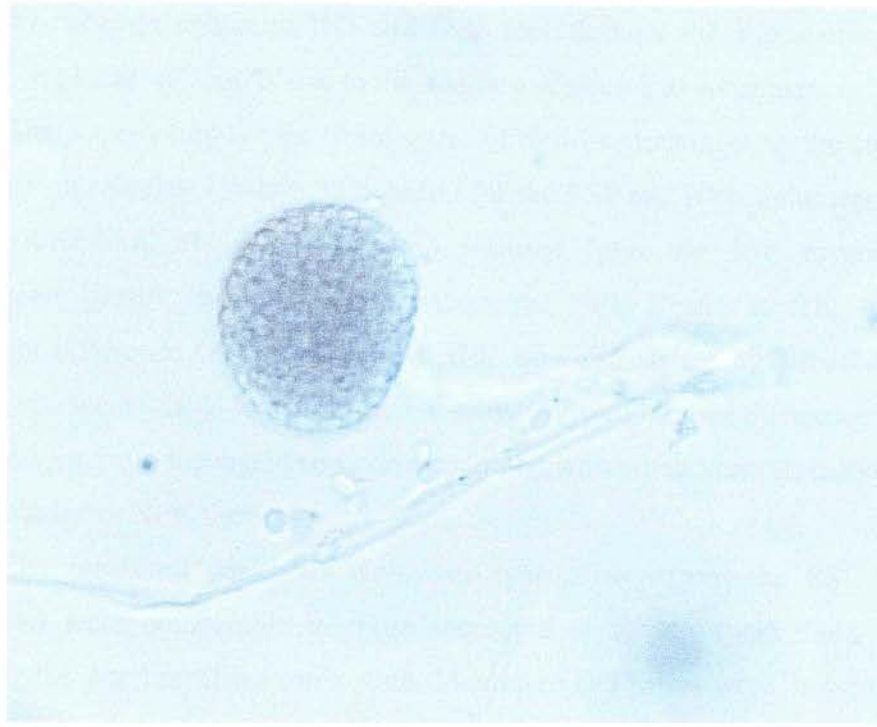

Plate 5.2 Magnified fungus found on the soil surface of the SSP cores – sporangium 36 μm in horizontal diameter

5.3.4 Discussion

5.3.4.1 Effect of fertiliser treatment on NO and N_2O emission

The application of both organic and inorganic fertilisers to repacked clay loam soil columns enhanced NO and N_2O loss through the stimulation of their production processes, largely due to the addition of microbial substrates.

There was a highly significant effect of fertiliser treatment on the cumulative NO emission calculated over a 32 d period for the SSP and NPK treatments (Figure 5.5). Furthermore, the cumulative NO released from the SSP treatment was significantly greater than that emitted from the NPK treatment. The lack of a significant difference between SSL and NPK was also shown by Tabachow *et al.* (2001), who were unable to demonstrate a statistically significant difference between NO emissions from incubated soil cores amended with either anaerobically digested sewage sludge or NPK fertiliser.

The measured peak NO emissions (particularly from the SSL and CS treatments) were comparable to those measured at the Cowpark field in 1999 following the April fertiliser application. Maximum NO losses were, however, *ca.* 3 and 4 times larger from the NPK and SSP treatments respectively. The emissions monitored from the CON and SR treatments were marginally larger than measured from the field site, although were still generally < 3 μg NO-N m^{-2} h^{-1} and < 6 μg NO-N m^{-2} h^{-1} respectively. This small rise in flux is likely to reflect the increased availability of mineral N, due to the absence of competition by an actively growing grass sward.

Nonetheless, emissions of NO were at the lower end of the ranges reported by Tabachow *et al.* (2001) in a comparable laboratory study using repacked soil columns. Despite a similar N application rate (112 kg N ha^{-1}) and sludge DM content (3.4%), losses in their study varied from 3 to 74 μg NO-N m^{-2} h^{-1} from unamended soils. NPK fertilised soils produced NO fluxes ranging from 9 to 520 μg NO-N m^{-2} h^{-1} and soils applied with a liquid anaerobically digested sewage sludge generated NO emissions spanning from 2 to 640 μg NO-N m^{-2} h^{-1}. The higher fluxes recorded by Tabachow *et al.* (2001) were probably the result of using a higher incubation temperature of 28 °C as compared to our temperature of *ca.* 17 °C, and the use of a

well aerated sandy loam soil. Furthermore, emissions were monitored from soils with a maximum WFPS of 40%, which had purposely been chosen to achieve a high NO flux rate. The method employed by Tabachow *et al.* (2001) may also have contributed to the very high NO fluxes. Air dried soil was firstly amended with the fertilisers, then re-wetted to achieve the target WFPS and subsequently incubated for only 24 h prior to the start of the experiment as opposed to our incubation of 7 days. Subsequent NO flux rate was only determined over a 30 min period. Stimulation of NO emissions has frequently been reported following the wetting up of dry soils (Anderson & Levine, 1987; Davidson *et al.* 1991; Davidson, 1992; Cárdenas *et al.* 1993; Hutchinson *et al.* 1993a; Meixner *et al.* 1997) and may, therefore, account for the large emissions measured.

The percentage of NO lost from total N and TAN added in fertiliser is valuable in the comparison of emissions from treatments that have been amended with different rates of N. The percentage of total N applied lost as NO over the 32 d incubation period (0.001-0.006%) (Table 5.3) was typically more than that lost following each of the 3 fertiliser additions in the field (0.0004-0.003%) (Tables 4.6 and 4.12). Nonetheless, the amount lost was still markedly less than that estimated by Veldkamp & Keller (1997) from inorganic N fertiliser (*ca.* 0.5%) and that reported from the laboratory based study of Paul *et al.* (1993). Over only a 6 day period, an average of 0.26% of the added total N in liquid dairy cattle and fresh solid beef manure was lost as NO (Paul *et al.* 1993). Assuming a constant NO emission rate based on 30 minutes data, Tabachow *et al.* (2001) estimated that 1.8% of the N applied would have been emitted from the soil as NO over a 90 day period. It is highly likely though that the NO emission would decrease significantly over this time period and, therefore a percentage loss of 1.8% would overestimate the actual loss.

In this study, due to the considerably larger total N application rate of the SSP treatment, the largest percentage of total N lost as NO was calculated from the NPK treatment. The magnitude of the percentage of applied TAN lost as NO essentially followed the cumulative NO emission pattern with a significantly larger loss from the SSP and NPK treatments (Table 5.3 and Figure 5.5).

Similar to the field results, the largest percentage loss was recorded from the SSP treatment (Table 5.3). The generation of NO by nitrification from the SSP treatment was potentially greater than from other treatments primarily because of the surface placement of the material combined with its composition. The slow release of mineral N into the well-aerated top few cm of the soil would reduce the gaseous diffusion pathway and hence the subsequent risk of NO loss via consumption. The importance of the surface application and slow release characteristics of the SSP fertiliser was reflected both in the soil NH_4^+-N and soil NO_3^--N concentrations measured in the soil cores (Figure 5.8). The substantial increase in mineral N levels over the study period in the SSP cores was only detected in the top 5 cm of the soil, whereas the concentration in the 5-10 cm layer was comparable to that in measured in the CON treatment. This was in contrast to the elevated 5-10 cm soil mineral N concentration of the NPK, SSL and CS treatments.

Interestingly, the pattern of both the cumulative emission and the percentage TAN lost as NO from the soil cores amended with NPK differed from that observed in the field. Losses of NO were considerably larger and second in magnitude only to those from the SSP treatment. It is likely that with neither the inhibiting effects of anaerobic conditions generated as a result of precipitation or the presence of an actively growing crop, more mineral N substrate was available for nitrification. Indeed the measured NH_4^+-N concentrations were markedly higher in the soil cores (12.0-115.8 μg g dry $soil^{-1}$) than from the soil samples taken from the field (1.8-26.3 μg g dry $soil^{-1}$).

The low emissions of NO from the SR treatment were not unexpected, because of the characteristics of the fertiliser, which permits only a small release of mineral N at any one time and were not statistically significant from background levels.

N_2O

A significant effect of fertiliser treatment on the cumulative N_2O emission calculated over a 32 d period immediately following fertiliser application was observed (Table 5.4). Losses of N_2O from the SSP treatment were significantly

larger than from either of the inorganic treatments i.e. SR and NPK. Similar results of higher N_2O emissions from organic manures that from synthetic fertiliser have been previously reported in the literature (Mosier *et al.* 1982; Christensen, 1983; Ellis *et al.* 1998), and are attributed to the stimulation of denitrification by the addition of organic carbon.

Peak N_2O fluxes measured from the soil columns from the SSL and CS treatments were comparable to those measured following the fertiliser application in 1998, whereas N_2O losses from the SSP treatment were similar to those detected after fertiliser amendment in both April and July 1999. In general though, peak N_2O fluxes monitored in the field were substantially larger than those measured from the soil cores. The reverse was demonstrated, however, with fluxes emitted from the CON treatment, whereby the maximum N_2O loss recorded in the laboratory experiment was 12 to 36 times larger than in the field.

The production of N_2O is believed to be predominantly the result of microbial anaerobic denitrification (Williams *et al.* 1998; Firestone & Davidson, 1989) and indeed this was the conclusion reached, with respect to the N_2O data collected in 1998 at the field site, by McTaggart *et al.* (1999). Fluxes of N_2O measured in the field from the NPK, CS and SSL treatments were all stimulated by the increase in anaerobicity caused by rainfall to produce the large peak emissions, although heavy rainfall appeared not to enhance the loss of N_2O from either the SR or SSP treatment (McTaggart *et al.* 1999). It is not unexpected therefore, that in the laboratory without periodic precipitation and the associated anaerobicity in the soil, peak N_2O fluxes were lower than measured in the field. Nonetheless, the cores did receive water in order to replace any lost via evaporation, although this was not added until after each gas sampling event, therefore, any resultant bursts of N_2O would not have been measured.

The markedly higher N_2O emissions monitored from the unfertilised soil cores were a consequence of intense production from 2 of the cores, whilst the third generated N_2O at a rate comparable to that measured in the field. It is suggested that because such stimulation was not observed with the measured NO emissions, the enhanced N_2O loss was the result of denitrifier 'hot-spot' activity, probably induced by the burst of microbial activity following re-wetting of the soil.

As a result of the atypical N_2O emission magnitude from the CON treatment, it was not feasible to calculate the percentage of N_2O lost from total N and TAN added in fertiliser from the SR and NPK treatments. Furthermore, loss rates derived from the SSL, SSP and CS emission data were liable to be underestimated.

The percentage of total N applied as organic waste, which was lost as N_2O over the 32 d incubation period (0.005-0.008%) was typically 1-3 orders of magnitude less than that lost over an approximately equivalent time period in the field (0.02-1.7%). Similarly, the percentage of applied TAN lost as N_2O from the soil cores (0.006-0.04%) was 1-2 orders of magnitude less than that measured in the field (0.1-2.7%).

5.3.4.2 Variation in NO and N_2O emission over the sampling period

The majority of peak NO emissions from the soil cores after fertiliser application followed the 'typical' pattern whereby peak emissions are measured soon after fertilisation and indeed they were measured only 1 hour later, although the maximum NO fluxes from surface placed SSP and NPK fertilisers occurred 14 and 31 days after fertilisation respectively (Figure 5.3).

Emissions of NO from the NPK treatment in the laboratory (Figure 5.3) were relatively consistent and were always > 13 μg NO-N m^{-2} h^{-1}. This emission pattern was strikingly different to that observed after each of the 3 fertiliser applications at the field site (Figures 4.4 and 4.13). The primary reason for the change was likely to be the absence of an actively growing crop in the laboratory, especially as grass possesses a high capacity to take up nitrogen (Whitehead, 1995) and adds organic carbon to the soil, stimulating activity (Pers. Comm., Dr. R. Rees, 2002). Unlike organic wastes, all of the N applied as NPK is readily available for uptake by plants and as such can be easily exploited. Subsequently, little mineral N substrate remains for use by the microbial biomass. Indeed, Opperman *et al.* (1989) reported that an observed decline in soil NH_4^+-N was accelerated by the presence of growing plants. Additionally, without precipitation falling onto the soil, the inorganic fertiliser N would not have been washed as far down into the bulk soil and, therefore, away from the topmost biologically active soil layers. Consequently, the mineral N may have potentially been available for use as a microbial substrate for a relatively longer time

period. Moreover, the soil cores incubated in the laboratory would not have experienced the inhibition of NO fluxes, which were observed in the field from the NPK treatment, as a result of the precipitation pattern around fertiliser application. Nonetheless, the enhanced NO emission from the NPK cores compared to the measured fluxes in the field, may simply be the result of spatial variation within the soil.

The production of NO from the SSP treatment approximately doubled from 2 to 4 days after fertiliser application and subsequently gradually increased to the measured peak emission (Figure 5.3). Accompanying the rise in NO loss was an increase in the NH_4^+-N concentration in the top 5 cm of the soil and the concomitant surface growth of fungi. It is hypothesised, therefore, that the fungi were responsible for the enhanced NH_4^+-N levels and the subsequent stimulation of NO fluxes. In support of this theory, the decline in NO production from the SSP treatment measured 31 days after fertilisation corresponded to a fall in the soil NH_4^+-N concentration and the virtual absence of fungi on the soil cores. Further evidence has been detailed in the literature. For example, Lerch *et al.* (1992) discussed the importance of fungi in the gradual decomposition of sewage sludge and Robinson & Polglase (2000) reported the mineralisation of organic N in surface applied, dewatered sewage sludge following re-wetting. The mineralisation was able to take place in the absence of soil, which they assumed was due to the presence of heterotrophic decomposers in the sewage sludge. However, unlike in this experiment, significant sewage sludge mineralisation did not occur until between 3 and 14 weeks after application, presumably because of a time lag for the generation of a heterotrophic population. In a laboratory study to investigate the source process responsible for N_2O production from andosols following amendment with organic material, white fungal hyphae had covered the soil surface following only 4 days of incubation (Inubushi *et al.* 1996). The time for the development of fungi was, therefore, comparable to that observed in this experiment.

The role of fungi as decomposers of organic N in the thermally dried sewage sludge pellets may well occur in the field. Although in decline, NO emissions were still highly elevated above those of the unfertilised CON treatment 31 days after fertilisation and would, therefore, account for the sustained measured field fluxes.

Similarly to the NPK treatment, emissions in the field were liable to be repressed due to inhibition of nitrification following the increase in anaerobicity of soil by precipitation, hence larger NO losses from the SSP treatment were observed in the laboratory.

Peak losses of NO from both of the simulated injection treatments were measured only 1 h after the application of the fertiliser and coincided with the maximum measured soil NH_4^+-N concentrations (Figures 5.3 and 5.7). Fluxes subsequently declined concomitantly with the NH_4^+-N content of the soil and therefore the drop in NO emission was probably the result of reduced microbial substrate availability. Immediately following the addition of the organic wastes, the microbial population would have been able to utilise the readily available NH_4^+-N, although later in the experiment, this source would have diminished and the availability of NH_4^+-N would have depended on the gradual mineralisation of the added manure organic N.

Emission minima from the NPK, SR, SSP and CON treatments were measured 2 days after fertilisation (Figure 5.3). It is more likely that the apparent emission decline was an artefact of enhanced microbial stimulation in the soil cores on day 0. Despite an incubation period of 1 week prior to the start of the experiment, the results obtained 3 days before fertiliser application demonstrated that NO emissions were elevated (e.g. an emission of 18.5 μg NO-N m^{-2} h^{-1} from the unfertilised CON treatment), presumably as a result of increased microbial activity following the re-wetting of dry soil (Davidson, 1992). Further evidence of the stimulation caused by re-wetting of the air-dried soil was exhibited by the soil NO_3^--N concentrations measured in the cores. The level of NO_3^--N in the soil was appreciably higher (*ca.* 40 μg g dry $soil^{-1}$) than that typically measured from the unfertilised CON plots at the Cowpark field site (< 1 μg g dry $soil^{-1}$) and is likely to have arisen from mineralisation followed by intense nitrification (Davidson, 1992; Hutchinson *et al.* 1993b).

The losses of NO from both the CON and SR treatments remained relatively consistent throughout the experimental period. For the SR treatment this reflects the gradual release of mineral N from the slow release fertiliser with little excess available for microbial release.

N_2O

The N_2O emission pattern from the soil cores differed to that displayed by the NO fluxes and also varied between treatments. Nonetheless, all treatments showed 14-31 or more days after fertiliser application.

The distribution pattern of N_2O fluxes from the CON treatment was greatly affected by the possible 'hot-spot' activity in 2 of the soil cores, which stimulated emissions much higher than would have been expected at the start of the experiment (Figure 5.4). Emissions did, however, decline to levels which were more typical of those measured from the Cowpark field site.

Similarly to the emission pattern from the CON treatment, the loss of N_2O from the NPK treatment peaked shortly after fertiliser application and subsequently diminished to a flux magnitude comparable to that exhibited by NO (Figures 5.3 and 5.4). Corresponding to the fall in N_2O emission over the study period was a decrease in the measured soil NH_4^+-N concentration, although the NO_3^--N content of the soil remained close to the initial level immediately following fertiliser application (Figures 5.7 and 5.8).

For the SR treatment fluxes of N_2O diminished throughout the experimental period (Figure 5.4), unlike the magnitude of the NO emissions which remained relatively consistent. It is possible that this reflects the lack of rainfall and/or an increase in air temperature in the laboratory which in the field would stimulate the release of mineral N from the SR granules and, therefore, provide a substrate for the microbial biomass. Indeed, there was no evidence, in the form of soil NH_4^+-N, for the release of such a substrate (Figure 5.7).

The loss of N_2O from the SSP treatment was comparable in magnitude to that measured from the SR treatment 1 h and 2 d after fertiliser application, but 4 d after fertilisation there was a dramatic 13 fold increase in the size of the emission for SSP cores (Figure 5.4). This considerable rise in the N_2O flux also coincided with a doubling of the NO emission (Figure 5.3) and the development of fungal growth on the surface of the soil cores.

The peak N_2O emissions from the CS and SSL treatments were measured 2 and 4 days after the fertiliser application respectively (Figure 5.4). A lag period between the addition of organic waste and the maximum flux was not apparent with

the emission of NO. Nevertheless, a lag period has frequently been reported in the literature e.g. Fine *et al.* (1989); Petersen (1992); Chadwick *et al.* (2000). Immediate immobilisation of added ammoniacal N following the application of organic wastes may account for such a lag period and has been documented in the literature (Comfort *et al.* 1988). It has been estimated that for immobilisation to occur, the C:N ratio of the organic material must be > 10 (Terry *et al.* 1981; Smith *et al.* 1998b). In this experiment, however, the C:N ratio of the CS and SSL were 7.8 and 7.9 respectively and similarly to studies by Epstein *et al.* (1978) and Chae & Tabatabai, (1986) the manures should not, therefore, be subjected to intense immobilisation.

Alternatively, the occurrence of a lag period before peak N_2O emissions has been attributed to the initial inhibition of nitrification, due to anaerobic conditions induced by the addition of organic wastes. Without significant nitrification the production of N_2O via denitrification would not be able to operate at appreciable levels. The length of the lag period, however, varies widely between studies e.g. Christensen (1983) measured a flush of N_2O following the application of cattle slurry to grassland within 5 days, whereas a completely anaerobic environment was reported for up to 3 weeks, following the injection of pig slurry (Weslien *et al.* 1998).

It is possible, although there is no evidence, that because the initial N_2O emissions measured from the CS and SSL treatments were elevated and there was only a relatively short period between fertiliser application and peak N_2O emission, that the denitrifiers were able to utilise the NO_3^--N already present in the soil. Indeed, Clemens & Huschka (2001) suggested that soil borne NO_3^--N had been used to induce N_2O emissions following the application of cattle slurry.

5.3.4.3 Controlling factors of NO and N_2O emission

The laboratory experimental set-up was designed so as to isolate the potential effects of fertiliser nitrogen on the emission of both NO and N_2O, through incubation at a constant air temperature and soil moisture content. It was no surprise consequently when statistically significant relationships were established between NO or N_2O emission and soil mineral N concentration.

Similar to the field results no correlation could be found between all collected NO data and the corresponding mineral N information. Furthermore, the loss of NO from the SR, CON, CS and SSP treatments did not show a significant relationship with the soil mineral N levels. It was likely that the fluxes emitted by the CON and SR treatments were so low as to mask the significance of any influential parameter by the natural variability of the soil environment. In support of this explanation, previously in the field and in other studies (Merino *et al.* 2001) it was shown that clearer relationships could be established if the range of emission magnitude/soil mineral N concentration was relatively wide and included enhanced fluxes/mineral N contents as a consequence of fertilisation. Furthermore, the constant temperature combined with the relatively short incubation period (compared to the length of the grass growing season) may have prevented the establishment of relationships between mineral N and NO emission such as were occasionally observed in the field.

A similar lack of a significant correlation between NO emitted from the SSP treatment and soil mineral N was also evident in the field. The implication is that the production of NO from the SSP treatment is not just based on a simple relationship with mineral N, as was frequently seen with the NPK and SSL treatments. This idea is supported through the evidence in the laboratory of the presence of fungi and their role in releasing available N for use by the soil microbial population. Consequently, the magnitude of NO emission was strongly influenced by the environmental factors which affect the growth and activity of the fungi.

In contrast to the NO emissions from the SSP treatment, those from the NPK treatment were apparently controlled relatively more straightforwardly. As observed in the field, a high percentage of the variation in the NO emission data (73-84%) could be accounted for by soil NO_3^--N in both the lower and upper soil core halves, respectively. The results of a stepwise regression of WFPS, soil NO_3^--N and soil NH_4^+-N were able to explain all (99.9%) the differences within the NO data.

Similar to the NO flux measured from the field plots amended with CS the strong influence of the soil NH_4^+-N concentration on NO production from the soil cores treated with SSL was clearly shown, such that NO emissions increased concomitantly with soil NH_4^+-N. The NH_4^+-N levels in both the top and bottom

halves of the core were able to account for virtually all of the variation (94-99%) in the NO data from the SSL treatment.

In the controlled environment of the laboratory most of the variation in the NO data from the SSL and NPK treatments has been explained. This demonstrates the importance of WFPS and high concentrations of soil mineral N as singular or combined factors. The fact that in the field less of the variation can be explained, points to the complex interactions within the soil and from fertiliser composition that influence the NO emission.

N_2O

Unlike the emission of NO, the loss of N_2O across all of the 6 treatments was apparently affected by the soil NO_3^--N concentration, as well as by the soil WFPS. N_2O fluxes were larger with a low NO_3^--N content and a wetter soil. Once more, the influence of WFPS in this soil should not be underestimated, since only a narrow range of WFPS was able to have a significant effect on emissions. The role of moisture content and precipitation on both the NO and N_2O emissions from the Cowpark field site, therefore, is potentially very substantial. In this laboratory experiment the potential controlling environmental variables of precipitation and crop activity were removed, although the stimulus of soil carbon may have been considerable (Firestone & Davidson, 1989).

On an individual treatment basis the significance of mineral N was demonstrated. The magnitude of N_2O fluxes was inversely correlated with the NO_3^--N concentration in the top 5 cm of soil from all but the SSP treatment. The importance of NH_4^+-N was exhibited in the loss of N_2O from both the CON and CS treatments, whereby emissions were enhanced with an increase in NH_4^+-N levels in the bottom half of the soil core.

It should be noted that due to the combination of relatively few data points and the lack of replication with respect to the soil variable data, the absence of a relationship between the emission of NO/N_2O and a particular soil variable does not necessarily indicate its unimportance.

5.3.4.4 Emission source

The primary aim of this experiment was to determine the process source of NO in the soil taken from the Cowpark field site. Within the discussion in chapter 4, it had previously been assumed that soil conditions were unfavourable for either heterotrophic nitrification or chemodenitrification and therefore production of NO was likely to be from autotrophic nitrification and/or anaerobic denitrification. Under the laboratory conditions established, air temperature and WFPS were controlled at predetermined levels and, therefore, isolated the effect of the application of either organic or synthetic fertilisers. Results from the Cowpark field experiment demonstrated the importance of mineral N in the added fertiliser, thus it was hoped that by investigating the relationship between mineral N and the emission of NO it would be possible to determine the relative importance of nitrification and denitrification as a source process. Additionally, further information to clarify the objective was gained from the measurement of N_2O from the same cores as used for NO monitoring. In particular, the ratio of N_2O:NO has been used by numerous researchers to indicate the relative contribution of nitrification and denitrification to the production of NO.

It was very clear that the addition of both organic and mineral fertilisers stimulated the emissions of both NO and N_2O. Results obtained from the Cowpark field site suggested that NO was primarily generated from nitrification, whereas the production of N_2O was likely to be from a combination of nitrification and denitrification. It is widely recognised that at the target WFPS of 60%, the dominance of nitrification and denitrification as the source of production changes, such that at < 60% WFPS nitrification dominates, but at > 60% WFPS denitrification is the major contributor to N-oxide production (Figure 5.10). In this laboratory experiment it was found that the WFPS of the soil cores generally varied over a 10% range i.e. from 55-65% and therefore, both nitrification and denitrification may have been possible sources.

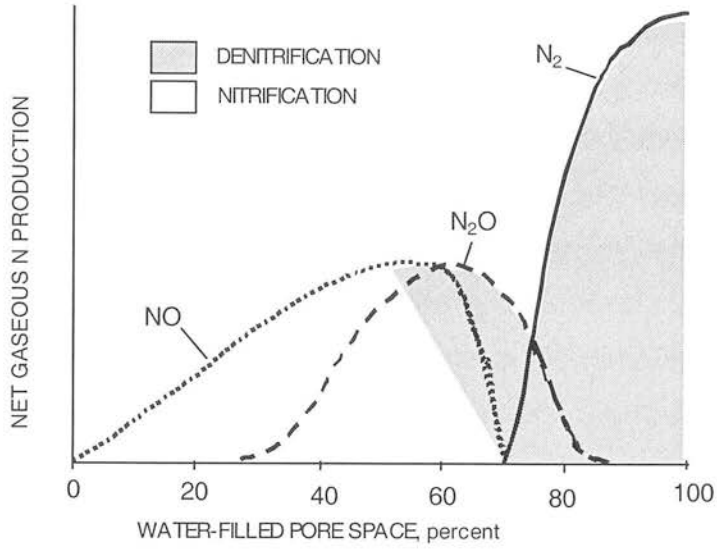

Figure 5.10 Model of the relationship between WFPS of soil and re fluxes of N gases. (From Davidson, 1991).

In this laboratory study, the WFPS was generally never high enough for intense denitrification, i.e. above 70%, and indeed the NO:N_2O ratios rarely show evidence of this (Table 5.6). Laboratory work has established that an NO:N_2O ratio < 0.01 indicates denitrification, whereas an NO:N_2O ratio > 1 is a sign of nitrification (Lipschultz *et al.* 1981; Anderson & Levine, 1986). Results from this experiment nearly always lie between the two defining ratios and as such suggest the operation of both nitrification and denitrification in neighbouring microsites. It is possible in this soil that, because of the WFPS values encountered, considerable N_2O may be produced from nitrification, since the process would be operating at its wetter range. It is thought that the production of N_2O from nitrification arises when O_2 becomes partially limiting in some microsites (Davidson, 1993) i.e. a situation which was likely in this experiment.

Results from this experiment clearly indicated the importance of nitrification. Overwhelming evidence of nitrification was demonstrated by the analysis of soil mineral N. In all treatments, despite a relatively high initial concentration, the content of soil NO_3^--N increased dramatically from that recorded at the start of the experiment to that measured 31 days after fertilisation. The increase of soil NO_3^--N

in such a laboratory-controlled system, without the effects of plants or wet and dry deposition, can only have arisen from nitrification (Brady & Weil, 1999).

Table 5.6 NO:N_2O ratios for losses measured from repacked soil cores amended with organic and inorganic fertilisers.

Treatment	Day after fertilisation							
	-3	0	2	4	7	10	14	31
NPK	0.10	0.08	0.06	0.34	0.89	0.84	1.48	1.27
SR	0.09	0.09	0.04	0.03	0.12	0.27	0.17	0.40
SSL	0.17	0.15	0.04	0.02	0.02	0.02	0.06	0.22
SSP	0.13	0.22	0.17	0.03	0.19	0.30	0.33	0.73
CS	0.07	0.05	0.01	0.07	0.21	0.16	0.48	0.51
CON	0.09	0.01	0.00	0.01	0.03	0.15	0.13	0.75

Correspondingly, the soil NH_4^+-N concentration from the NPK, SSL and CS treatments declined over the duration of the experiment. There are, however, a number of mechanisms that can reduce the level of NH_4^+-N in the soil. Uptake by plants can be discounted as no plants were grown, although a small number of tiny seedlings were removed from the soil columns. Immobilisation of NH_4^+-N may occur, but was earlier discounted in this study due to a C:N ratio of < 10 for the organic fertilisers applied. The loss of fertiliser NH_4^+-N as NH_3, particularly from organic wastes (animal and sewage sludge), has been frequently reported (Terry *et al.* 1981; Adamsen & Sabey, 1987; Quemada *et al.* 1998; Weslien *et al.* 1998; Fine *et al.* 1989; Robinson & Polglase, 2000). Nonetheless, NH_3 losses have been demonstrated to be reduced with injection (Weslien *et al.* 1998; Chadwick *et al.* 2001) and from the NPK treatment are not expected to be large at < 2% of added TAN lost, especially in the slightly acidic soil (pH 6.7) (Harrison & Webb, 2001).

Numerous authors have reported a decline in soil NH_4^+-N following the addition of organic wastes and a subsequent rise in soil NO_3^--N concentration as a result of nitrification (King, 1973; Comfort *et al.* 1988; Petersen, 1992; Abbasi & Adams, 2000; Chadwick *et al.* 2000; Chantigny *et al.* 2001). In a laboratory experiment, following the incorporation of anaerobically digested sewage sludge to a

loamy sand, Smith *et al.* (1998c, 1998d) reported the decrease in NH_4^+-N concentration concomitantly with the formation of soil NO_3^--N. Over a 12 week incubation of liquid sewage sludge applied to a sandy loam, the soil NO_3^--N content increased, such that at the end of the experiment 96% of the mineral N was in the NO_3^--N form (Adamsen & Sabey, 1987). It was estimated that 2 weeks after the injection of anaerobically digested sewage sludge to a silty clay loam, 72% of the NH_4^+-N in the sludge had been converted to NO_3^--N (Stamatiadis *et al.* 1999). Lindemann and Cardenas (1984) reported that nitrification was rapid following sludge amendment of sandy and clay soils, whilst nitrification accounted for the rapid decrease in NH_4^+-N coupled with the increase in NO_3^--N after the application of dairy cattle manure to a loam soil (Lessard *et al.* 1996).

In this experiment the measured emission of NO from both of the injected treatments peaked 1 h after fertiliser application and coincided with the maximum soil NH_4^+-N values. The NH_4^+-N would have diffused away from the anaerobic injection slit and into the more aerated soil matrix, where nitrification could have proceeded. If denitrification contributed to the majority of the NO production from the CS and SSL treatments, then one would not expect to see the immediate flux stimulation which followed the simulated injection. Both organic fertilisers would have contained a negligible amount of NO_3^--N and would therefore not provide the necessary substrate. Potentially, however, this may have been available in the soil core as evidenced by the enhanced NO_3^--N levels measured compared to that in the field.

The stimulation of N_2O fluxes in the SSL and CS treatments above those of the inorganic fertiliser treatments were almost certainly the result of a combination between nitrification and denitrification. The added carbon in the manures not only provides a necessary substrate for denitrification, but also reduces O_2 levels in the soil during its decomposition. The anaerobic conditions were further enhanced due to the extra liquid added in the slurries, which was reflected in an increase of WFPS to *ca.* 70%. The increase in N_2O fluxes over the first couple of days would have corresponded to the generation of NO_3^--N by nitrification and subsequent diffusion into the anaerobic zone, immediately adjacent to the injection slit for potential use in denitrification (Nielsen *et al.* 1996).

Significantly, there was a positive relationship between the N_2O emission from the CS and the soil NH_4^+-N concentration in the bottom half of the soil core i.e. where the bottom of the injection slit was located. The NH_4^+-N concentration measured on the day of application had roughly doubled 2 days after fertiliser application, presumably because of mineralisation of added organic N. Over the same period the N_2O emission increased, but so did the soil NO_3^--N concentration, although at a lower rate to the increase in soil NH_4^+-N. Such a discrepancy between the rates of NH_4^+-N loss and NO_3^--N formation suggest that even though nitrification is proceeding, denitrification is continuing at a faster rate (Abbasi & Adams, 2000). Nonetheless, it is probable that nitrification did contribute to the N_2O fluxes, a phenomenon reported by Lessard *et al.* (1996) following the application of cattle manure to a maize crop.

The NO emission measured from the NPK treatment showed a strong relationship to soil NO_3^--N, such that NO fluxes were larger when NO_3^--N concentrations were also higher. Such a relationship may indicate the production of NO_3^--N from nitrification, or the use of NO_3^--N as a substrate in denitrification. However, as previously mentioned the soil NH_4^+-N concentration decreased and a loss by any other mechanism except nitrification was discounted. The emission of NO was able to be sustained even with a declining NH_4^+-N concentration because of the large initial ammoniacal-N input, consequently levels were still relatively high at the end of the experiment. The inverse relationship established between NO emission from the NPK treatment and WFPS strengthens the evidence for production by nitrification. Losses of NO were larger when the WFPS was lower, as a result of increased aeration, a factor favoured for nitrification.

The case for nitrification is further strengthened when the NO:N_2O ratio is examined. Table 5.6 shows that for the NPK treatment an NO:N_2O ratio > 1 was calculated 14 and 31 days after fertilisation and thus suggests nitrification was the dominant source process. Although the ratio from early measurement dates was < 1, at no time was the ratio < 0.01, a sign of denitrification. Because the emission of NO from the NPK treatment was relatively consistent throughout the experiment, it appeared that the magnitude of the N_2O flux determined the NO:N_2O ratio. Despite the elevated NO_3^--N content, the emission of N_2O from the NPK treatment declined

over time, presumably as a result of lack of soil carbon for denitrification and/or as a response to the reduction in NH_4^+-N. It is probable that both denitrification and nitrification contributed to the emission of N_2O, since although nitrification was obviously occurring as indicated by the NH_4^+-N reduction, the increase in NO_3^--N was not as much as was expected. Certainly, compared to all the other treatments, the NPK treatment demonstrated the smallest NO_3^--N increase over the duration of the study.

The relationship between NO evolution from the SSP treatment and mineral N was not as simple as that with the NPK, CS and SSL treatments. The primary reason appears to be the association of a fungal growth with the sewage sludge. This fungus initiated the mineralisation of sludge organic N and subsequently the release of NH_4^+-N. The evidence collected from the field study indicated that the NO produced had originated during nitrification and despite no statistical relationship between the emission of NO and soil mineral N, the theory was substantiated by the results generated from this laboratory experiment. An increase in soil NH_4^+-N concentration over the study period was only measured from the SSP treatment and generally mirrored the increase in NO emission. Indeed, the approximate halving of the NO emission from 14 to 31 days after fertilisation was concurrent with an approximate halving of the soil NH_4^+-N concentration. It is possible that the measured NO and N_2O may have arisen from denitrification of the NO_3^--N produced during nitrification. If substantial denitrification was occurring, however, it would be expected that the soil NO_3^--N concentration would decrease. In fact, out of all of the treatments the increase in NO_3^--N over the study period was the greatest from the SSP, presumably reflecting intense nitrification of mineralised NH_4^+-N.

It seems likely that in the laboratory conditions the production of N_2O from the SSP treatment was also predominantly from nitrification. There was a significant positive relationship of N_2O emission and the soil NH_4^+-N concentration i.e. N_2O fluxes increased with a rise in NH_4^+-N. It may be that the relationship was a surrogate for an association with the subsequent NO_3^--N produced from nitrification and it was this NO_3^--N that was important for emission of N_2O from denitrification. However, similar to the NO emission, production of N_2O decreased concomitantly with the NH_4^+-N concentration 31 days after fertilisation. If denitrification was the

main mechanism for production of N_2O, one would expect to see the largest fluxes when the NO_3^--N concentration was the highest, which in fact was when the N_2O flux was the lowest measured. Nonetheless, the NO:N_2O ratios do not indicate the dominance of nitrification and it is therefore likely that N_2O was produced from both nitrification and denitrification or that more N_2O than NO was produced from nitrification. Studies involving the use of inhibitors (e.g. C_2H_2, nitrapyrin etc.) would have clarified this issue.

No relationships could be established between NO emission from the SR and CON treatments and mineral N, however inverse relationships were established between the N_2O fluxes and soil NO_3^--N i.e. fluxes declined as the soil NO_3^--N increased. Furthermore, a positive relationship between N_2O emission and the soil NH_4^+-N concentration in the 5-10 cm soil layer suggested a stimulation of production with an increase in NH_4^+-N. It is therefore suggested that nitrification mainly contributed to both the emission of NO and N_2O. A low level nitrifier population may have been established as a result of the re-wetting of the soil prior to the start of the experiment, which may not necessarily be present to such a degree in the field. The existence of denitrifier 'hot-spot' activity in two of the three replicate soil cores from the CON treatment was supported by the NO:N_2O ratios. In particular, the NO:N_2O ratios calculated for the measurement occasions 1 hour, 2 days and 4 days after fertilisation indicated denitrification i.e. the ratio was ≤ 0.01. This denitrifier activity did not however, prevent the accumulation of NO_3^--N over the study period, although similarly to the NPK treatment the NO_3^--N concentration did not increase as much as in other treatments.

The results suggested that the emission of NO from the repacked soil cores fertilised with organic or inorganic fertilisers was generally produced from nitrification and support the data collected during the 2 year field campaign. Inhibitor studies would have provided clarification of the source process for NO production. It should be noted, though, that disruption of anaerobic aggregates, which may have been important sites for denitrification, may have been destroyed in the sieving and subsequent re-packing of the soil cores. Furthermore, re-packing may have introduced more O_2 into the soil than would naturally have been present.

5.3.5 Conclusion

- In this laboratory study we demonstrated that the application of slow release fertiliser, cattle slurry, liquid sewage sludge and no fertiliser significantly reduced NO emissions over NPK mineral fertiliser and sewage sludge pellets applications by 70-95%. Emissions of NO from slow release fertilisers were not significantly different to those from the unfertilised treatment.
- This study confirms the findings from the field experiment that emissions of NO were largest (although not significant) from soil fertilised with thermally dried sewage sludge pellets. Cumulative NO fluxes were statistically larger (2-24 times) from cores amended with pellets than from all other treatments including NPK mineral fertiliser.
- In contrast to the field results the percentage loss of total N and total ammoniacal N applied lost as NO was not significantly different from cores fertilised with NPK mineral fertiliser and sewage sludge pellets. It is suggested that in the field the large amount of mineral N applied to the soil is rapidly taken up by the crop and is washed deeper into the soil matrix by precipitation resulting in an increased potential of NO loss by denitrification. In this laboratory study without these factors the availability of mineral N for microbial use is increased.
- Similar to the field experiment no one variable could adequately explain all the variation in the NO data across treatments. The influence of soil mineral N on the NO emissions from the NPK and SSL treatments was, however, able to account for the majority of the variation and demonstrated the importance of interacting variables in the field on the magnitude of NO emissions.
- The surface growth of a soil saprophytic fungus was important in the mineralisation of organic N from the surface applied sewage sludge pellets and the subsequent release of NH_4^+-N for nitrification and contributed to the high sustained NO fluxes during the measurement period.
- Significant correlations between emissions and soil ammonium, soil nitrate and soil WFPS as well as the mineral N and emission trends over time were useful indicators of the source process for the emission of NO and N_2O.
- The results suggest that nitrification was the primary source of NO production in this clay loam soil following organic or inorganic fertiliser application, whereas

both denitrification and nitrification were probably source processes for the production of N_2O. Additional clarification could be provided by the use of inhibitors.

5.4 Thermally dried biosolids experiment

5.4.1 Introduction

The results collected from both the laboratory experiment and the field campaigns demonstrated the high potential of thermally dried sewage sludge (biosolids) pellets (SSP) to release NO into the atmosphere. The surface placement of the pellets appeared to be highly significant in the generation of the NO flux. It was hypothesised that the application of the pellets to the surface would result in the gradual release of NH_4^+-N into the well aerated, most biologically active, upper few cm of the soil. Consequently, incorporation of the pellets into a greater soil depth may reduce NO emissions, since the nitrification potential would be reduced (less aeration) and the risk of NO consumption would increase with a longer diffusion pathway. The aim of this laboratory experiment was, therefore, to investigate the effect of biosolid placement on the magnitude of the NO emission.

5.4.2 Sites and treatments

The soil used in this study was collected, prepared and incubated as in the organic and inorganic fertiliser laboratory experiment. For a more detailed history of the field site see chapter 4.

The cores were arranged in a randomised block design with 3 blocks for cores used in the gas sampling and 3 blocks for cores used in the destructive sampling (Figure 5.11). Cores were either amended with thermally dried biosolid pellets (which were either spread on the soil surface as in the field experiment at Cowpark (SUF) or mixed into the top 7 cm of the soil (MIX)), or received no nitrogen fertiliser (CON). Soil was initially added to the column to a depth of 3 cm and subsequently was made up to a depth of 10 cm with soil which had previously been comprehensively mixed with the sewage sludge pellets. These were incorporated into the soil to a depth of 7 cm, because this was also the depth to which the liquid sewage sludge and cattle slurry were injected to in the field.

The cores received one fertiliser addition at a target rate of 120 kg available N ha^{-1} (i.e. corresponding to that used at the Cowpark site) one hour prior to the first gas sampling event. The actual application of total and ammoniacal nitrogen is

equivalent to that applied in the organic and inorganic laboratory experiment (Table 5.1). Directly after fertiliser application, all soil core surfaces were slightly moistened with 2 sprays of deionised water from a spray bottle.

Losses of both NO and N_2O were quantified on day 0, 1, 7 and 14. Available soil NH_4^+-N, NO_3^--N and moisture content were ascertained on only 1 soil core per treatment, which was removed on day 1, 7 and 14.

The layout of the soil cores by treatment is indicated in Figure 5.11.

3	MIX	SUF	CON
1	CON	MIX	SUF
B	CON	MIX	SUF
2	SUF	CON	MIX
C	SUF	MIX	CON
A	MIX	CON	SUF

Figure 5.11 A plan view of the soil core layout as positioned in the controlled temperature room. Rows A, B and C represent the 3 treatment blocks used for gas sampling and rows 1, 2 and 3 represent the cores periodically removed for destructive soil analysis.

5.4.3 Results

The measured NO fluxes emitted from the fertilised soil cores are illustrated in Figure 5.12 and varied from 4 to 27 μg NO-N m^{-2} h^{-1} for the fertilised treatments and from -0.2 to 1.5 μg NO-N m^{-2} h^{-1} for the unfertilised, CON treatment.

There was a highly significant effect of time ($P < 0.001$) on the emission of NO from the soil cores and a highly significant interaction between time and treatment (Table 5.7), such that peaks of NO emission were observed at different times from all 3 treatments. The SUF and MIX treatments showed the maximum loss 14 and 7 days after fertilisation respectively, whereas peak emission from the CON treatment was recorded 1 hour after fertilisation of the other 2 treatments and steadily decreased. Emissions of NO from the CON treatment were consistently small throughout the experiment period and never increased above 1.5 μg NO-N m^{-2} h^{-1}.

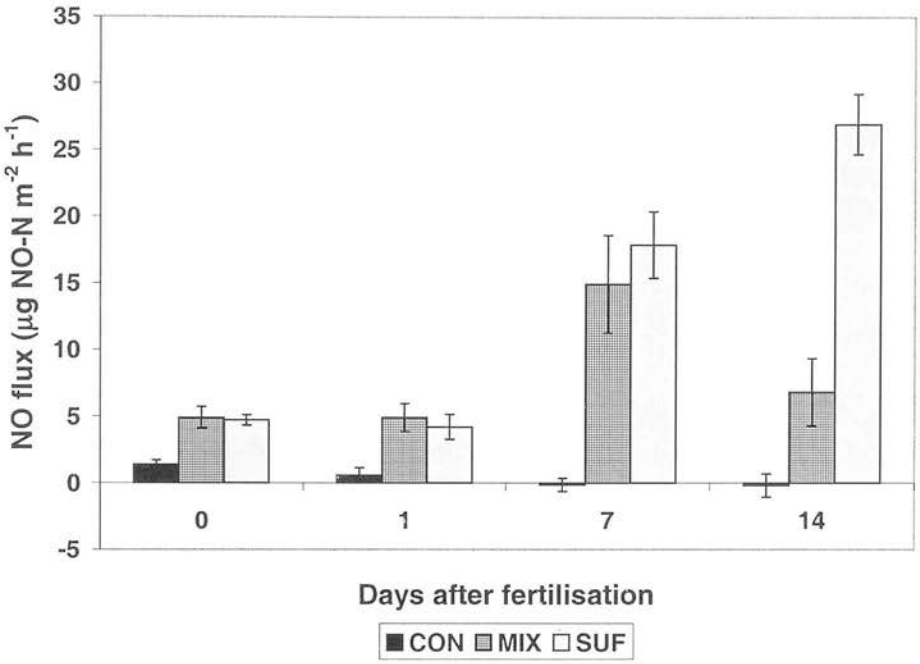

Figure 5.12 Mean NO flux measured from repacked soil cores amended with thermally dried biosolids. Error bars represent ± one standard error of the mean (N=3).

Table 5.7 Table of mean values (treatment and time) produced as a result of repeated measures ANOVA of NO flux (μg NO-N m^{-2} h^{-1}) and mean values (time) of N_2O (μg N_2O-N m^{-2} h^{-1}).

Flux type	Treatment			Degrees of freedom	Standard error of differences of means
	CON	MIX	SUF		
NO	0.4	7.9	13.4	4	2.4

Flux type	Time - Days after fertilisation				Degrees of freedom	Standard error of differences of means
	0	1	7	14		
NO	3.7	3.2	10.9	11.2	18	0.9
N_2O	15.9	21.0	87.4	34.6	18	18.4

Emissions from both the SUF and MIX treatments were initially only slightly stimulated to about 5 μg NO-N m^{-2} h^{-1} and did not increase further at the next measurement occasion, 1 day after fertilisation. Losses from the SUF treatment continued to rise until the measured emission maximum of 27 μg NO-N m^{-2} h^{-1} at day 14, while NO emissions from the MIX treatment declined following the peak emission of *ca.* 15 μg NO-N m^{-2} h^{-1} on day 7.

There was a significant effect of time on the emission of N_2O ($P < 0.05$) as well as a significant interaction between time and treatment ($P < 0.05$). The N_2O emission pattern from the fertilised treatments differed to that measured for NO (Figure 5.12). Unlike NO loss, no stimulation above the level emitted from the CON treatment (18 μg N_2O-N m^{-2} h^{-1}) was apparent from the fertilised treatments 1 hour after application. The loss of N_2O from the CON treatment, however, decreased over the duration of the experiment, similarly to the NO emission. The N_2O emission from the MIX treatment had risen 1 day after fertilisation and peaked at 79 μg N_2O-N m^{-2} h^{-1}, 7 days after fertilisation. In contrast, the measured emission of N_2O from the SUF treatment did not alter from day 0 to 1 day after fertilisation, however, there was a marked increase to the peak emission of 175 μg N_2O-N m^{-2} h^{-1} 7 days after fertilisation. The decline in measured N_2O emission from day 7 to day 14, may have been the result of a mechanical failure, which resulted in a rise in the laboratory temperature to *ca.* 20 °C for 4 days. Even though the cores were watered daily, the rise in temperature may have been sufficient to dry the soil surface and to favour NO production over N_2O. Emissions from both of the fertilised treatments remained elevated above those monitored from the CON treatment at the end of the experiment, 14 days after fertilisation.

A repeated measures ANOVA showed that there was a statistically significant ($P < 0.05$) effect of the fertilisation treatment on the emission of NO. The soil cores amended with thermally dried biosolids stimulated emissions such that they emitted significantly more NO than the unfertilised, CON soil cores (Table 5.7). There was no significant difference ($P > 0.05$), however, in the emission of N_2O between treatments.

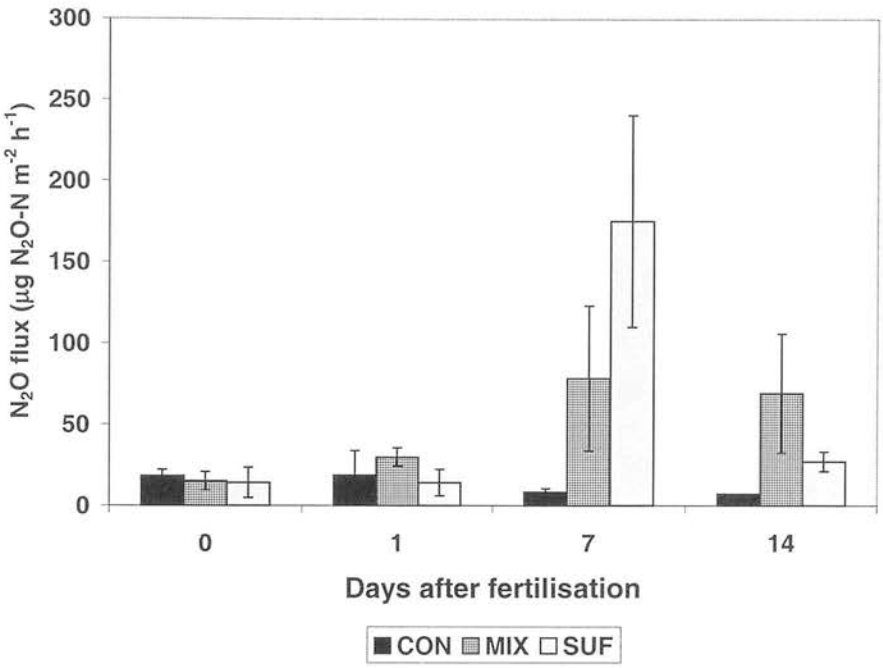

Figure 5.13 Mean N_2O flux measured from repacked soil cores amended with thermally dried biosolids. Error bars represent ± one standard error of the mean (N=3).

Measured NO fluxes were used to calculate potential cumulative losses over a 15 d period following fertilisation. Cumulative NO loss ranged from 2.5 – 230 µg NO-N m^{-2}, with the lowest emission measured from the CON treatment and the highest observed from the SUF treatment (Table 5.8).

The cumulative emission from the MIX and the SUF cores were 58 and 93 times the value from the CON cores respectively. There was a significant effect of fertilisation treatment ($P < 0.05$, d.f = 4) on the emission of NO from the soil cores. Losses of NO were substantially higher from the fertilised cores than from the CON treatment. However, there was only a statistically significant difference between the MIX and CON cores. The percentage of both the applied total and ammoniacal (TAN) nitrogen lost as NO was extremely small and was not significantly different between treatments.

Table 5.8 Cumulative NO losses following the application of thermally dried biosolids and the subsequent percentage of fertiliser nitrogen (total and TAN) lost as NO from the soil cores over the 15 d sampling period

Treatment	Cumulative NO emission ($\mu g\ NO\text{-}N\ m^{-2}$)		Total N applied lost as NO (%)	TAN applied lost as NO (%)
	Total	Net		
CON	2.51 (8.3)	-	-	-
MIX	145.62 (64.3)	143.11	0.0003	0.001
SUF	232.37 (26.4)	229.87	0.0004	0.002

Table 5.9 Cumulative N_2O losses following the application of thermally dried biosolids and the subsequent percentage of fertiliser nitrogen (total and TAN) lost as N_2O from the soil cores over the 15 d sampling period

Treatment	Cumulative N_2O emission ($\mu g\ N_2O\text{-}N\ m^{-2}$)		Total N applied lost as N_2O (%)	TAN applied lost as N_2O (%)
	Total	Net		
CON	171.57 (49.0)	-	-	-
MIX	881.01 (413.5)	709.43	0.001	0.006
SUF	1306.40 (479.3)	1134.82	0.002	0.010

Cumulative N_2O loss varied from 170 – 1300 $\mu g\ N_2O\text{-}N\ m^{-2}$, with the highest emission measured from the SUF treatment and the lowest monitored from the CON treatment (Table 5.9). The cumulative emission from the SUF and the MIX cores were 5 and 8 times the value from the CON cores respectively. Measured N_2O

emissions were used to calculate potential cumulative losses over a 15 d period following fertiliser application, although due to the large variability there was not a statistically significant difference between treatments. The percentage of both the applied total and ammoniacal (TAN) nitrogen lost as N_2O from the SUF treatment was approximately double that lost from the MIX treatment, although there was no significant difference between treatments (Table 5.9).

The reported results from the analysis of the soil variables, WFPS, available NO_3^--N and available NH_4^+-N are only from 1 soil core, therefore, no calculation of significance could be carried out and caution should be taken in their interpretation. Table 5.10 shows the calculated WFPS of the soil cores, which were initially adjusted to a target WFPS of 60%. The WFPS values were relatively consistent in that they did not greatly vary between and within cores. Indeed the mean WFPS over all treatments and both depths was 60%, with an associated CV of only 6%.

Table 5.10 WFPS measured at 2 depths (0-5 cm and 5-10 cm), in repacked soil cores.

Treatment	Depth (cm)	Days after fertilisation		
		1	7	14
CON	0-5	63.4	60.7	50.8
	5-10	61.3	59.1	55.1
MIX	0-5	63.9	56.3	66.5
	5-10	58.9	56.7	65.3
SUF	0-5	61.8	61.1	60.4
	5-10	60.3	57.2	61.8

Table 5.11 demonstrates that during the 2 week experiment the soil NH_4^+-N concentration in the CON treatment was consistently low, below 7 μg g dry soil^{-1} and with no appreciable difference between the top and bottom half of the soil core. The incorporation of thermally dried biosolid pellets to the top 7 cm of soil in the cores increased the NH_4^+-N concentration to *ca.* 20 μg g dry soil^{-1} in the top 5 cm of soil. The concentration continued to rise and peaked at 31 μg g dry soil^{-1} and 10 μg g dry soil^{-1} in the upper and lower soil layers respectively 7 days after fertilisation. The

surface application of the thermally dried biosolid appeared to have little influence on the NH_4^+-N concentration 1 day after fertilisation, since levels throughout the core were < 10 μg g dry $soil^{-1}$. Nevertheless, concentrations rapidly increased in the top 5 cm of soil to > 30 μg g dry $soil^{-1}$ 7 days after fertilisation and remained at this level when measured at 14 days after fertilisation. In contrast, the NH_4^+-N concentrations of SUF in the lower 5 cm of soil were comparable to those in the CON treatment 7 and 14 days after fertilisation.

Table 5.11 Soil NH_4^+-N measured at 2 depths (0-5 cm and 5-10 cm), in repacked soil cores.

Treatment	Depth (cm)	Days after fertilisation		
		1	7	14
CON	0-5	2.2	7.1	1.7
	5-10	2.4	3.7	1.5
MIX	0-5	19.6	30.8	5.9
	5-10	5.9	9.9	3.3
SUF	0-5	7.5	34.2	34.6
	5-10	6.5	2.4	1.5

Similar to the NH_4^+-N concentrations measured in the soil cores, throughout the study period, the soil NO_3^--N levels in the CON treatment were low at < 7 μg g dry $soil^{-1}$, with no discernible difference in concentration between the 2 halves of the soil core (Table 5.12). In both of the biosolid amended treatments, there was no clear evidence of elevated soil NO_3^--N concentrations in either the upper of lower soil layers 1 day after fertilisation, since NO_3^--N levels were measured at *ca.* 7 μg g dry $soil^{-1}$. However, in both the MIX and SUF treatments soil NO_3^--N levels in the top 5 cm of soil steadily increased to a peak, 14 days after fertilisation. At both the measurement occasions 7 and 14 days after fertilisation, this NO_3^--N concentration in the MIX treatment was more than twice as high as that in the SUF treatment. Furthermore, the NO_3^--N concentration in the bottom 5 cm of the soil core measured from the MIX treatment similarly increased to a peak 14 days after fertilisation,

whereas that in the lower half of the SUF treatment remained at a comparable concentration to that measured in the CON treatment.

Table 5.12 Soil NO_3^--N measured at 2 depths (0-5 cm and 5-10 cm), in repacked soil cores.

Treatment	Depth (cm)	Days after fertilisation		
		1	7	14
CON	0-5	5.1	5.9	7.1
	5-10	5.1	6.1	5.7
MIX	0-5	7.9	36.1	71.2
	5-10	6.0	13.1	33.0
SUF	0-5	6.8	14.9	29.7
	5-10	6.7	7.7	6.8

5.4.4 Discussion

Cumulative NO fluxes were 93 and 1.6 times larger from the surface applied thermally dried biosolids (SUF) than from the unfertilised (CON) and incorporated thermally dried biosolids (MIX) treatments respectively. Nevertheless, there was only a statistically significant effect of fertiliser treatment between the SUF and CON treatments. A similar emission pattern was evident for the loss of N_2O from the soil cores, i.e. emissions were in the order SUF > MIX > CON. There was, however, no significant difference between the cumulative N_2O emissions from any of the treatments, presumably because of the large variability (CVs ranged from 3-140%).

It may have been possible to detect significant differences with more data points and with an increased study period. With only 4 measurement occasions the NO emission at each occasion was strongly weighted in the calculation of the cumulative flux and therefore, the interpretation between points may not have been entirely reliable. As was shown in the previous laboratory experiment, the SSP treatment demonstrated enhanced fluxes at 31 days after fertiliser application. This experiment only continued until 14 days after fertilisation, but the discrepancy in emissions may have become more pronounced, especially if the NO loss from the

MIX treatment continued to decline. In support of this idea, the emission of NO from the SUF treatment increased throughout the experiment, whereas that from the MIX treatment approximately halved from 7 to 14 days after fertilisation.

It is hypothesised that the emissions of NO from the SUF treatment have the potential to be larger than from the MIX treatment because the release of NH_4^+-N from the mineralisation of biosolid organic N was concentrated in the upper, most aerated and biologically active few cm of the soil. It had previously been suggested that nitrification was the probable source process for NO emission from the pellets, consequently losses of NO would increase from sites with a continuous NH_4^+-N substrate resource, a high nitrifier population, a good O_2 supply and a short diffusion pathway. In contrast the biosolid in the MIX treatment was distributed throughout the top 7 cm, which would increase the diffusion path length for both incoming O_2 and outgoing NO. Indeed the analysis of NH_4^+-N from the soil SUF cores indicated that levels were only enhanced in the top 5 cm, whilst the concentration in the bottom 5 cm did not appear to be any different to that in the CON treatment. Ammoniacal-N in the MIX treatment, however, increased in the lower 5 cm 7 days after biosolid application.

Notably, the soil NH_4^+-N concentration in the surface of the MIX treatment had apparently increased only 1 day after fertiliser application, whereas that in the SUF treatment did not increase to values seen in the field until measurement 7 days after fertilisation. It is suggested that the pellets incorporated into the soil would be in more favourable conditions for decomposition than those on the surface. The pellets mixed into the soil would be contained within a moist environment which is ideal for decomposition, whereas those on the soil surface would be subjected to a much drier environment, particularly without precipitation. In the field, however, the effect of rainfall on surface applied pellets may be at least partially offset by the desiccating action of wind and solar radiation.

The soil mineral N analyses supported the suggestion that indeed decomposition, hence mineralisation of organic N proceeded at a far greater rate in the MIX treatment than in the SUF treatment. In the MIX treatment not only were the soil NH_4^+-N levels elevated only 1 day after biosolid application, but the concentration had dramatically declined to *ca.* background levels 14 days after

fertilisation. Over the same period, the soil NO_3^--N content in both the top and bottom halves of the soil cores significantly increased, such that at 7 and 14 days after fertilisation the concentration of NO_3^--N measured in the MIX treatment was > twice that recorded in the SUF treatment. The large NO_3^--N accumulation would suggest a high nitrification rate of the mineralised NH_4^+-N and hence a higher NO emission would be expected from the MIX treatment. This was not the case and implies consumption of NO during its diffusion through the soil to the surface.

A greater, more rapid accumulation of NO_3^--N over 18 weeks was also reported following the incorporation of anaerobically digested sewage sludge, compared to that from sludge which had been surface applied (King, 1973). The results obtained in this experiment and by King (1973) differed to those recorded by Quemada *et al.* (1998) and Terry *et al.* (1979). Quemada *et al.* (1998) reported that apparent mineralisation of anaerobically digested sewage sludge was 1.5 times greater from surface placement rather than incorporated, consequently more NO_3^--N was reported in the surface applied treatments. This difference was attributed to more favourable moisture and temperature conditions at the surface (5°C higher than in this experiment). Also in a laboratory study undertaken by Terry *et al.* (1979), surface application of liquid synthetic sludge decomposed more rapidly than incorporation of freeze-dried synthetic sludge. They concluded was that intimately mixing the sludge with soil reduced decomposition due to the association of sludge organic material with soil and inorganic constituents. Interestingly, no comment was made with respect to the differing composition of the sludge used in the 2 placements.

A smaller NO_3^--N concentration in the SUF treatment may suggest higher denitrification rates. In the well oxygenated soil surface, however, lower denitrification rates would be expected compared to a treatment where the biosolids were incorporated. The soil environment in the MIX treatment would potentially be less aerated and with a higher decomposition rate providing denitrifier substrates and reducing O_2 levels, an anaerobic site would be likely to form around each pellet. A higher denitrification rate in the MIX treatment was, however, not reflected in the emission of N_2O.

Table 5.13 NO:N_2O ratios for losses measured from repacked soil cores amended with thermally dried biosolids.

Treatment	Day after fertilisation			
	0	1	7	14
CON	**0.08**	**0.03**	**-0.02**	**-0.03**
MIX	**0.32**	**0.16**	**0.19**	**0.10**
SUF	**0.33**	**0.30**	**0.10**	**0.99**

The ratio NO:N_2O suggests that in this experiment both nitrification and denitrification were in operation (Table 5.13), although the accumulation of NO_3^--N over the experiment indicated that nitrification was highly important. What is more, in the MIX treatment the rate of soil NH_4^+-N decline measured 7 and then 14 days after fertilisation was approximately balanced by the increase in NO_3^--N, which does not suggest intense denitrification. Nonetheless, N_2O emissions appeared to be larger from the MIX treatment compared to the SUF treatment 14 days after biosolid application, although the difference was not statistically significant. In the organic and inorganic fertiliser laboratory experiment it was observed that peak N_2O emissions from injected organic wastes were not as large as those measured in the field. This was attributed to the absence of precipitation and the associated formation of anaerobic sites suitable for intense denitrification. It is suggested that a higher decomposition rate providing denitrifier substrates after incorporation of thermally dried sewage sludge pellets in a field situation may induce bursts of N_2O production following increased soil anaerobicity as a result of rainfall. Furthermore, with an increased concentration of NO_3^--N in the soil following incorporation of the pellets, leaching of this ion may increase. It is possible that incorporation may result in 'pollution swapping', with decreased emissions of NO and potentially NH_3, but a rise in N_2O production and NO_3^--N leaching. The N dynamics resulting from incorporation of thermally dried sewage sludge pellets, therefore, need to be investigated further.

5.4.5 Conclusion

- This laboratory study showed that unfertilised soil cores significantly reduced NO emissions over cores applied on the surface with thermally dried sewage sludge pellets by 99%. This confirmed previous laboratory and field studies which demonstrated the stimulating effect of sewage sludge pellets on NO emission. There was no significant difference in NO emissions from cores with surface applied sludge pellets and cores with sludge pellets mixed into the top 7 cm of soil.
- There was no significant difference in the percentage of total N or total ammoniacal N applied lost as NO and as such no definite conclusions can be reached on the effect of placement of sewage sludge pellets in/on the soil.
- A more intense NO sampling frequency over a longer period may have shown a significant difference in NO emission between the surface placed and mixed treatments or between mixed and control treatments.
- A preliminary suggestion may be that the incorporation of thermally dried biosolids may reduce the long-term NO emission and early indications are that such a strategy would not significantly increase N_2O fluxes, although the potential for N_2O emission is liable to increase in a field situation. More research on potential 'pollution swapping' as a result of pellet incorporation as a possible NO abatement technique is needed to provide firm conclusions.

5.5 Water-filled pore space (WFPS) experiment

5.5.1 Introduction

The property of water filled pore space (WFPS) is a highly useful and relevant soil variable to calculate in relation to gaseous emissions of NO and N_2O. As its name suggests, WFPS indicates the water content of the soil, but with reference to the amount of soil air also present. This is crucial in the context of NO and N_2O emissions as it indicates the potential anaerobicity of the soil environment and the ease of gaseous diffusion into and out of the soil. A soil which has a relatively high WFPS will contain more water, hence anaerobic conditions are liable to predominate. Furthermore, gaseous diffusion of O_2 into the soil will be restricted, as will diffusion of NO and N_2O, out of the soil into the atmosphere. Consequently, the source process of NO and N_2O will be heavily influenced, with nitrification dominant in more aerobic soils and denitrification as the major process in more anaerobic soils.

Field studies carried out in 1998 and 1999 at the arable Beechgrove site highlighted the potential importance of WFPS in strongly influencing the emissions of both NO and N_2O. It was not always possible, however, to isolate the potential effect of WFPS on NO emission from the effect of soil mineral nitrogen levels and crop growth. The aim of this study was, therefore, to use laboratory cores to clarify the role of WFPS in influencing the magnitude of NO emission and its source process in this agricultural soil typical of the Midland Valley of Scotland.

5.5.2 Sites and Treatments

The soil used in this study was collected from the top 10 cm of an unfertilised, recently ploughed research plot located at the Beechgrove (BG) experimental site, Bush Estate, *ca.* 15 km south of Edinburgh (NT243628). For a more detailed history of the field see chapter 3. Initial analysis indicated a soil pH of 6.3, and an exchangeable soil NH_4^+-N and NO_3^--N concentration of 1.28 (0.11) and 0.38 (0.08) $\mu g\ g^{-1}$ dry soil respectively (numbers in parentheses represent the standard error of the mean).

The soil cores were adjusted to yield six treatments of either 50, 60, 65, 70, 78 or 90% WFPS (Figure 5.14). This range of WFPS was selected to mirror the extent of the moisture conditions measured during the field experiments at the Beechgrove site (Chapter 3). The WFPSs of 78% was chosen as it represents field capacity at the Beechgrove site and 65% was chosen as it represents an apparently important marker, identified from the Beechgrove field trials (Chapter 3), between the process of nitrification or denitrification as the dominant source of emission. Throughout the experiment the soil cores were kept in a controlled environment room at a temperature of between 15 and 16 °C (target temperature of 15 °C), which corresponds to the mean surface temperature measured at the time of NO sampling at Beechgrove in 1998.

All cores were amended with a solution of NH_4NO_3 to yield an application rate of 80 kg N ha^{-1}, i.e., equivalent to that used at the Beechgrove site (Chapter 3). The solution was applied by pipette one hour before the first gas sampling occasion. The use of a pipette was shown to be more accurate than a spray bottle, where much of the solution landed on the inside walls of the column and induced an edge effect.

B	70	65	78	90	60	50
1	90	78	65	70	60	50
3	65	70	90	50	78	60
2	65	70	90	60	78	50
C	60	78	50	70	65	90
A	90	50	65	78	60	70

Figure 5.14 A plan view of the soil core layout (treatments shown are a WFPS of 50, 60, 65, 70, 78 and 90%) as positioned in the controlled temperature room. Rows A, B and C represent the 3 treatment blocks used for gas sampling and rows 1, 2 and 3 represent the cores periodically removed for destructive soil analysis.

Emissions of both NO and N_2O were measured on day 0, 1, 2, 4, 7 and 16. Available soil NH_4^+-N, NO_3^--N and moisture content were determined on 1 soil core per treatment, which were removed on day 0, 1, 4 and 16. On day 16, however,

following the last gas sampling, these cores were themselves used for the soil analysis determinations. Consequently, the results were derived from 3 cores per treatment. The layout of the soil cores is shown by treatment in Figure 5.14.

5.5.3 Results

The NO fluxes ranged from -0.1 to 22 μg NO-N m^{-2} h^{-1} (Figure 5.15). The measured NO emission peaked from all six treatments 1 hour after fertilisation and generally, steadily declined until the last measurement occasion, 16 days after fertilisation. Negative NO fluxes were observed twice and only from the 90% treatment, furthermore fluxes from this treatment were consistently detected at < 1.0 μg NO-N m^{-2}.

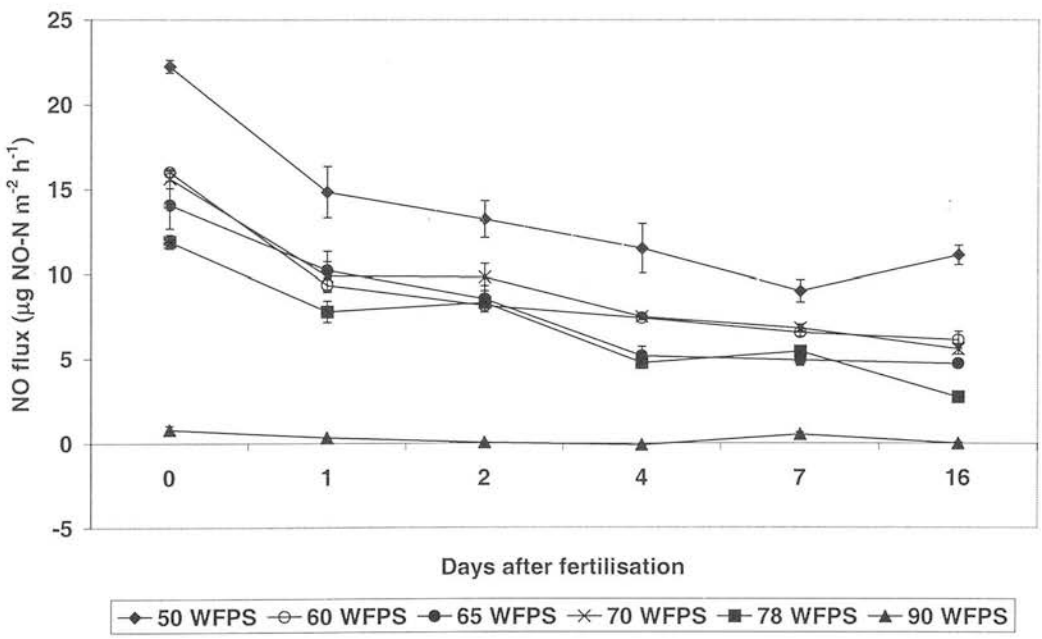

Figure 5.15 Mean NO flux measured from repacked soil cores adjusted to a specific WFPS. Error bars represent one standard error of the mean (N=3).

Losses of N_2O were measured from the identical soil cores to those used for the NO emission determination. The N_2O fluxes are shown in Figure 5.16 and varied from -3 to 530 μg N_2O-N m^{-2} h^{-1}. Peak N_2O emissions were substantially larger than

the peak NO fluxes, such that maximum N_2O emissions were approximately 24 times bigger.

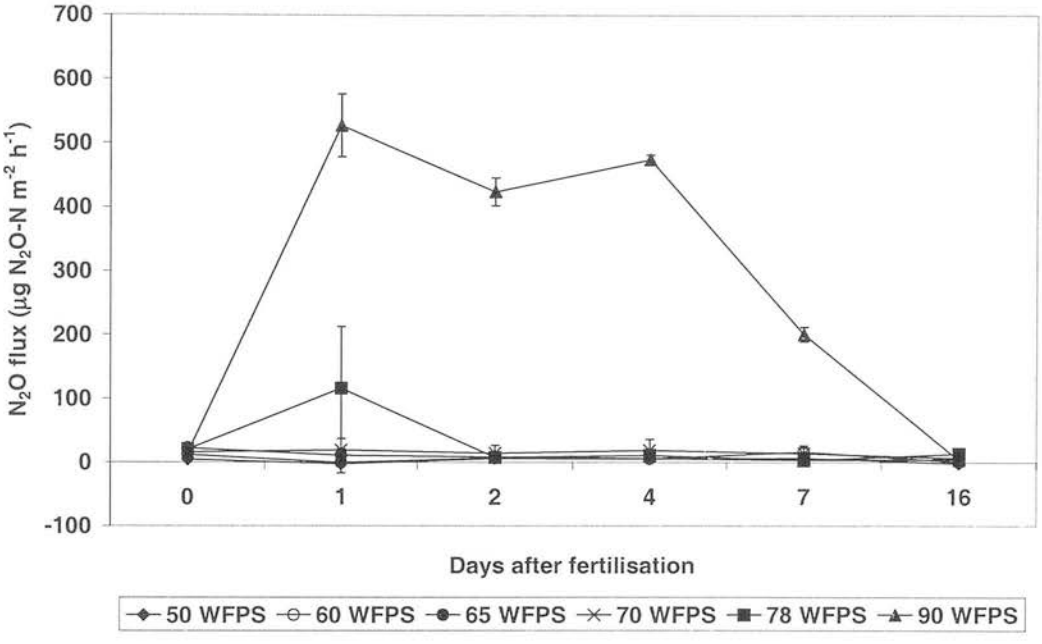

Figure 5.16 Mean N_2O flux measured from repacked soil cores adjusted to a specific WFPS. Error bars represent one standard error of the mean (N=3).

In contrast to the NO fluxes, there was little uniformity in the pattern of measured peak N_2O emissions from each of the 6 treatments. Negative N_2O fluxes were observed on 3 measurement occasions from the 50% and 60% treatments, which coincided with emission minima and also the last measurement occasion from the 50% treatment (Figure 5.16).

Cumulative NO loss ranged from 4 – 195 µg NO-N m^{-2}, with the lowest emission measured from 90% WFPS treatment and the highest observed from the 50% WFPS treatment (Table 5.14). The cumulative emission between these 2 extremes of the WFPS range studied differed by a factor of *ca.* 45. A highly significant effect of WFPS ($P < 0.001$, d.f = 10) on the NO flux was recorded, such that emissions of NO from 78%, 70%, 65%, 60% and 50% were significantly larger than from the 90% treatment, and losses from the 50% were also significantly greater than from the 78%, 70%, 65% and 60% treatments (Tukey test, $P < 0.05$).

Table 5.14 Cumulative NO losses over the 17 d sampling period from repacked soil cores adjusted to confer various WFPS

WFPS (%)	Cumulative NO emission (μg NO-N m^{-2})	Standard error of the mean
50	195.6	24.8
60	126.0	2.6
65	105.0	15.0
70	131.4	7.4
78	90.6	7.9
90	4.3	1.5

Cumulative N_2O emissions varied from 60 – 3600 μg N_2O-N m^{-2}, so that the lowest cumulative emission measured from the 50% WFPS treatment was more than 60 times smaller than the highest monitored emissions from the 90% treatment (Table 5.15).

Table 5.15 Cumulative N_2O losses over the 17 d sampling period from repacked soil cores adjusted to confer various WFPS

WFPS (%)	Cumulative N_2O emission (μg N_2O-N m^{-2})	Standard error of the mean
50	59.2	28.2
60	146.6	49.2
65	112.5	33.5
70	218.9	96.2
78	232.5	72.4
90	3590.9	65.1

There was a highly significant effect of the WFPS level ($P < 0.001$, d.f = 10) on the emission of N_2O from the soil surface of the cores. The results generated from the Tukey multiple comparison test, however, demonstrated that there was only a

statistically significant difference between the 90% treatment and all the others ($P < 0.05$) (Table 5.15).

Cumulative emissions of NO and N_2O were comparable in magnitude from the 60% and 65% treatments, whereas NO emissions were larger from the 50% treatment and N_2O losses were greater (although not significantly) from the 70% and 78% treatments, and (specifically) much higher from the 90% treatment (Tables 5.14 and 5.15).

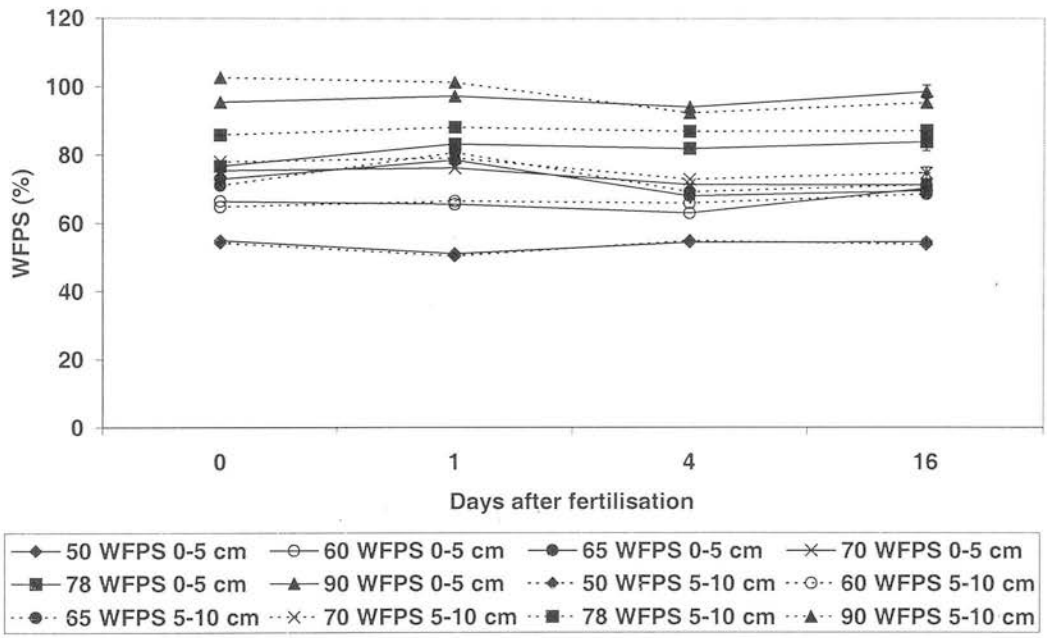

Figure 5.17 WFPS measured at 2 depths (0-5 cm and 5-10 cm), in repacked soil cores adjusted to confer various WFPS. Error bars (only for 16 days after fertilisation) represent one standard error of the mean (N=3).

Figure 5.17 demonstrates the variability in WFPS between soil cores from the same treatment. It was noticeable that typically the measured WFPS was approximately 5% wetter than the target WFPS. In general, the difference in WFPS between 0-5 cm and 5-10 cm core depth was less than the variability between cores, although frequently the WFPS of the upper soil layer is drier than the lower due to evaporation and downward percolation of water.

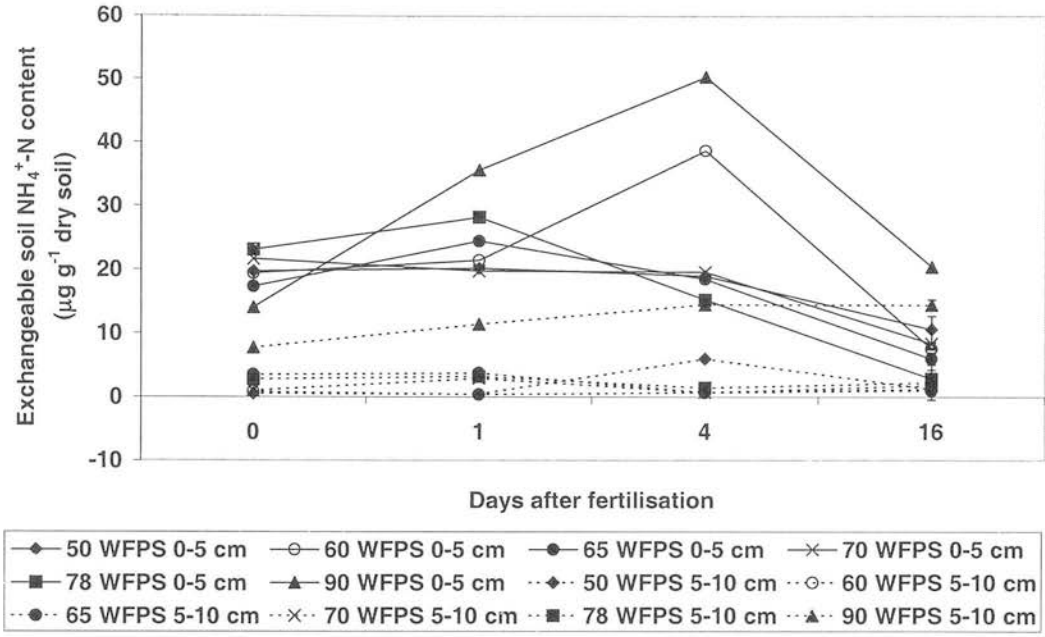

Figure 5.18 Soil NH_4^+-N measured at 2 depths (0-5 cm and 5-10 cm), in repacked soil cores adjusted to confer various WFPS. Error bars (only for 16 days after fertilisation) represent one standard error of the mean (N=3).

As with both the WFPS and the soil NO_3^--N, the soil NH_4^+-N concentration values reported (except from the last sample occasion) are only from 1 soil core and, therefore, caution should be taken since they may not be truly representative. Nonetheless, the data in figure 5.18 indicated that for the first 5 days of the experiment, the soil NH_4^+-N concentration in the top 5 cm of the cores remained relatively constant, but had diminished by > half at the last sampling occasion, 16 days after fertilisation. The NH_4^+-N concentration in the 90% treatment, however, did not show the substantial decline of the other treatments. Moreover, the NH_4^+-N concentration in the bottom 5 cm of the soil cores from the 90% treatment was maintained at *ca.* 10 µg g dry soil^{-1}, whereas the concentration in the other treatments was generally < 4 µg g dry soil^{-1}.

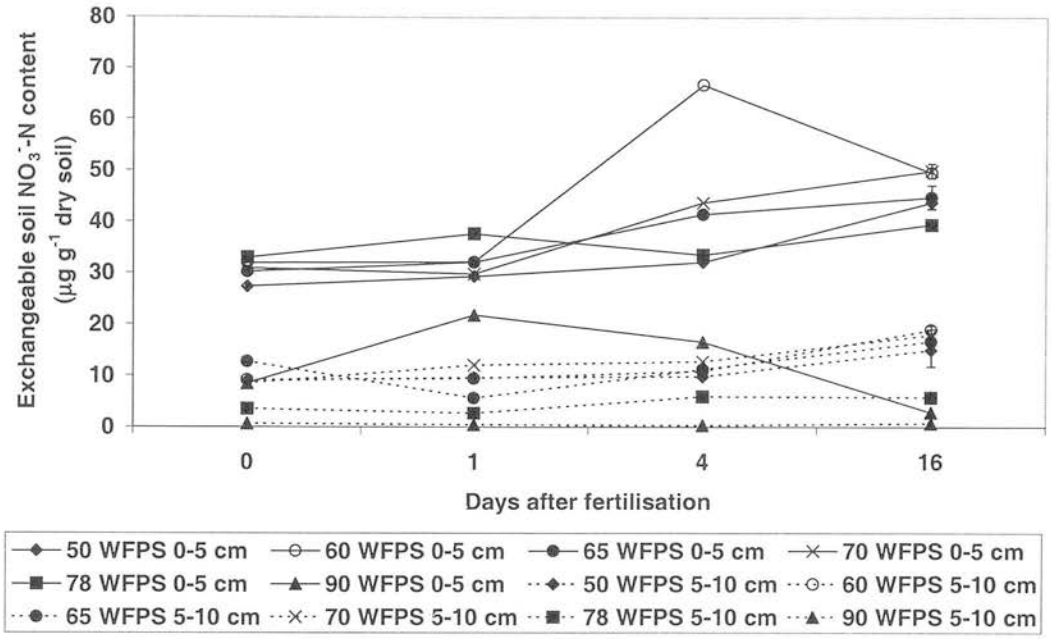

Figure 5.19 Soil NO_3^--N measured at 2 depths (0-5 cm and 5-10 cm), in repacked soil cores adjusted to confer various WFPS. Error bars (only for 16 days after fertilisation) represent one standard error of the mean (N=3).

The soil NO_3^--N concentration in the soil cores from the 50%-70% treatments exhibited a typical pattern directly opposite to that shown with the soil NH_4^+-N concentration (Figure 5.19). Over the length of the experiment the soil NO_3^--N concentration commonly increased from about 30-40 µg g dry soil^{-1} and 10-20 µg g dry soil^{-1} in the upper and lower halves of the cores respectively. The soil NO_3^--N concentration in both the upper and lower soil layers of the 78% treatment appeared to remain relatively constant throughout the experiment, although at < 6 µg g dry soil^{-1} in the bottom half of the cores. The 90% cores demonstrated a different pattern in soil NO_3^--N concentration to either of the other treatments. In the top half of the cores the levels were never greater than 22 µg g dry soil^{-1} and showed a significant drop between 4 and 16 days after fertilisation to < 3 µg g dry soil^{-1}. Furthermore, the soil NO_3^--N concentration in the lower half of these cores was never > 1µg g dry soil^{-1}.

There was a highly statistically significant ($P < 0.001$) effect of WFPS on the NO flux (Table 5.16). The more oxygenated, drier soil cores with a lower WFPS

stimulated emissions such that significantly more NO was released than from the wetter soil cores. There was a highly statistically significant ($P < 0.001$) effect of WFPS on the emission of N_2O (Table 5.16). The wetter soil cores with a higher WFPS stimulated emissions such that they emitted significantly more N_2O than the drier soil cores. There was also a highly statistically significant ($P < 0.001$) effect of time on both the NO and N_2O emissions (Table 5.16), as well as a highly significant interaction between time and WFPS indicating that the effect of time on NO/N_2O emission was not consistent between WFPS treatments.

Table 5.16 Table of means (treatment and time) produced as a result of repeated measures ANOVA of NO (μg NO-N m^{-2} h^{-1}) and N_2O (μg N_2O-N m^{-2} h^{-1}) emitted over 16 days from repacked soil cores adjusted to a specific WFPS.

Flux type	Treatment - WFPS (%)						Degrees of freedom	Standard error of differences of means
	50	60	65	70	78	90		
NO	13.7	8.9	7.9	9.2	6.8	0.3	10	0.9
N_2O	3.0	6.7	9.3	14.3	29.8	277.1	10	10.8
Flux type	**Time - Days after fertilisation**						**Degrees of freedom**	**Standard error of differences of means**
	0	**1**	**2**	**4**	**7**	**16**		
NO	13.4	8.7	8.0	6.0	5.5	5.0	60	0.5
N_2O	14.7	110.4	77.8	88.8	44.2	4.3	49	7.6

The analysis of replicated cores for WFPS and soil NH_4^+-N and NO_3^--N concentration on the last measurement occasion showed a highly statistically significant ($P < 0.001$) effect of WFPS (Table 5.17). There was also a highly significant ($P < 0.001$) effect of soil depth on the NH_4^+-N and NO_3^--N concentrations, although not on WFPS (Table 5.17). Additionally, a significant

interaction was observed between WFPS treatment and soil depth, such that at a higher WFPS there was a larger influence of depth on the soil NO_3^--N concentration ($P < 0.001$), conversely at a lower WFPS soil NH_4^+-N was more strongly affected by soil depth ($P < 0.05$) (Table 5.17).

Table 5.17 Summary of ANOVA results (*P* values and associated level of significance) for soil NO_3^-, soil NH_4^+ and WFPS at 16 days after fertilisation

Data	Treatment	*P*-value	Significance level
Soil available NO_3^-	WFPS	<0.001	***
	Depth	<0.001	***
	WFPS x Depth	<0.001	***
Soil available NH_4^+	WFPS	<0.001	***
	Depth	<0.001	***
	WFPS x Depth	0.027	*
WFPS	WFPS	<0.001	***
	Depth	NS	-
	WFPS x Depth	NS	-

*** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$; NS = not significant

A highly significant ($P < 0.001$) relationship between measured WFPS in the top 5 cm of the soil cores and both NO ($r^2 = 0.53$) and ln N_2O ($r^2 = 0.46$) emission from all treatments was observed. Figure 5.20 demonstrates that as the WFPS increased so did the N_2O emission and the NO emission decreased.

No significant relationships ($P > 0.05$) were demonstrated between NO emission from all the treatments and either soil NH_4^+-N or soil NO_3^--N in the top 5 cm of soil. Nonetheless, a combination of soil NO_3^--N and WFPS was able to explain 62% of the measured variation in the NO data ($P < 0.001$). A significant ($P < 0.001$) positive relationship was observed, however, between ln N_2O from all treatments and soil NH_4^+-N concentration in the top 5 cm of soil ($r^2 = 0.40$). A greater proportion of

the variation in the N_2O data ($r^2 = 0.67$) could be explained by a combination of soil NH_4^+-N and WFPS ($P < 0.001$).

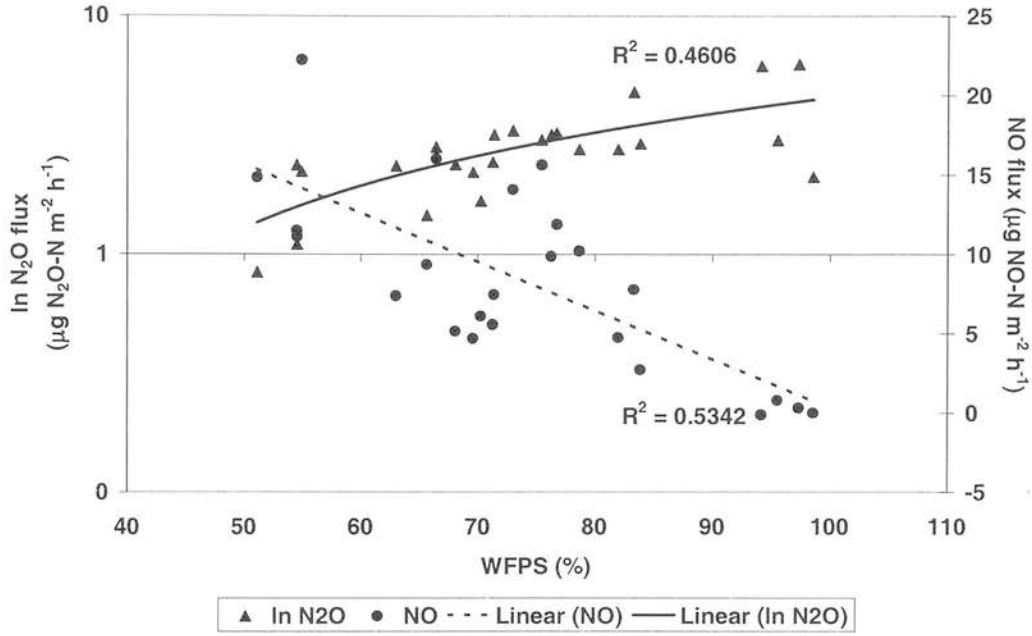

Figure 5.20 The relationship between both NO and N_2O (ln transformed) emission and soil WFPS measured from the top 5 cm of repacked soil cores adjusted to a specific WFPS (N=3). Note the log scale of the y axis.

An inverse relationship was established between NO ($r^2 = 0.92$; $P < 0.05$) emission and soil NO_3^--N from the 65% WFPS treatment. In contrast a statistically significant ($P < 0.05$) positive correlation ($r^2 = 0.93$) was observed between soil NO_3^--N and ln transformed N_2O emission from the 90% treatment.

Soil NO_3^--N, NH_4^+-N and WFPS from the bottom 5 cm of the soil cores exhibited comparable, although generally weaker relationships with NO and N_2O as those formed with the top 5 cm of soil. A highly significant ($P < 0.01$) negative correlation ($r^2 = 0.99$) was also observed between NO emission and soil NH_4^+-N from the 90% treatment.

On all four of the sampling occasions when soil analyses were carried out, soil WFPS was able to account for 83-95% of the variation in measured NO emissions from all treatments ($P < 0.05$ to $P < 0.01$). All of these relationships were

linear and negative, that is the NO emission decreased as the WFPS increased. In contrast, only on the second and third days after fertilisation was there a significant positive relationship between ln transformed N_2O emission and WFPS across all the treatments, such that 91% ($P < 0.01$) and 68% ($P < 0.05$) of the variation in the data could be accounted for respectively.

5.5.4 Discussion

There was a statistically significant effect of WFPS on both the emission of NO and N_2O and regression analysis demonstrated that NO emissions decreased with an increase in WFPS, whilst emissions of N_2O decreased. The magnitude of cumulative NO fluxes also generally declined with an increase in WFPS, consequently the largest emission was recorded from the 50% treatment and the smallest from the 90% treatment. In contrast, cumulative N_2O emissions typically increased with an increase in the WFPS, accordingly the smallest emission was measured from the 50% treatment and the biggest from the 90% treatment.

The observed difference in emission was expected, since NO commonly decreases with an increase in moisture content over the WFPS range studied (Davidson, 1993). Nitrification is commonly believed to be the dominant source process for the production of NO, but it requires O_2 in order to function. Consequently, with an increase in WFPS, there is a simultaneous decrease in the quantity of air and therefore O_2 in the soil. The opposite is usually true with respect to the production of N_2O. Anaerobic denitrification is typically regarded as the major contributor to the generation of N_2O, therefore, an increase in soil WFPS will concomitantly reduce the soils' aeration and favour denitrifier activity (Davidson, 1993).

The results of several studies support the results of this experiment. Using both a clay loam and a sandy loam in, a laboratory incubation, it was demonstrated that the ratio of NO/N_2O was a function of the soil water content with more NO evolved in drier soils and relatively more N_2O generated in wetter soils (Drury *et al.* 1992). Gödde & Conrad (1998) recorded that an increase in the soil water content from 30 to 60% maximum water-holding capacity caused a decrease in the contribution of nitrification to the production of NO. Similarly, the emission of NO

from a sandy loam soil rapidly diminished as the WFPS increased from 43% to 90% (Ormeci *et al.* 1999).

In this soil it is apparent that emissions of NO and N_2O do not dramatically differ between the WFPS treatments of 60-78% (Figure 5.15 and Table 5.16), which approximately corresponded to 65-80% WFPS in the soil cores (Figure 5.17). However, the lack of a significant difference may be the result of the difficulties experienced in achieving the target WFPS, which can be attributed to a combination of the variability still found within the repacked soil cores and an inaccurate balance. Nonetheless, it is likely that the lack of a significant difference was related to the source processes dominant at each WFPS treatment.

Not only did the soil NH_4^+-N concentration decline and the soil NO_3^--N content increase over the study period from the 50% treatment, but similar changes in the mineral N content were shown in the 50-70% WFPS treatments. The soil NO_3^--N concentration from the 78% treatment, however, did not appear to alter over the study period. This general trend in the soil mineral N content is typically indicative of nitrification and would therefore suggest that nitrification did operate in this sandy loam soil at least up to a true WFPS value of 80% i.e. field capacity. Similarly, Gödde & Conrad (1998) recorded the presence of nitrification at a water holding capacity of 80% and Abbasi & Adams (2000) reported nitrification at 84% WFPS.

The calculated NO:N_2O ratios (Table 5.18) also support the suggestion that nitrification was taking place in the soil. Laboratory cultures of nitrifiers have shown that an NO:N_2O ratio > 1 is a sign of gaseous N production by nitrification (Lipschultz *et al.* 1981; Anderson & Levine, 1986). Such a condition was fulfilled on every measurement occasion from the 50% treatment, which would account for the high NO and the low N_2O emissions recorded. Furthermore, nitrification generally appeared to be a highly significant gaseous N production process for both the 60% and 65% treatments, although similar to the majority of the results calculated from the 70% and 78% treatments, several ratios were < 1. Consequently, the suggestion is that both nitrification and denitrification were operating in these 4 WFPS treatments.

The NO:N_2O ratio calculated using the emissions from the 90% treatment signified the presence of intense denitrification (Table 5.18). An NO:N_2O ratio of < 0.01 has been shown to indicate that denitrification is the main source process for N

gas production (Lipschultz *et al.* 1981; Anderson & Levine, 1986). With the exception of the measurement taken only 1 hour after the addition of fertilisation, when the denitrifier population may not have been fully active, the NO:N_2O ratio was well below < 0.01.

Table 5.18 NO-N:N_2O-N ratios for losses measured from repacked soil cores adjusted to a specific WFPS.

Treatment	Day after fertilisation					
	0	1	2	4	7	16
50	5.2	-5.5	2.2	2.0	1.4	-5.6
60	1.4	-13.0	1.2	1.4	0.4	18.6
65	0.6	1.0	1.0	0.9	1.5	1.1
70	1.0	0.5	0.7	0.4	0.5	0.9
78	0.6	0.1	1.1	0.4	1.9	0.2
90	0.0516	0.0006	0.0001	-0.0002	0.0026	-0.0050

Further evidence for intense denitrification was demonstrated by the soil mineral N analyses. Unlike the other WFPS treatments, there was no apparent difference between the soil NH_4^+-N concentration measured 1 hour after fertilisation and that measured 16 days after the addition of NH_4NO_3 fertiliser. This would suggest the absence of nitrification in the 90% treatment. In contrast the soil NO_3^--N concentration was approximately 6 times larger 4 days after fertilisation than 16 days after fertiliser application. It seems that 16 days after fertilisation, the soil NO_3^--N concentration had declined to such a small level that denitrification became substrate limited. The reliance of N_2O production from the 90% treatment on soil NO_3^--N was demonstrated by the significant positive relationship obtained between the 2 parameters, such that the magnitude of the N_2O emission increased with a rise in the NO_3^--N concentration.

It was not surprising that in this laboratory study, the soil WFPS would be a highly influential variable on the magnitude of both the NO and N_2O fluxes. Indeed, WFPS was able to explain 83-95% of the variation in the NO data obtained across all treatments per measurement occasion, such that the NO emission was stimulated to a

greater extent in the drier soils than the wetter ones. Similarly, WFPS could account for 68-91% of the variation in the N_2O flux generated from all treatments on each sampling day. The strong controlling nature of WFPS, however, was not apparent 1 hour or 16 days after fertiliser application.

At the Beechgrove field site, where the soil used in this laboratory experiment was collected from, the results from the 1998 field campaign indicated a large decrease in the emission of NO mid-way through the experiment. Concurrent with this NO decline was a marked decline in the soil NO_3^--N concentration, a rise in the WFPS and continued uptake of mineral N by the growing barley crop. It was suggested that the primary reason for the change in NO production was the result of the increase in WFPS from *ca.* 55 to *ca.* 70%. The results from this laboratory study support this idea, as it has been shown that there is a significant difference between NO emissions from the 50% treatment and that at 70%. Furthermore, the small and occasionally negative NO fluxes measured in 1999 from the direct drilled plots with a WFPS of *ca.* 90% were mirrored in this experiment and can be attributed to intense denitrifier activity.

It is possible that the importance of nitrification in this soil was exaggerated compared to the situation which may exist in the field. During soil core formation, larger anaerobic soil aggregates were likely to have been destroyed, thus the soil would have become more aerated as a consequence of directly exposing more soil to the atmosphere. Consequently, it was likely that the potential for aerobically driven processes e.g. nitrification increased, whereas the capacity for anaerobically controlled processes e.g. denitrification may be lower than in the field situation.

5.5.5 Conclusion

- In this laboratory study we have discovered that a soil WFPS of 90% significantly reduces NO emission over a WFPS of 50% by 98%. However, the 'price' is an increase in the emission of the potent greenhouse gas N_2O of nearly 6000%.

- Ratios of NO:N_2O and soil mineral N trends over the experimental period demonstrated that NO and N_2O fluxes from the soil cores adjusted to 50% WFPS were generated entirely from the nitrification process.
- Ratios of NO:N_2O and soil mineral N trends over the experimental period demonstrated that NO and N_2O fluxes from the soil cores adjusted to 90% WFPS were generated entirely from the denitrification process.
- The results show that there was no significant difference in the magnitude of NO or N_2O emissions from the 60-78% WFPS treatments, which appeared to be produced by a combination of nitrification and denitrification.
- This laboratory study confirms the field data that over the typical range of WFPSs encountered in the field at Beechgrove, both nitrification and denitrification were proceeding simultaneously and contributed to NO and N_2O production. A marked increase or decrease in the production of NO would only occur out with this range i.e. $\leq$ 50% WFPS e.g. in the ploughed plots in 1998 and $\geq$ 90% WFPS e.g. in the direct drilled soils in 1999.

Chapter 6. Discussion

In both field experiments (i.e. at Beechgrove and Cowpark) the stimulating effect of nitrogenous fertiliser, be it organic or synthetically produced, was shown. Cumulative losses of NO were 2-255 times and 4-42 times larger from plots amended with fertiliser N than unfertilised plots at the Beechgrove and Cowpark sites respectively. The increase in NO emissions following fertiliser application is consistent with the numerous studies where NO flux has been measured. As in this work, the magnitude and the duration of the elevated fluxes reported in the literature varies enormously between sites and even at the same location. This temporal and spatial discrepancy encountered is primarily the consequence of variability associated with the environmental factors which significantly influence the microbial production processes of nitrification and denitrification.

At both the Beechgrove arable site and the Cowpark ungrazed grassland site statistically significant relationships were established between NO emission and the soil variables of NH_4^+-N, NO_3^--N and soil moisture (WFPS), although no one variable was able to explain the variation in the data across individual treatments at each site or even from the same treatment in different years. The influence of soil moisture, which is reported in this work in relation to gas diffusivity i.e. as WFPS, was considerable and in both field studies an increase in WFPS reduced the emission of NO.

The reduction in NO emission as a response to increased soil moisture was particularly evident as a result of the direct drilling management regime. Typically the recorded WFPS from the direct drilled soil was consistently more than 7% higher than measured in the ploughed soil. Consequently, the decrease in oxygenation of the soil and the concurrent increase in anaerobic denitrification resulted in NO emissions which were 2-283 times smaller in the direct drilled plots than in those which had been ploughed.

No previous studies have been found in the literature which have compared the effect of zero-tillage and ploughing on the pattern of NO emission, although there are numerous reports with respect to N_2O and N_2 loss e.g. Aulakh *et al.*, (1982); Rice & Smith (1982); Linn & Doran (1984); Lemke *et al.*, (1999). These studies all record

the presence of substantial denitrification in the soil under zero-tillage and indeed this was suggested as the primary production process occurring in the direct drilled tillage system at the Beechgrove site. This is supported by results obtained from the supporting laboratory experiment where repacked soil cores were adjusted to a range of target WFPS values. At equivalent WFPS values (90%) to those measured from the direct drilled plots at the Beechgrove field site, the relationship between N_2O emission and particularly soil NO_3^--N, as well as the NO:N_2O ratio all indicated that intense denitrification was proceeding.

In contrast both nitrification and denitrification were found to be taking place simultaneously in the repacked soil cores at WFPS values similar to those found in the ploughed plots (i.e. 60-78%). However, at the drier end of the WFPS range (i.e. 50%) data from the laboratory experiment suggested that nitrification was the dominant mechanism for the generation of NO and may therefore account for the relatively higher fluxes measured from the ploughed plots. Nonetheless, the elevated NO fluxes following ploughing may be as a result of the ploughing action itself stimulating mineralisation.

Results from the Beechgrove field site indicated that a WFPS of 65% (field capacity) marked a distinct shift between NO emissions < 50 μg NO-N m^{-2} h^{-1} (WFPS < 65%) and those < 15 μg NO-N m^{-2} h^{-1} (WFPS > 65%). At a WFPS < 65% the large emissions measured were generally emitted from fertilised ploughed plots. It seems that there is an interaction between WFPS, nitrogen and potentially the action of ploughing, which permits fluxes to increase. Data obtained from the WFPS laboratory experiment fitted into the limits imposed, such that no emissions > 15 μg NO-N m^{-2} h^{-1} were emitted at a WFPS above 65%. The evidence for another interacting variable other than N fertiliser was strengthened since even though the repacked cores were amended with the equivalent fertiliser rate to that of the field site, at a WFPS of 50% the highest NO flux measured was only 20 μg NO-N m^{-2} h^{-1}.

It would appear that a suitable abatement strategy to reduce NO loss from arable systems would be to move away from a tillage regime of ploughing to one of zero-tillage. Results from the field work demonstrated that the mean percentage of total N applied lost as NO-N extrapolated over 1 year was estimated as 0.04% for conventional ploughing and 0.02% for direct drilling. This would suggest that

widespread take-up of direct drilling would halve the loss of NO-N produced. A move to zero-tillage, particularly in the moist climate of Scotland, is liable to incur financial penalties in terms of crop yield as well as favour 'pollution swapping' with the associated 300% increase in N_2O production. Furthermore, direct drilling requires additional herbicide use compared to conventional ploughing and for this reason Skiba *et al.* (1997) discounted a move to direct drilling as a suitable mitigation option. However, with a change in direction of policy away from intensive production and towards a more sustainable system of agriculture, the benefits of zero-tillage to the soil could be utilised in a rotational system with conventional ploughing. With appropriate research, such an idea may have potential, especially in the drier parts of south-east Britain, where the prospect for denitrification would be lower than in the wetter west and northern areas of Britain.

In the laboratory experiments the absence of precipitation was suggested as the cause for the significantly high NO fluxes emitted from repacked soil cores amended with surface applied NPK mineral fertiliser or thermally dried sewage sludge pellets, as well as reduced N_2O peaks compared to those in the field. This highlighted the influence of rainfall in impeding NO emission in the field. In both field studies the pattern of rainfall, especially around fertiliser application was observed to significantly affect the magnitude of emissions. At Beechgrove for example, emissions of NO appeared to be inhibited by the lack of precipitation following fertiliser application until the first precipitation event washed the surface-applied prills into the soil matrix. At Cowpark the release of NO following the surface application of both NPK and SSP fertilisers was potentially diminished by rainfall which induced anaerobic conditions and subsequently retarded nitrification. Nonetheless, Skiba *et al.*, (1997) suggest that the timing of fertiliser application to coincide with periods of precipitation and hence wet soils, does not represent a viable opportunity as a successful mitigation option, because it is too difficult to predict the weather to enough accuracy. Furthermore, even if NO emissions were reduced, stimulation of N_2O loss by anaerobic denitrification is highly probable through increased soil anaerobicity.

In this study, the relative importance of nitrification and denitrification to the emissions of NO at the Beechgrove and Cowpark sites were inferred from only the

relationships established between the NO flux and the soil variables of mineral N and WFPS and additional support was provided by the NO:N_2O ratios. In the field rarely were both NO and N_2O measured on the same day and therefore this calculation provided only limited evidence. In an attempt to clarify the inference reached from the Cowpark field results that nitrification was the primary source of NO production, a laboratory study was established using repacked soil cores. Both NO and N_2O were monitored from each core and although the NO:N_2O ratios generated did not indicate the exclusive production of both gases from nitrification, the trends in the soil mineral N content over the experimental period combined with significant linear regression calculations did indicate that indeed the predominant production of NO was likely to be from nitrification.

It is possible, however, that during the re-packing procedure, anaerobic aggregates were destroyed and/or oxygen was introduced to the soil, which would not have been present in such high concentrations in the field. These actions would thus reduce the potential for denitrification and simultaneously increase the opportunity for nitrification. Furthermore, particularly in the field, bulk soil analyses collected to a depth of 10 cm may have provided a poor measure of the soil variables used to indicate denitrifying microsite activity e.g. the measurement of WFPS may not have reflected the potential for anaerobic niches in the soil. Moreover, a standard, representative bulk density value was calculated for the field and subsequently applied to all soil samples. Within the naturally heterogeneous field the bulk density will have varied, which would not have been reflected in the calculated WFPS values. The use of various inhibitors (e.g. acetylene, N-serve, DCD) and more recently ^{15}N labelled fertiliser have been used to determine the relative contribution of the two processes to the emission of NO (Remede & Conrad, 1991b; Schuster & Conrad, 1992; Skiba *et al*, 1993a). The use of such indicators, particularly in a laboratory study, may be extremely useful in reconciling the primary source process responsible for the emission of NO from the individual treatments at both Beechgrove and Cowpark.

In both of the field experiments, the amount of NO lost from the applied fertiliser N (0.0004-0.03%) was substantially less than that recorded in most other studies. Indeed, the mean (geometric) percentage loss based on a wide range of

studies from all over the world was calculated as 0.3% (Skiba *et al.*, 1997). This estimate was later used in a guidebook created to aid in the production of country wide NO emission inventories under the EMEP/CORINAIR programmes (Simpson *et al.*, 1999). The much lower percentage loss calculated in my research was probably the result of the prevailing climate of Scotland, since denitrification would be favoured over nitrification and a significant production of NO. The wetter soils were less well aerated and therefore, the process of nitrification would be inhibited, furthermore enhanced denitrification would result in the consumption of NO produced prior to its release from the soil surface. Nevertheless, the percentage loss of fertiliser N as NO was considerably smaller than that observed from other temperate sites. The majority of experiments in the literature were also carried out using a sandy soil. The larger pore dimensions of sandy soils are known to encourage oxygenation and hence promote nitrification and the subsequent production of NO. Moreover, the risk of NO consumption during anaerobic denitrification is appreciably reduced in a well aerated soil.

It is possible that the lower than average percentage of fertiliser N lost as NO from both field studies may have been the result of a limited data set and consequently highly influential large emission peaks were not measured and not reflected in the cumulative emission. A more accurate and valid estimate of the cumulative NO emission may well have occurred with an improved sampling frequency i.e. more intense sampling particularly around fertiliser application and harvest, which continued over the whole year. Nonetheless, following the addition of either organic of inorganic fertilisers to repacked soil cores in the laboratory, a relatively more concentrated sampling pattern did not result in a marked increase in cumulative fluxes. The percentage of fertiliser N lost as NO ranged from 0.001-0.006%, although this only covered a 32 d sampling period and on the final measurement occasion, fluxes were still highly elevated from the NPK inorganic fertiliser and from the thermally dried sewage sludge pellet treatments.

As seen with the choice of tillage regime, the use of sewage sludge and its management can significantly affect the gaseous loss of NO. Increasingly it seems, that as a result of various EU directives, the recycling of sewage sludge to land will become more frequent and as such must be regarded as a beneficial source rather

than merely as a route of disposal. As with the use of animal manures, it is imperative that sewage sludge should be managed so as to minimise the risk of environmental pollution including that of gaseous N loss. There are very few studies in the literature that have reported NO emission as a result of application of either animal manures or sewage sludge.

This range of NO emissions generated as a result of organic waste addition highlights the requirement for further research. Specific influences remain unexamined which have the potential to significantly stimulate NO emissions. In this field study only the influence of cattle slurry was investigated with respect to animal manures. Both pig slurry and poultry manure are liable to favour nitrification, due to their very high percentage of readily available NH_4^+-N e.g. pig slurry is estimated to contain 60% TAN compared to only 50% in cattle slurry. A search of the literature shows that the role of grazing cattle has rarely been investigated. The application of a large amount of NH_4^+-N substrate for nitrification to the well aerated soil surface may potentially encourage large fluxes of NO, although initially nitrification may be inhibited as a consequence of urine addition.

Data collected from both the Cowpark field experiment and the supporting laboratory study emphasised the considerable potential source of NO following the surface application of thermally dried sewage sludge pellets. Results from the field work demonstrated that the mean percentage of TAN applied lost as NO-N extrapolated over 1 year was estimated as 0.02% for sewage sludge amended soil and 0.009% for NPK mineral N amended soil. This would suggest that a widespread move to sewage sludge pellet application would double the loss of NO-N from the soil. The requirement for advanced treated sludge has meant that water companies have heavily invested in equipment for thermal drying. In England & Wales only 0.2% of the sewage sludge produced in 1996/97 and applied to agricultural land underwent thermal drying to pellets, however this is estimated to increase to 18% in 2005/06 (Gendebien *et al.*, 1998). The rise in production is predicted to be even greater in Scotland, from no production of thermally dried pellets in 1996/97 to 57% of the sewage sludge recycled to agricultural land in this way in 2005/06 (Gendebien *et al.*, 1998). Furthermore, the use of thermally dried sewage sludge pellets has proved to be highly beneficial in land reclamation and with generally thin soils and

relatively little initial competition from an actively growing crop, the potential for the availability of NH_4^+-N in a well aerated medium is high. It is possible, therefore, that the production of NO from thermally dried sewage sludge pellets may markedly increase NO emitted to the atmosphere in rural areas and strategies to reduce emissions should be investigated further, particularly the suggested role of incorporation.

Several factors require exploration which are common to both application of animal wastes and sewage sludge. The effect of organic fertiliser addition on the microbial soil community has briefly been investigated, although a more in-depth review of the influence of nitrifiers and denitrifiers following organic waste addition may help to predict the magnitude and pattern of NO emissions. The results from this research indicates that the application method may be crucial in controlling the NO emission. In particular, the surface application of sewage sludge pellets appeared to enhance emissions above that of those that had been incorporated in the soil, although no significant difference was seen. Sewage sludge that is applied to grazing land must be deep injected (ADAS, 2001), whereas this research was carried out using only a shallow injection technique. One might expect that with an increased diffusion path length emissions of NO may be curtailed following deep injection, although 'pollution swapping' may occur with an increase in the loss of N_2O.

This research underlined the potential of the organic waste composition to significantly affect the emission of NO. Specifically, dry matter content was implied as a factor which may have strongly influenced emissions in the field (Chapter 4). The highly organic slow release properties of the thermally dried sewage sludge pellets combined with their surface placement provide an explanation for the large NO losses observed from this treatment. The supporting laboratory experiment was able to increase the understanding of the controlling factor for this release of a steady supply of mineral N. A fungus was shown to heavily colonise the pellets and seemed to be associated with the magnitude of the NO emissions.

The effect of composition needs to be reconciled from both animal manures and sewage sludges, particularly as no work has been carried out on the possible influence of either dewatered sludges (DM of *ca.* 25%) or lime stabilised sludge. The study at Cowpark was only carried out over 2 years, which is unlikely to be of a long

enough duration to demonstrate the possible effect of residual organic N from repeated applications of organic waste on the emission of NO. It is imperative that long term studies are commissioned which aim to specifically examine the mineralisation of organic N from wastes and the subsequent availability of mineral N to the crop and microbial soil population. If there is a policy move to a more sustainable system of agricultural, the use of both animal manures and sewage sludge may increase. It is therefore essential that the role of organic wastes in the production of gaseous emissions including NO has been established. Furthermore, there needs to be a drive to overcome the considerable problems of efficient utilisation of manure N, both in the year of application and in subsequent seasons.

Several general questions resulted from the field studies at both the arable site at Beechgrove and the ungrazed grassland site of Cowpark. Namely, would a move to a much sandier soil and/or a drier climate yield larger emissions of NO, as a result of increased aeration and the subsequent stimulation of nitrification. Furthermore, would comparable results be obtained with the use of different crops, particularly with the application of organic wastes to arable soils?

Accurate and reliable emission inventories are highly important if the UK is to comply with International and European policies and protocols. Nonetheless, there still exists a large degree of uncertainty, especially with the contribution of soil derived NO from agriculture. With increased measurements of NO, the robustness of emission estimates can only improve and may highlight specific areas which may benefit from mitigation.

The mean NO-N flux calculated from all the emission data from the two field sites, one grassland and one arable was calculated as 5 μg NO-N m^{-2} h^{-1}. The annual flux was estimated as 0.44 kg NO-N ha^{-1}, which was extrapolated to UK agricultural land (17000,000 ha) to give an annual estimated total flux of 0.007 Tg of NO-N. This value is similar to that reported by Yamulki *et al.* (1995) of 0.008 Tg of NO-N, although this amount was estimated to be emitted from UK arable land and not from the total UK agricultural land area. Based on field data also collected in Scotland Skiba *et al.* (1992) predicted that the annual NO emission from only fertilised crop land and fertilised grassland in the UK would be between 0.013 and 0.038 Tg NO-N. It is likely that compared to the studies by Yamulki *et al.* (1995) and Skiba *et al.*

(1992) the lower estimate produced from this study is the result of a lower initial daily flux rate, a consequence of a lighter textured soil and/or a more favourable climate and soil conditions for nitrification and NO production. It is estimated that in the UK fossil fuels annually emit 500 kt of NO_x-N, therefore based on an annual total of 0.007 Tg of NO-N and assuming that NO is equivalent to NO_x, the estimated NO_x-N production from UK agricultural land is approximately 1.5% of the total amount. Based on the evidence collected from the 2 field studies the emission of NO from agricultural soils in the UK is not significant in terms of its contribution to the NO-N total. However, agricultural soils may emit NO to the atmosphere and produce localised concentrations high enough (e.g. after fertiliser application) to generate harmful levels of tropospheric O_3.

Skiba *et al.*, (1997) suggest that current potential abatement strategies are likely to be expensive and to require a dramatic change in agricultural practice. Furthermore, they estimate that only a 4% decrease in the total global biogenic NO emissions would result and thus the most promising method of reduction would be by curbing the non-biogenic NO emissions caused by vehicles and fossil fuel burning (Skiba *et al.*, 1997). This is particularly likely to be the case in the UK and Europe, where the majority of NO (> 99%) is produced by non-biogenic processes (Simpson *et al.*, 1999).

References

Abbasi M. K. & Adams W. A. (2000) Gaseous N emission during simultaneous nitrification-denitrification associated with mineral N fertilisation to a grassland soil under field conditions. *Soil Biology and Biochemistry* **32**: 1251-1259.

Adamsen F. J. & Sabey B. R. (1987) Ammonia volatilisation from liquid digested sewage sludge as affected by placement in soil. *Soil Society of America Journal* **51**: 1080-1082.

ADAS (2001) The safe sludge matrix - Guidelines for the application of sewage sludge to agricultural land. ADAS, 3rd edition, April, 2001.

Aitken M. N. (1997) *Use of Sewage Sludge on Agricultural Land.* SAC Technical Note T450, SAC, Edinburgh.

Anderson I. C. & Levine J. S. (1986) Relative rates of nitric oxide and nitrous oxide production by nitrifiers, denitrifiers and nitrate respirers. *Applied and Environmental Microbiology* **51**: 938-945.

Anderson I. C. & Levine J. S. (1987) Simultaneous field measurements of biogenic emissions of nitric oxide and nitrous oxide. *Journal of Geophysical Research* **92**: 965-976.

Anderson I. C., Levine J. S., Poth M. A. & Riggan P. J. (1988) Enhanced biogenic emissions of nitric oxide and nitrous oxide following biomass burning. *Journal of Geophysical Research* **93**: 3893-3898.

Aneja V. P., Robarge W. & Holbrook B. D. (1995) Measurements of nitric oxide flux from an upper coastal plain, North Carolina agricultural soil. *Atmospheric Environment* **29**: 3037-3042.

Anon. (1997) *Climate change the United Kingdom programme: The United Kingdom's second report under the Framework Convention on Climate Change.* The Stationary Office, London.

Anon (2001) Organic contaminants in sewage sludge for agricultural use. European Commission.

Arah J. R. M. & Smith K. A. (1989) Steady-state denitrification in aggregated soils: a mathematical model. *Journal of Soil Science* **40**: 139-149.

Arah J. R. M. & Smith K. A. (1990) 'Factors influencing the fraction of the gaseous products of soil denitrification evolved to the atmosphere as nitrous oxide' In: Bouwman A. F. (ed.) *Soils and The Greenhouse Effect*, pp 475 – 480. John Wiley & Sons Ltd., Chichester, UK.

Aulakh M. S., Rennie D. A. & Paul E. A. (1982) Gaseous nitrogen losses from cropped and summer fallowed soils. *Canadian Journal of Soil Science* **62**: 187-195.

Aulakh M. S., Rennie D. A. & Paul E. A. (1984a) Gaseous nitrogen losses from soils under zero-till as compared with conventional-till management systems. *Journal of Environmental Quality* **13**: 130-136.

Aulakh M. S., Rennie D. A. & Paul E. A. (1984b) The influence of plant residues on denitrification rates in conventional and zero tilled soils. *Soil Science Society of America Proceedings* **48**: 790-794.

Bacon J. R., Campbell C. D., Coull M. C., Chambers B. J., Gibbs P. A., Chaudri A., McGrath S. P., Carlton-Smith C. & Aitken M. (2001) A long-term study of the effects of heavy metals in sewage sludge on soil fertility and soil microbial activity. In: Dhir R. K., Limbachiya M. C. & McCarthy M. J. (eds.) *Recycling and Reuse of Sewage Sludge*. pp 87-96. Thomas Telford, London.

Ball B. C. (1994) Experience with minimum and zero tillage in Scotland. In: Tebrügge F. & Böhrnsen A. (eds.) *Experience with the applicability of no-tillage crop production in the West-European countries*, Concerted Action (No. Air 3-CT 93-1464), Proceedings of the EC-Workshop-1- Giessen, 27-28 June, 1994, pp 15-24. Wissenschafterlicher Fachverlag, Gießen, Germany.

Ball B. C., Parker J.P. & Scott A. (1999a) Soil and residue management effects on cropping conditions and nitrous oxide fluxes under controlled traffic in Scotland. 2. Nitrous oxide, soil N status and weather. *Soil and Tillage Research* **52**: 191-201.

Ball B. C., Scott A. & Parker J.P. (1999b) Field N_2O, CO_2 and CH_4 fluxes in relation to tillage, compaction and soil quality in Scotland. *Soil and Tillage Research* **53**: 29-39.

Baumgärtner M. & Conrad R. (1992) Role of nitrate and nitrite for production and consumption of nitric oxide during denitrification in soil. *FEMS Microbiology Ecology* **101**: 59-65.

Bibby J., Douglas H. A., Thomasson A. J. & Robertson J. S (1982) Land capability classification for agriculture. Macaulay Institute for Soil Research, Aberdeen, UK.

Blackmer A. M. & Cerrato M. E. (1986) Soil properties affecting the formation of nitric oxide by chemical reactions of nitrite. *Soil Society of America Journal* **50**: 1215-1218.

Bouwman A. F. (1990). 'Part II, Background.' In: Bouwman A. F. (ed.) *Soils and The Greenhouse Effect*, pp 25 – 192. John Wiley & Sons Ltd., Chichester, UK.

Brady N. C. & Weil R. R. (1999) *The Nature and Properties of Soils*. (12th Ed.). Prentice-Hall, Inc., New Jersey, USA.

Byrnes B. H., Christianson C. B., Holt L. S. & Austin E. R. (1990) 'Nitrous oxide emissions from the nitrification of nitrogen fertilisers' In: Bouwman A. F. (ed.) *Soils and The Greenhouse Effect*, pp 489 – 495. John Wiley & Sons Ltd., Chichester, UK.

Cameira M. C., Magalhães M. C. & Pato R. L. (1996) Nitrogen dynamics on a soil with different tillage systems. In: Van Cleemput O., Hofman G. & Vermoesen A. (eds.) *Progress in Nitrogen Cycling Studies*, pp 31-35. Kluwer Academic Publishers, Netherlands.

Cárdenas L., Rondón A., Johansson C. & Sanhueza E. (1993) Effects of soil moisture, temperature, and inorganic nitrogen on nitric oxide emissions from acidic tropical savannah soils. *Journal of Geophysical Research* **98**: 14,783-14,790.

Carter M. R. & Rennie D. A. (1984) Nitrogen transformations under zero shallow tillage. *Soil Science Society of America Journal* **48**: 1077-1081.

CEC. (1986) Commission of the European Communities. Council Directive of 12 June 1986 on the protection of the environment, and in particular the soil, when sewage sludge is used in agriculture (86/278/EEC). *Official Journal of the European Communities* No. L 181.

CEC. (1991) Commission of the European Communities. Council Directive of 21 May 1991 concerning urban wastewater treatment (91/271/EEC). *Official Journal of the European Communities* No. L 135/40-52.

Chemlab (1982) Continuous flow analysis - ammonia, Chemlab Instruments Ltd., Hornchurch, Essex.

Chemlab (1988) Continuous flow analysis - total oxidised nitrogen, Chemlab Instruments Ltd., Hornchurch, Essex.

Chadwick D. R., Pain B. F. & Brookman S. K. E. (2000) Nitrous oxide and methane emissions following application of animal manures to grassland. *Journal of Environmental Quality* **29**: 277-287.

Chadwick D. R. & Harrison R. (2001) DEFRA project code WA0654, Emissions of nitric oxide from agricultural soils. Final Report to DEFRA, LMID, London.

Chadwick D. R., Martinez J., Marol C. & Béline F. (2001) Nitrogen transformations and ammonia loss following injection and surface application of pig slurry: a laboratory experiment using slurry labelled with ^{15}N-ammonium. *Journal of Agricultural Science* **136**: 231-240.

Chae Y. M. & Tabatabai M. A. (1986) Mineralisation of nitrogen in soils amended with organic wastes. *Journal of Environmental Quality* **15**: 193-198.

Chalmers A., Hounsome B., Lang B., Renwick A. & Rush C. (2000) *The British Survey of Fertiliser Practice: Fertiliser Use on Farm Crops for Year 1999.* Fertiliser Manufacturers' Association, Peterborough, UK.

Chantigny M. H., Rochette P. & Angers D. A. (2001) Short-term C and N dynamics in a soil amended with pig slurry and barley straw: a field experiment. *Canadian Journal of Soil Science* **81**: 131-137.

Christensen S. (1983) Nitrous oxide emission from a soil under permanent grass: Seasonal and diurnal fluctuations as influenced by manuring and fertilisation. *Soil Biology and Biochemistry* **15**: 531-536.

Christensen S. & Tiedje J. M. (1990) Brief and vigorous N_2 production by soil at spring thaw. *Journal of Soil Science* **41**: 1-4.

Christensen S., Ambus P., Arah J. R. M. Clayton H., Galle B., Griffith D. W. T., Hargreaves K. J., Klemedtsson L., Lind A.-M., Maag M., Scott A., Skiba U., Smith K. A., Welling M. & Wienhold F. G. (1996) Nitrous oxide emissions from an agricultural field: comparison between measurements by flux chamber and micrometeorology techniques. *Atmospheric Environment* **30**: 4183-4190.

Christian, D. G. (1994) Experience with direct drilling and reduced cultivation in England. In: Tebrügge F. & Böhrnsen A. (eds.) *Experience with the applicability of no-tillage crop production in the West-European countries*, Concerted Action (No. Air 3-CT 93-1464), Proceedings of the EC-Workshop-1- Giessen, 27-28 June, 1994, pp 25-31. Wissenschafterlicher Fachverlag, Gießen, Germany.

Christian, D. G. & Ball, B. C. (1994) Reduced Cultivation and Direct Drilling for cereals in Great Britain. In: Carter, M. R. (ed.) *Conservation Tillage in Temperate Agroecosystems,* pp 117-140. Lewis Publishers, CRC Press, Florida.

Clayton H., Arah J. R. M. & Smith, K. A. (1994) Measurement of nitrous oxide from fertilised grassland using closed chambers. *Journal of Geophysical Research* **99**: 16,599-16,607.

Clemens J. & Huschka A. (2001) The effect of biological oxygen demand of cattle slurry and soil moisture on nitrous oxide emissions. *Nutrient Cycling in Agroecosystems* **59**: 193-198.

Clemitshaw K. C., Carpenter L. J., Penkett S. A. & Jenkin M. E. (1997) A calibrated peroxy radical chemical amplifier for ground-based tropospheric measurements. *Journal of Geophysical Research* **102**: 25,405-25,416.

CLRTAP (1999) Convention on Long-Range Transboundary Air Pollution, UN/ECE. http://www.unece.org/env/lrtap

Cole V., Cerri C., *et al.*, (1996) Agricultural options for mitigation of greenhouse gas emissions. In: Climate Change 1995. *Impacts, Adaptations and Mitigation of Climate*

Change: Scientific-Technical Analyses, pp 745-771. IPCC Working Group II. Cambridge University Press.

Comfort S. D., Kelling K. A., Keeney D. R. & Converse J. C. (1988) The fate of nitrogen from injected manure in a slit loam soil. *Journal of Environmental Quality* **17**: 317-322.

Conrad R. (1990) 'Flux of NO_x between soil and atmosphere: importance and soil microbial metabolism.' In: Revsbech N. P. & Sørensen J. (eds.) *Denitrification in Soil and Sediment*, pp 105-128. Plenum Press, New York.

Conrad R. (1994) Compensation concentration as a critical variable for regulating the flux of traces gases between soil and atmosphere. *Biogeochemistry* **27**: 155-170.

Conrad R. (1995) Soil microbial processes involved in production and consumption of atmospheric trace gases. In: Gwynfryn Jones. J. (ed.) *Advances in Microbial Ecology,* **14**: 207-250 Plenum Press, New York.

Crutzen P. J. (1988) 'Tropospheric ozone: An overview.' In: Isaksen I. S. A. (ed.) *Tropospheric Ozone-Regional and Global Scale Interactions.* NATO ASI Series C, vol. 227. Reidel. Boston, USA.

Davidson E. A. (1991) 'Fluxes of nitrous oxide and nitric oxide from terrestrial ecosystems.' In: Rogers J. E. & Whitman W. B. (eds.) *Microbial Production and Consumption of Greenhouse Gases: Methane, Nitrogen oxides and Halomethanes.* pp 219-235. American Society for Microbiology, Washington D.C. USA.

Davidson E. A. (1992) Sources of nitric oxide and nitrous oxide following wetting of dry soil. *Soil Science Society of America Journal* **56**: 95-102.

Davidson E. A. (1993) 'Soil water content and the ratio of nitrous oxide to nitric oxide emitted from soil.' In: Oremland R. S. (ed.) *Biogeochemistry of Global Change – Radiatively Active Trace Gases.* pp 219-235. Chapman and Hall, Inc. New York.

Davidson E. A., Swank W. T. & Perry T. O. (1986) Distinguishing between nitrification and denitrification as sources of gaseous nitrogen production in soil. *Applied and Environmental Microbiology* **52**: 1280-1286.

Davidson E. A., Vitousek P. M., Matson P. A., Riley R., García-Méndez & Maass J. M. (1991) Soil emissions of nitric oxide in a seasonally dry tropical forest of Mexico. *Journal of Geophysical Research* **96**: 15,439-15,445.

Davidson E. A., & Schimel J. P. (1995) 'Microbial processes of production and consumption of nitric oxide, nitrous oxide and methane.' In: Matson P. A., & Harriss R. C. (eds.) *Biogenic Trace Gases: Measuring Emissions from Soil and Water.* pp 327-357. Blackwell Science Ltd., Oxford.

Davidson E. A. & Kingerlee W. (1997) A global inventory of nitric oxide emissions from soils. *Nutrient Cycling in Agroecosystems* **48**: 37-50.

Davies M. G., Smith K. A. & Vinten (2001) The mineralisation and fate of nitrogen following ploughing of grass and grass-clover swards. *Biology and Fertility of Soils* **33**: 423-434.

Delany A. C., Fitzjarrald., Lenschow D. H., Pearson R., Wendel G. J. & Woodruff B. (1986) Direct measurements of nitrogen oxides and ozone fluxes over grassland. *Journal of Atmospheric Chemistry* **4**: 429-444.

DeLuca T. H., Keeney D. R. & McCarty G. W. (1992) Effect of freeze-thaw events on mineralisation of soil nitrogen. *Biology and Fertility of Soils* **14**: 116-120.

Dick W. A. (1983) Organic carbon, nitrogen, and phosphorus concentrations and pH in soil profiles as affected by tillage intensity. *Soil Science Society of America Journal* **47**: 102-107.

Dickson J. W. & Ritchie R. M. (1996a) Zero and reduced ground pressure traffic systems in an arable rotation .1. Cultivation power requirement. *Soil and Tillage Research* **38**: 71-88.

Dickson J. W. & Ritchie R. M. (1996b) Zero and reduced ground pressure traffic systems in an arable rotation .2. Soil and crop responses. *Soil and Tillage Research* **38**: 89-113.

Dobbie K. E., McTaggart I. P. & Smith K. A. (1999) Nitrous oxide emissions from intensive agricultural systems: Variations between crops and seasons, key driving variables, and mean emission factors. *Journal of Geophysical Research* **104**: 26,891-26,899.

Doran J. W. (1980) Soil microbial and biochemical changes associated with reduced tillage. *Soil Science Society of America Journal* **44**: 765-771.

Doran J. W. (1987) Microbial biomass and mineralisable nitrogen distributions in no-tillage and plowed soils. *Biology and Fertility of Soils* **5**: 68-75.

Douglas B. F. & Magdoff F. R. (1991) An evaluation of nitrogen mineralisation indices for organic residues. *Journal of Environmental Quality* **20**: 368-372.

Dowdell R. J. & Cannell R. Q. (1975) Effect of ploughing and direct drilling on soil nitrate content. *Journal of Soil Science* **26**: 53-61.

Drury C. F., McKenney D. J. & Findlay W. I. (1992) Nitric oxide and nitrous oxide production from soil: water and oxygen effects. *Soil Society of America Journal* **56**: 766-770.

Dunfield P. F. & Knowles R. (1998) Organic matter, heterotrophic activity and NO consumption in soils. *Global Change Biology* **4**: 199-207.

Duxbury J. M., Harper L. A., & Mosier A. R. (1993) 'Contributions of agroecosystems to global climate change.' In: *Agricultural Ecosystem Effects on Trace Gases and Global Climate Change*. pp 1-18. ASA Special Publication no. 55. American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America.

Dyson P. W. (1992) *Fertiliser allowances for manures and slurries*. SAC Technical Note T309, SAC, Edinburgh.

Edwards A. C. & Cresser M. S. (1992) Freezing and its effects on chemical and biological properties of soil. *Advances in Soil Science* **18**: 59-79.

Ellis F. B., Elliott J. G., Pollard F., Cannell R. Q. & Barnes B. T. (1979) Comparison of direct drilling, reduced cultivation and ploughing on the growth of cereals. 3. Winter wheat and spring barley on a calcareous clay.

Ellis S., Yamulki S., Dixon E., Harrison R., & Jarvis S. C. (1998) Denitrification and N_2O emissions from a UK pasture soil following the early spring application of cattle slurry and mineral fertiliser. *Plant and Soil* **202**: 15-25.

Epstein E., Keane D. B., Meisinger J. J. & Legg J. O. (1978) Mineralisation of nitrogen from sewage sludge and sludge compost. *Journal of Environmental Quality* **7**: 217-221.

Erickson H. E. & Keller M. (1997) Tropical land use change and soil emissions of nitrogen oxides. *Soil Use and Management* **13**: 278-287.

Fine P., Mingelgrin U. & Feigin A. (1989) Incubation studies of the fate of organic nitrogen in soils amended with activated sludge. *Soil Society of America Journal* **53**: 444-450.

Finlayson-Pitts B. J. & Pitts J. N. (1986) *Atmospheric Chemistry: Fundamentals and Experimental Techniques*. John Wiley & Sons, Inc., New York.

Firestone M. K., & Davidson E. A. (1989) 'Microbiological basis of NO and N_2O production and consumption in soil.' In: Andreae M. O & Schimel D. S. (eds.) *Exchange of Trace Gases between Terrestrial Ecosystems and the Atmosphere*. John Wiley & Sons. Chichester.

Fowler D. & Duyzer J. H. (1989) 'Micrometeorological techniques for the measurement of trace gas exchange.' In: Andreae M. O & Schimel D. S. (eds.) *Exchange of Trace Gases between Ecosystems and the atmosphere*, pp 189-207. John Wiley & Sons Ltd.

Fowler D., Skiba U. & Hargreaves K. J. (1997) 'Emissions of nitrous oxide from grasslands.' In: Jarvis S. C. & Pain B. F. (eds.) *Gaseous Nitrogen Emissions from Grasslands*, pp147-164. CAB International.

Galbally I. E. (1989) 'Factors controlling NO_x emissions from soils' In: Andreae M. O & Schimel D. S. (eds.) *Exchange of Trace Gases between Ecosystems and the atmosphere*, pp 23 – 37. John Wiley & Sons Ltd.

Gardner W. H. (1986) 'Water content.' In: Klute A. (ed.) *Methods of Soil Analysis, Part 1. Physical and Mineralogical Methods*-Agronomy Monograph no. 9. American Society of Agronomy-Soil Science Society of America, Madison, USA.

Gasche R. & Papen H. (1999) A 3-year continuous record of nitrogen trace gas fluxes from untreated and limed soil of a N-saturated spruce and beech forest ecosystem in Germany 2. NO and NO_2 fluxes. *Journal of Geophysical Research* **104**: 18,505-18,520.

Gendebien A., Carlton-Smith C., Izzo M. & Hall J. E. (1998) UK Sludge Survey - National Presentation, Environment Agency Final Technical Report, pp 165.

Gödde M. & Conrad R. (1998) Simultaneous measurement of nitric oxide production and consumption in soil using a simple static incubation system and the effect of soil water content on the contribution of nitrification. *Soil Biology and Biochemistry* **30**: 433-442.

Gödde M. & Conrad R. (1999) Immediate and adaptational temperature effects on nitric oxide production and nitrous oxide release from nitrification and denitrification in two soils. *Biology and Fertility of Soils* **30**: 33-40.

Goodroad L. L. & Keeney D. R. (1985) Site of nitrous oxide production in field soils. *Biology and Fertility of Soils* **1**: 3-7.

Hargreaves K., Wienhold F. G. & Klemedtsson L. (1996) Measurement of nitrous oxide emission from agriculture land using micrometeorological methods. *Atmospheric environment* **30**: 1563-1571.

Harrison R. & Webb J. (2001) A review of the effect of N fertiliser type on gaseous emissions. *Advances in Agronomy* **73**: 65-108.

Hudson H. J. (1972) Fungal Saprophytism. *Studies in Biology No. 32*. Edward Arnold, London.

Hutchinson G. L. & Brams E. A. (1992) NO versus N_2O from an NH_4^+ amended Bermuda grass pasture. *Journal of Geophysical Research* **97**: 9889-9896.

Hutchinson G. L. & Davidson E. A. (1993) 'Processes for production and consumption of gaseous nitrogen oxides in soil.' In: *Agricultural Ecosystem Effects on Trace Gases and Global Climate Change*. ASA Special Publication no. 55.

American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America.

Hutchinson G. L., Livingston G. P. & Brams E. A. (1993a) 'Nitric and nitrous oxide evolution from managed subtropical grassland.' In: Oremland R. S. (ed.) *Biogeochemistry of Global Change – Radiatively Active Trace Gases*. pp 290-316. Chapman and Hall, Inc. New York.

Hutchinson G. L., Guenzi W. D. & Livingston G. P. (1993b) Soil water controls on aerobic soil emission of gaseous nitrogen oxides. *Soil Biology and Biochemistry* **25**: 1-9.

Inubushi K., Naganuma H. & Kitahara S. (1996) Contribution of denitrification and autotrophic and heterotrophic nitrification to nitrous oxide production in andosols. *Biology and Fertility of Soils* **23**: 292-298.

IPCC-Intergovernmental Panel on Climate Change. (1995) *Climate Change 1994-Radiative Forcing of Climate Change*. University Press, Cambridge.

IPCC-Intergovernmental Panel on Climate Change. (1997) Guidelines for national greenhouse gas inventories, OECD, Paris.

Jaffe D. A. (1992) 'The nitrogen cycle.' In: Butcher S. S., Charlson R. J., Orians G. H. & Wolfe G. V. (eds.) *Global Biogeochemical Cycles*. Academic Press, London.

Jarvis S.C. & Macduff J. H. (1989) Nitrate nutrition of grasses from steady-state supplies in flowing solution culture following nitrate deprivation and/or defoliation. *Journal of Experimental Botany* **40**: 965-975.

Johansson C. (1984) Field Measurements of emission of nitric oxide from fertilised and unfertilised forest soils in Sweden. *Journal of Atmospheric Chemistry* **1**: 492-442.

Johansson C. & Galbally I. E. (1984) Production of nitric oxide in loam under aerobic and anaerobic conditions. *Applied and Environmental Microbiology* **47**: 1284-1289.

Johansson C. & Granat L. (1984) Emission of nitric oxide from arable land. *Tellus* **36B**: 25-37.

Johansson C. & Sanhueza E. (1988) Emission of NO from savanna soils during the rainy season. *Journal of Geophysical Research* **93**: 14,193-14,198.

Johansson C., Rodhe H. & Sanhueza E. (1988) Emission of NO in a tropical savanna and cloud forest during the dry season. *Journal of Geophysical Research* **93**: 7180-7192.

Killham K. (1986) 'Heterotrophic nitrification.' In: Prosser J. I. (ed.) *Nitrification*. The society for general microbiology, pp 117-126, IRL Press, Oxford, UK,

Killham K. (1994) *Soil Ecology*. Cambridge University Press, Cambridge, UK.

King L. D. (1973) Mineralisation and gaseous loss of nitrogen in soil-applied liquid sewage sludge. *Journal of Environmental Quality* **2**: 356-358.

Klemedtsson L., Svensson B. H. & Rosswall T. (1988a) A method of selective inhibition to distinguish between nitrification and denitrification as sources of nitrous oxide in soil. *Biology and Fertility of Soil* **6**: 112-119.

Klemedtsson L., Svensson B. H. & Rosswall T. (1988b) Relationship between soil moisture content and nitrous oxide production during nitrification and denitrification. *Biology and Fertility of Soil* **6**: 106-111.

Klemedtsson L, Hansson G. & Mosier A. (1990) 'The use of acetylene for the quantification of N_2 and N_2O production from biological processes in soil.' In: Revsbech N. P. & Sørensen J. (eds.) *Denitrification in Soil and Sediment*, pp 167-180. Plenum Press, New York.

Lammel G. & Graßl H. (1995) Greenhouse effect of NO_x. *Environmental Science and Pollution Research* **2**: 40-45.

Lemke R. L., Izaurralde R. C., Nyborg M. & Solberg E. D. (1999) Tillage and n source influence soil-emitted nitrous oxide in the Alberta Parkland region. *Canadian Journal of Soil Science* **79**: 15-24.

Lerch R. N., Barbarick K. A., Sommers L. E. & Westfall D. G. (1992) Sewage sludge proteins as labile carbon and nitrogen sources. *Soil Science Society of America Journal* **56**: 1470-1476.

Lessard R., Rochette P., Gregorich E. G., Pattey E. & Desjardins R. L. (1996) Nitrous oxide fluxes from manure-amended soil under maize. *Journal of Environmental Quality* **25**: 1371-1377.

Levine J. S., Winstead E. L., Parsons D. A. B., Scholes M. C., Scholes R. J., Cofer W. R., Cahoon D. R. & Sebacher D. I. (1996) Biogenic soil emissions of nitric oxide (NO) and Nitrous oxide (N_2O) from savannas in South Africa: The impact of wetting and burning. *Journal of Geophysical Research* **101**: 23,689-23,697.

Lindemann W. C. & Cardenas M. (1984) Nitrogen mineralisation potential and nitrogen transformations of sludge-amended soil. *Soil Science Society of America Journal* **48**: 1072-1077.

Linn D. M. & Doran J. W. (1984) Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and non-tilled soils. *Soil Society of America Journal* **48**: 1267-1272.

Lipschultz F., Zafiriou O. C., Wofsy S. C., McElroy M. B., Valois F. W. & Watson S. W. (1981) Production of NO and N_2O by soil nitrifying bacteria. *Nature* **294**: 641-643.

Livingston G. P. & Hutchinson G. L. (1995) 'Enclosure-based measurement of trace gas exchange: applications and sources of error.' In: Matson P. A. & Harriss R. C. (eds.) *Methods in Ecology – Biogenic Trace Gases: Measuring Emissions from Soil and Water*, pp. 14 – 51. Blackwell Science Ltd.

Logan J. (1988) 'The ozone problem in rural areas of the United States.' In: Isaksen I. S. A. (ed.) Tropospheric Ozone-Regional and Global Scale Interactions. NATO ASI Series C, Vol. 227. Reidel, Boston, USA.

MAFF (2000). *Fertiliser Recommendations for Agricultural and Horticultural Crops. Reference Book 209, 7th ed.* HMSO, London.

Maag M. (1989) 'Denitrification losses from soil receiving pig slurry.' In: Hansen J. A. & Henriksen K. (eds.) *Nitrogen in Organic Wastes Applied to Soils*, pp 235-246. Academic Press, London.

Maag M. & Vinther F. P. (1996) Nitrous oxide emission by nitrification and denitrification in different soil types and at different soil moisture contents and temperatures. *Applied Soil Ecology* **4**: 5-14.

Maggiotto S. R., Webb J. A., Wagner-Riddle C. & Thurtell G. W. (2000) Nitrous and nitrogen oxide emissions from turfgrass receiving different forms of nitrogen fertiliser. *Journal of Environmental Quality* **29**: 621-630.

Marshall T. J., Holmes J. W. & Rose C. W. (1996) *Soil Physics*. Cambridge University Press, Cambridge, UK.

McKenney D. J. & Drury C. F. (1997) Nitric oxide production in agricultural soils. *Global Change Biology* **3**: 317-326.

McTaggart I. P. & Smith K. A. (1996) Nitrous Oxide emissions from arable and grassland soils: Effect of crop type and nitrification inhibitors. *Transactions of the 9th Nitrogen workshop*. pp 523-526. Braunschweig, September, 1996.

McTaggart I. P., Clayton H., Parker J., Swan L. & Smith K. A. (1997) Nitrous oxide emissions from grassland and spring barley following N fertiliser application with and without nitrification inhibitors. *Biology and Fertility of Soils* **25**: 261-268.

McTaggart I. P., Scott A. & Ball B. C. (1999) Emissions of nitrous oxide from a grass soil amended with organic wastes. *Proceedings of the 10th Nitrogen Workshop*, Copenhagen, Denmark.

Meixner F. X. (1994) Surface exchange of odd nitrogen oxides. *Nova Acta Leopoldina NF 70* **288**: 299-348.

Meixner F. X., Fickinger Th., Marufu L., Serça D., Nathaus F. J., Makina E., Mukurumbira L., Andreae M. O. (1997) Preliminary results on nitric oxide emission from a southern African savanna ecosystem. *Nutrient Cycling in Agroecosystems* **48**: 123-138.

Melander B. (1994) Impact of non-inversion tillage on weeds in temperate regions. In: Tebrügge F. & Böhrnsen A. (eds.) *Experience with the applicability of no-tillage crop production in the West-European countries*, Concerted Action (No. Air 3-CT 93-1464), Proceedings of the EC-Workshop-1- Giessen, 27-28 June, 1994, pp 49-58. Wissenschafterlicher Fachverlag, Gießen, Germany.

Merino M. P., Estavillo J. M., Pinto M., Rodríguez M., Duñabeitia M. K. & González-Murua C. (2001) Nitrous oxide emissions from grassland in an intensive dairy farm in the Basque country of Spain. *Soil Use and Management* **17**: 121-127.

Mosier A. R. & Hutchinson G. L. (1981) Nitrous oxide emissions from cropped fields. *Journal of Environmental Quality* **10**: 169-173.

Mosier A. R., Hutchinson G. L., Sabey B. R. & Baxter J. (1982) Nitrous oxide emissions from barley plots treated with ammonium nitrate or sewage sludge. *Journal of Environmental Quality* **11**: 78-81.

Mosier A. R. (1990) 'Gas flux measurement techniques with special reference to techniques suitable for measurements over large ecologically uniform areas.' In: Bouwman A. F. (ed.) *Soils and The Greenhouse Effect*, pp 289 – 301. John Wiley & Sons Ltd., Chichester, UK.

Nägele W. & Conrad R. (1990) Influence of pH on the release of NO and N_2O from fertilised and unfertilised soil. *Biology and fertility of soils* **10**: 139-144.

NEGTAP (2001) National Expert Group on Transboundary Air Pollution. http://www.nbu.ac.uk/negtap/

Nieder R., Kersebaum K. C. & Richter J. (1995) Significance of nitrate leaching and long term immobilisation after the plough layers for the N regime of arable soils in N.W. Germany. *Plant and Soi*l **173**: 167-175.

Nielsen T. H., Nielsen L. P. & Revsbech N. P. (1996) Nitrification and coupled nitrification-denitrification associated with a soil-manure interface. *Soil Society of America Journal* **60**: 1829-1840.

Nikitas C., Clemitshaw K. C., Oram D. E. & Penkett S. A. (1997) Measurements of PAN in the polluted boundary layer and free troposphere using a luminol-NO_2 detector combined with a thermal converter. *Journal of Atmospheric Chemistry* **28**: 339-359.

Oenema O., Velthof G. L., Yamulki S. & Jarvis S. C. (1997) Nitrous oxide emissions from grazed grassland. *Soil Use and Management* **13**: 288-295.

Ormeci B., Sanin S. L. & Pierce J. J. (1999) Laboratory study of NO flux from agricultural soil: Effects of soil moisture, pH and temperature. *Journal of Geophysical Research* **104**: 1621-1629.

O'Sullivan M. F., Vinten A. J. A. & Ball B. C. (2001) 'Carbon and nitrogen losses after ploughing out grass and grass-clover swards.' In: Rees R. M., Ball B. C., Campbell C. D. & Watson C. A. (eds.) *Sustainable Management of Soil Organic Matter*, pp 142-149. CABI Publishing, Oxon, UK.

Otter L. B., Yang W. X., Scholes M. C. & Meixner F. X. (1999) Nitric oxide emissions from a Southern African savanna. *Journal of Geophysical Research* **104**: 18,471-18,485.

Papen H., Von Berg R., Hinkle I., Thoene B. & Rennenberg H. (1989) Heterotrophic nitrification by *Alcaligenes faecalis*: NO_2^-, NO_3^-, N_2O and NO production in exponentially growing cultures. *Applied and Environmental Microbiology* **55**: 2068-2072.

Papen H., Hellman B., Papke H. & Rennenberg H. (1993) 'Emission of N-oxides from acid irrigated and limed soils of a coniferous forest in Bavaria.' In: Oremland R. S. (ed.) *Biogeochemistry of Global Change – Radiatively Active Trace Gases*. Chapman and Hall, Inc. New York.

Parker C. F. & Sommers L. E. (1983) Mineralisation of nitrogen in sewage sludges. *Journal of Environmental Quality* **12**: 150-156.

Parkin T. B. (1987) Soil microsites as a source of denitrification variability. *Soil Science Society of America Journal* **51**: 1194-1199.

Parkin T. B., Sexstone A. J. & Tiedje J. M. (1985) Adaptation of denitrifying populations to low soil pH. *Applied and Environmental Microbiology* **49**: 1053-1056.

Paul J. W., Beauchamp E. G. & Zhang X. (1993) Nitrous and nitric oxide emissions during nitrification and denitrification from manure-amended soil in the laboratory. *Canadian Journal of Soil Science* **73**: 539-553.

Paustian K., Andrén O., Janzen H. H., Lal R., Smith P., Tian G., Tiessen H. & Van Noordwijk M. (1997) Agricultural soils as a sink to mitigate CO_2 emissions. *Soil Use and Management* **13**: 230-244.

Payne W. J. (1981) *Denitrification*. John Wiley & Sons, Inc. USA.

Petersen S. O. (1992) Nitrification and denitrification after direct injection of liquid cattle manure. *Acta Agriculture Scandinavia* **42B**: 94-99.

Photochemical oxidants review group (PORG). (1997) Ozone in the United Kingdom. Fourth Report of the Photochemical Oxidants Review Group.

Prather M., Derwent R., Ehhalt D., Fraser P., Sanhueza E., & Zhou X. (1995) 'Other trace gases and atmospheric chemistry.' In: *Climate Change 1994 - Radiative Forcing of Climate Change*, pp76 – 126. Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK.

Prinz B. (1988) 'Ozone effects on vegetation.' In: Isaksen I. S. A (ed.) Tropospheric Ozone-Regional and Global Scale Interactions. NATO ASI Series C, Vol. 227. Reidel, Boston, USA.

Quemada M., Lasa B., Lamsfus C. & Aparicio-Tejo P. M. (1998) Ammonia volatilisation from surface or incorporated biosolids by the addition of Dicyandiamide. *Journal of Environmental Quality* **27**: 980-983.

Rasmussen K. J. (1994) Experiments with no-inversion tillage systems in Scandinavia – Impacts on crop yields, soil structure and fertilisation. In: Tebrügge F. & Böhrnsen A. (eds.) *Experience with the applicability of no-tillage crop production in the West-European countries*, Concerted Action (No. Air 3-CT 93-1464), Proceedings of the EC-Workshop-1- Giessen, 27-28 June, 1994, pp 39-48. Wissenschafterlicher Fachverlag, Gießen, Germany.

Reicosky D. C. (1997) Tillage-induced CO_2 emission from soil. *Nutrient Cycling in Agroecosystems* **49**: 273-285.

Remde A., Slemr F. & Conrad R. (1989) Microbial production and uptake of nitric oxide in soil. *FEMS Microbiology Ecology* **62**: 221-230.

Remde A. & Conrad R. (1991a) Metabolism of nitric oxide in soil and denitrifying bacteria. *FEMS Microbiology Ecology* **85**: 81-94.

Remde A. & Conrad R. (1991b) Role of nitrification and denitrification for NO metabolism in soil. *Biogeochemistry* **12**: 189-205.

Rice C. W. & Smith M. S. (1982) Denitrification in no-till and plowed soils. *Soil Science Society of America Journal* **46**: 1168-1173.

Robertson G. P. & Tiedje J. M. (1987) Nitrous oxide sources in aerobic soils: nitrification, denitrification and other biological processes. *Soil Biology and Biochemistry* **19**: 187-193.

Robertson L. A. & Kuenen J. G. (1990) 'Physiological and ecological aspects of aerobic denitrification, a link with heterotrophic nitrification? In: Revsbech N. P. & Sørensen J. (eds.) *Denitrification in Soil and Sediment*, pp 91-104. Plenum Press, New York.

Robinson M. B. & Polglase P. J. (2000) Volatilisation of nitrogen from dewatered biosolids. *Journal of Environmental Quality* **29**: 1351-1355.

Roelle P., Aneja V. P., O'Conner J., Robarge W., Kim D. & Levine J. S. (1999) Measurement of nitrogen oxide emissions from an agricultural soil with a dynamic chamber system. *Journal of Geophysical Research* **104**: 1609-1619.

Rondón. A., Johansson C. & Sanhueza E. (1993) Emission of nitric oxide from soils and termite nests in a Trachypogon savanna of the Orinoco Basin. *Journal of Atmospheric Chemistry* **17**: 293-306.

Rudolph J., Koschorreck M. & Conrad R. (1996) Oxidative and reductive microbial consumption of nitric oxide in a heathland soil. *Soil Biology and Biochemistry* **28**: 1389-1396.

Ryden J. C. (1981) N_2O exchange between a grassland soil and the atmosphere. *Nature* **292**: 235-237.

Sahrawat K. L. & Keeney D. R. (1986). Nitrous oxide emission from soils. *Advances in Soil Science* **4**: 103-148.

Sanhueza E., Hao W.M., Scharffe D., Donoso L. & Crutzen P. J. (1990) N_2O and NO emissions from soils of the Northern part of the Guayana Shield, Venezuela. *Journal of Geophysical Research* **95**: 22,481-22,488.

Schimel J. P., Firestone M. K. & Killham K. S. (1984) Identification of heterotrophic nitrification in a Sierran forest soil. *Applied and Environmental Microbiology* **48**: 802-806.

Schuster M. & Conrad R. (1992) Metabolism of nitric oxide and nitrous oxide during nitrification and denitrification in soil at different incubation conditions. *FEMS Microbiology Ecology* **101**: 133-143.

Scott A., Ball B. C., Crichton I. & Aitken M. N. (2000) Nitrous oxide emissions from grassland amended with sewage sludge. *Soil Use and Management* **16**: 36-41.

Sexstone A. J., Revsbech N. P., Parkin T. B., Tiedje J. M. (1985) Direct measurement of oxygen profiles and denitrification rates in soil aggregates. *Soil Science Society of America Journal* **49**: 645-651.

Shepherd M. F., Barzetti S. & Hastie D. R. (1991) The production of atmospheric NO_x and N_2O from a fertilised agricultural soil. *Atmospheric Environment* **25A**: 1961-1969.

Sierra J. (1997) Temperature and soil moisture dependence of N mineralisation in intact soil cores. *Soil Biology and Biochemistry* **29**: 1557-1563.

Simpson D. (1993) Biogenic VOC emissions in Europe: Modelling the implications for ozone control strategies. *AWMA Conference*, Nov. 8-12, 1993, San Diego, California.

Simpson D. (1995) Biogenic emissions in Europe 2. Implications for ozone control strategies. *Journal of Geophysical Research* **100**: 22,891-22, 906.

Simpson D., Guenther A., Hewitt C. N. & Steinbrecher R. (1995) Biogenic emissions in Europe 1. Estimates and uncertainties. *Journal of Geophysical Research* **100**: 22,875-22,890.

Simpson D., Winiwarter W., Börjesson G., Cinderby S., Ferreiro A., Guenther A., Hewitt C. N., Janson R., Khalil M. A. K., Owen S., Pierce T. E., Puxbaum H., Shearer M, Skiba U., Steinbrecher R., Tarrasón L. & Öquist M. G. (1999). Inventorying emissions from nature in Europe. *Journal of Geophysical Research* **104**: 8,113-8,152.

Skiba U., Hargreaves K. J., Fowler D. & Smith K. A. (1992) Fluxes of nitric and nitrous oxides from agricultural soils in a cool temperate climate. *Atmospheric Environment* **26A**: 2477-2488.

Skiba U., Smith K. A. & Fowler D. (1993) Nitrification and denitrification as sources of nitric oxide and nitrous oxide in a sandy loam soil. *Soil Biology and Biochemistry* **25**: 1527-1536.

Skiba U., Fowler D. & Smith K. A. (1997) Nitric oxide emissions from agricultural soils in temperate and tropical climates: sources, controls and mitigation options. *Nutrient Cycling in Agroecosystems* **48**: 139-153.

Skiba U.M., Van Dijk S., Ball B. C. (2002) The influence of tillage on spring NO and N_2O fluxes under spring and winter barley in Scotland. *Soil Use and Management* (in press).

Slemr F. & Seiler W. (1984) Field measurements of NO and NO_2 emissions from fertilised and unfertilised soils. *Journal of Atmospheric Chemistry* **2**: 1-24.

Slemr F. & Seiler W. (1991) Field study of environmental variables controlling the NO emissions from soil and the NO compensation point. *Journal of Geophysical Research* **96**: 13,017-13,031.

Smith K. A. (1980) A model of the extent of anaerobic zones in aggregated soils, and its potential application to estimates of denitrification. *Journal of Soil Science* **31**: 263-277.

Smith K. A. (1997) The potential for feedback effects induced by global warming on nitrous oxide by soils. *Global Change Biology* **3**: 327-338.

Smith K. A., Scott A., Galle B. & Klemedtsson L. (1994) Use of long-path infrared gas monitor for measurement of nitrous oxide from soil. *Journal of Geophysical Research* **99**: 16,585-16,592.

Smith J. B., Strzepek K. M., Kalkstein L. S., Nicholls R. J., Smith T. M., Riebsame W. E., & C Rosenzweig. (1995b). 'Executive summary.' In: Strzepek K. M., & Smith J. B (eds.) *As Climate Changes-International Impacts and Implications*. Cambridge University Press, Cambridge.

Smith K. A., Thomson P. E., Clayton H., McTaggart I. P. & Conen F. (1998a) Effects of temperature, water content and nitrogen fertilisation on emissions of nitrous oxide by soils. *Atmospheric Environment* **32**: 3301-3309.

Smith S. R., Woods V. & Evans T. D. (1998b) Nitrate dynamics in biosolids-treated soils. I. Influence of biosolid type and soil type. *Bioresource Technology* **66**: 139-149.

Smith S. R., Woods V. & Evans T. D. (1998c) Nitrate dynamics in biosolids-treated soils. II. Thermal-time models of the different nitrogen pools. *Bioresource Technology* **66**: 151-160.

Smith S. R., Woods V. & Evans T. D. (1998d) Nitrate dynamics in biosolids-treated soils. III. Significance of the organic nitrogen, a twin pool exponential model for nitrogen management and comparison with the nitrate production from animal wastes. *Bioresource Technology* **66**: 161-174.

Spoor. G. (1994) Machinery experiences with direct drilling in the United Kingdom. In: Tebrügge F. & Böhrnsen A. (eds.) *Experience with the applicability of no-tillage crop production in the West-European countries*, Concerted Action (No. Air 3-CT 93-1464), Proceedings of the EC-Workshop-1- Giessen, 27-28 June, 1994, pp 9-13. Wissenschafterlicher Fachverlag, Gießen, Germany.

Stamatiadis S., Doran J. W. & Kettler T. (1999) Field and laboratory evaluation of soil quality changes resulting from injection of liquid sewage sludge. *Applied Soil Ecology* **12**: 263-272.

Stohl A., Williams E., Wotawa G., & Kromp-Kolb H. (1996) A European inventory of soil nitric oxide emissions and the effect of these emissions on the photochemical formation of ozone. *Atmospheric Environment* **30**: 3741-3755.

Sullivan L. J., Moore T. C., Aneja V. P., Robarge W. P., Pierce T. E., Geron C. & Gay B. (1996) Environmental variables controlling nitric oxide emissions from agricultural soils in the Southeast United States. *Atmospheric Environment* **30**: 3573-3582.

Swift G. & Vipond J. E. (1991) The Beechgrove sheep mixture: three years' results 1988-1990. *Scottish Agricultural College technical note* **T274**. The Scottish Agricultural Colleges, Edinburgh.

Swift G., Vipond J. E., Cleland A. T. & Hunter E. A. (1993) 'A sustainable grass-clover sward for sheep.' In: Hopkins A. & Younie D. (eds.) Forward with grass into Europe. *Occasional Symposium of the British Grassland Society* **27**: 170-172.

Tabachow R. M., Peirce J. J. & Jousset S. (2001) Nitric oxide emissions from fertilised and biosolids-amended soil. *Journal of Environmental Engineering* **127**: 517-523.

Terry R. E., Nelson D. W. & Sommers L. E. (1979) Decomposition of anaerobically digested sewage sludge as affected by soil environmental conditions. *Journal of Environmental Quality* **8**: 342-347.

Terry R. E., Nelson D. W. & Sommers L. E. (1981) Nitrogen transformations in sewage sludge - Amended soils as affected by soil environmental factors. *Soil Science Society of America Journal* **45**: 506-513.

Thompson R. B. & Pain B. F. (1989) 'Denitrification from cattle slurry applied to grassland.' In: Hansen J. A. & Henriksen K. (eds.) *Nitrogen in Organic Wastes Applied to Soils*, pp 247-260. Academic Press, London.

Thornton F. C. & Valente R. J. (1996) Soil emissions of nitric oxide and nitrous oxide from no-till corn. *Soil Society of America Journal* **60**: 1127-1133.

Thornton F. C., Bock B. R. & Tyler D. D. (1996) Soil emissions of nitric oxide and nitrous oxide from injected anhydrous ammonium and urea. *Journal of Environmental Quality* **25**: 1378-1384.

Tortoso A. C. & Hutchinson G. L. (1990) Contributions of autotrophic and heterotrophic nitrifiers to soil NO and N_2O emissions. *Applied and Environmental Microbiology* **56**: 1799-1805.

Tottman D. R. (1987) The decimal code for the growth stages of cereals, with illustrations. *Annals of Applied Biology* **110**: 441-454.

Umarov M. M. (1990) ' Biotic sources of nitrous oxide (N_2O) in the context of the global budget of N_2O' In: Bouwman A. F. (ed.) *Soils and The Greenhouse Effect*, pp 263 – 285. John Wiley & Sons Ltd., Chichester, UK.

UNFCCC (1994) 'Understanding Climate Change: A beginners guide to the United Nations Framework Convention.' UNEP/WMO Information Unit on Climate Change, December 1994. www.unfccc.de/resource/beginner.html

Valente R. J., & Thornton F. C. (1993) Emissions of NO from a soil at a rural site in central Tennessee. *Journal of Geophysical Research* **98**: 16,745-16,753.

Van Breemen N. & Feijtel T. C. J. (1990) ' Soil processes and properties involved in the production of greenhouse gases, with special reference to soil taxonomic systems.' In: Bouwman A. F. (ed.) *Soils and The Greenhouse Effect*, pp 195 – 223. John Wiley & Sons Ltd., Chichester, UK.

Van Dijk S. M. & Duyzer J. H. (1999) Nitric oxide emissions from forest soils. *Journal of Geophysical Research* **104**: 15,955-15,961.

Veldkamp E. & Keller M. (1997) Fertiliser-induced nitric oxide emissions from agricultural soils. *Nutrient Cycling in Agroecosystems* **48**: 69-77.

Veldkamp E., Keller M. & Nuñez M. (1998) Effects of pasture management on N_2O and NO emissions from soils in the humid tropics of Costa Rica. *Global Biogeochemical Cycles* **12**: 71-79.

Velthof G. L. & Oenema O. (1995a) Nitrous oxide fluxes from grassland in the Netherlands: I. Statistical analysis of flux-chamber measurements. *European Journal of Soil Science* **46**: 533-540.

Velthof G. L. & Oenema O. (1995b) Nitrous oxide fluxes from grassland in the Netherlands: II. Effects of soil type, nitrogen fertiliser application and grazing. *European Journal of Soil Science* **46**: 541-549.

Verhagen F. J. M., Duyts H. & Laanbroek H. J. (1992) Competition for ammonium between nitrifying and heterotrophic bacteria in continuously percolated soil columns. *Applied and Environmental Microbiology* **58**: 3303-3311.

Vinten A. J. A., Ball B. C., O'Sullivan M. F., Henshall J. K., Howard R., Wright F. & Ritchie R. (2001) The effects of cultivation method and timing, previous sward and fertiliser level on subsequent crop yields and the fate of organic C and N, following cultivation of long term grazed grass and grass-clover swards. *Journal of Agricultural Science* – in press.

Vipond J. E., Swift G., Cleland A. T., Fitzimons J. & Hunter E.A. (1997) A comparison of diploid and tetraploid perennial ryegrass and tetraploid ryegrass/white clover swards under continuous sheep stocking at controlled sward heights. 3. Sward characteristics and animal output, years 4-8. *Grass and Forage Science* **52**: 99-109.

Vlasenko A. N., Sharkov I. N., Sineshchekov V. E. & Prozorov A. S. (2001) Reduced tillage and nitrogen mineralisation. *Eurasian Soil Science* **34**: 993-998.

Watanabe T., Osada T., Yoh M. & Tsuruta H. (1997) N2O and NO emissions from grassland soils after the application of cattle and swine excreta. *Nutrient Cycling in Agroecosystems* **49**: 35-39.

Weber P. & Rennenberg H. (1996) Exchange of NO and NO_2 between wheat canopy monoliths and the atmosphere. *Plant and Soil* **180**: 197-208.

Weslien P., Klemedtsson L., Svensson., Galle B., Kasimir-Klemedtsson Å. & Gustafsson A. (1998) Nitrogen losses following application of pig slurry to arable land. *Soil Use and Management* **14**: 200-208.

Whitehead D. C. (1995) *Grassland Nitrogen*. CAB International, Wallingford, UK.

Williams E. J., Parrish D. D., Buhr M. P., Fehsenfeld F. C., & Fall R. (1988) Measurement of soil NO_x emissions in central Pennsylvania. *Journal of Geophysical Research* **93**: 9539-9546.

Williams E. J., & Fehsenfeld F. C. (1991) Measurement of soil nitrogen oxide emissions at three North American ecosystems. *Journal of Geophysical Research* **96**: 1033-1042.

Williams E. J., Guenther A., & Fehsenfeld F. C. (1992) An inventory of nitric oxide emissions from soils in the United States. *Journal of Geophysical Research* **97**: 7511-7519.

Williams P. H., Jarvis S. C. & Dixon E. (1998) Emission of nitric oxide and nitrous oxide from soil under field and laboratory conditions. *Soil Biology and Biochemistry* **30**: 1885-1893.

Williams J. R., Chambers B. J., Bhogal A., Chadwick D. R. & Pain B. F. (1999) Field studies of farm manure organic mineralisation. *Proceedings of the 8th International Conference of the FAO Escorena Network on Recycling of Agricultural, Municipal and Industrial Residues in Agriculture*. Eds. Martinez J. & Maudet M. N. Food and Agricultural Organisation of the United Nations, Rome and Cemagref, Antony, France, pp 101-113.

Yamulki S., Goulding K. W. T., Webster C. P. & Harrison R. M. (1995) Studies on NO and N_2O fluxes from a wheat field. *Atmospheric Environment* **29**: 1627-1635.

Yienger, J. J, & Levy II H. (1995) Empirical model of global soil-biogenic NO_x emissions. *Journal of Geophysical Research* **100**: 11,447-11,464.

Young I. M. & Ritz K. (2000) Tillage, habitat space and function of soil microbes. *Soil and Tillage Research* **53**: 201-213.