LEABHARLANN CHOLÁISTE NA TRÍONÓIDE, BAILE ÁTHA CLIATH | TRINITY COLLEGE LIBRARY DUBLIN
Ollscoil Átha Cliath | The University of Dublin

Terms and Conditions of Use of Digitised Theses from Trinity College Library Dublin

Copyright statement

All material supplied by Trinity College Library is protected by copyright (under the Copyright and Related Rights Act, 2000 as amended) and other relevant Intellectual Property Rights. By accessing and using a Digitised Thesis from Trinity College Library you acknowledge that all Intellectual Property Rights in any Works supplied are the sole and exclusive property of the copyright and/or other IPR holder. Specific copyright holders may not be explicitly identified. Use of materials from other sources within a thesis should not be construed as a claim over them.

A non-exclusive, non-transferable licence is hereby granted to those using or reproducing, in whole or in part, the material for valid purposes, providing the copyright owners are acknowledged using the normal conventions. Where specific permission to use material is required, this is identified and such permission must be sought from the copyright holder or agency cited.

Liability statement

By using a Digitised Thesis, I accept that Trinity College Dublin bears no legal responsibility for the accuracy, legality or comprehensiveness of materials contained within the thesis, and that Trinity College Dublin accepts no liability for indirect, consequential, or incidental, damages or losses arising from use of the thesis for whatever reason. Information located in a thesis may be subject to specific use constraints, details of which may not be explicitly described. It is the responsibility of potential and actual users to be aware of such constraints and to abide by them. By making use of material from a digitised thesis, you accept these copyright and disclaimer provisions. Where it is brought to the attention of Trinity College Library that there may be a breach of copyright or other restraint, it is the policy to withdraw or take down access to a thesis while the issue is being resolved.

Access Agreement

By using a Digitised Thesis from Trinity College Library you are bound by the following Terms & Conditions. Please read them carefully.

I have read and I understand the following statement: All material supplied via a Digitised Thesis from Trinity College Library is protected by copyright and other intellectual property rights, and duplication or sale of all or part of any of a thesis is not permitted, except that material may be duplicated by you for your research use or for educational purposes in electronic or print form providing the copyright owners are acknowledged using the normal conventions. You must obtain permission for any other use. Electronic or print copies may not be offered, whether for sale or otherwise to anyone. This copy has been supplied on the understanding that it is copyright material and that no quotation from the thesis may be published without proper acknowledgement.

Nitrous Oxide Emissions associated with Land Use Change to Bioenergy Crops *Miscanthus x giganteus* and *Phalaris arundinacea*.

PhD Thesis

2013

Dominika Joanna Krol

Supervisors: Dr. Mike Williams

Dr. Gary Lanigan

School of Natural Sciences

Trinity College

University of Dublin

TRINITY COLLEGE
- 4 MAR 2014
LIBRARY DUBLIN
Thesis 10317

Declaration

I declare that this thesis has not been submitted as an exercise for a degree at this or any other university and it is entirely my own work.

I agree to deposit this thesis in the University's open access institutional repository or allow the Library to do so on my behalf, subject to Irish Copyright Legislation and Trinity College Library conditions of use and acknowledgement.

Summary

Bioenergy crops are an increasingly popular alternative to fossil fuels for electricity and heat generation due to their substantial yields combined with low inputs of resources. As these crops are characterised by low nutrient requirements, they can facilitate mitigation of fertiliser-induced gaseous emissions and consequently aid meeting the greenhouse gas (GHG) reduction targets posed by various policies. However very little research on GHG emissions associated with the cultivation of the energy crops is available. Nitrous oxide (N_2O) emissions dominate the GHG budget of tillage and pastoral soils (Rees and Ball, 2010) therefore monitoring of N_2O from these soils is crucial. This study investigated the impact of land-use change (LUC) from pasture to two energy grasses (*Miscanthus x. giganteus* and *Phalaris arundinacea*) on N_2O emissions through a field trial and two lysimeter studies focused on investigating the effects of ploughing technique, fertiliser management, soil type and crop type.

All three experiments were conducted at the Teagasc Johnstown Castle Environmental Research Centre, Co. Wexford. A field trial commenced in March 2009 and took place over 24 months. The effects of LUC were examined by weekly measurements of N_2O emissions by a static chamber method from two *Miscanthus* plots and two fallow grassland plots during the first year, which were sown with Reed Canarygrass in the second year. Soil temperature and water filled pore space were measured at the same time as N_2O. In addition, measurements of soil available nitrogen (ammonium and nitrate), soil physical characteristics and grass yields were performed. Similarly the effects of ploughing using alternative ploughing techniques were examined by monitoring N_2O emissions, leached N and soil temperature and moisture content during a lysimeter experiment between June and November 2009. Finally, a second lysimeter study examined the effects of fertilisation on N_2O and dinitrogen (N_2) emissions, leached N losses, plant uptake and soil mineralisation/immobilisation.

Ploughing and fertilisation of the lysimeter soils caused a significant increase in gaseous and leached N fluxes; however, the response to each of these practices differed. Ploughing using conventional (CT) or minimum (MT) tillage resulted in a high, short-lived increase of N_2O emissions, whereas fertilisation was associated with lower N_2O

fluxes than post-ploughing, but over a long period. Most of the N_2O emissions were attributed to fertilisation rather than ploughing (over 61% on poorly-drained conventionally tilled soil up to 92% on moderately-drained minimum tilled soil). N_2O emissions were higher from both minimum-tilled and also from poorly-drained soils whereas leached N losses were higher from well-drained soils. Whilst alterations in tillage and soil type affected the balance between gaseous and leached N, there was no significant difference in total N losses.

Following the field-scale conversion to biomass crops the first year post-LUC exhibited high N_2O fluxes with the highest losses from the well-drained *Miscanthus* plot which exhibited emissions comparable to those of managed pasture systems. Emissions in the subsequent year were lower due to increases in both shoot and rhizome density. By contrast, emissions from newly established Reed canarygrass plots did not differ significantly compared to fallow grassland plots.

Fertiliser application to lysimeters containing established crops resulted in substantial quantitative and qualitative differences in both N_2 and N_2O losses from both grassland and biomass crop systems. N_2O emissions were primarly produced through nitrification and nitrifier de-nitrification in sandy soils and denitrification on heavy soils. Higher rates of total de-nitrification (quantified by N_2 fluxes) were also observed on heavy clay and loam soils compared to sandy soils. Gaseous N emissions were generally lowest for *Miscanthus*, due to greater N uptake associated with the extensive rhizome system.

Energy crops are more and more widely utilised in heat and electricity production as a cheap, indigenous alternative to fossil fuels. Land use change into energy crops can also be reported as a sink activity under article 3.4 of the Kyoto Protocol therefore helping to reduce greenhouse gas (GHG) emissions. However the impact of these crops on GHG emissions is strongly influenced by the production of trace gases such as N_2O. N_2O emissions observed during this study were substantial immediately following LUC into energy crops but were comparable to intensively managed pasture; therefore, no negative impacts on GHG balance is expected in medium to long term. Low N requirement together with a high N_2:N_2O ratio supports the hypothesis that energy crops, especially *Miscanthus,* could be a viable energy production and GHG mitigation option.

Acknowledgements

First, I would like to thank my supervisors for giving me the opportunity to conduct this research project. I owe my deepest gratitude to Dr. Gary Lanigan, Teagasc, for his mentoring throughout the last four years, for his tremendous support and patience, and contagious enthusiasm for science. Dr. Mike Williams, Trinity College Dublin, for his knowledge, insight, and expert advice on all aspects of this study.

I would like to extend my gratitude to the Research Stimulus Fund, administered by the Department of Agriculture, Fisheries and Food, Ireland for financially supporting this research under the National Development Plan.

I would like to thank Prof. Mike Jones, Dr. John Finnan, Dr. Kees-Jan van Groeningen, Dr. Karl Richards, Dr. Eddy Minet, Stan Lalor, and the late Dr. Saul Otero for providing me with invaluable help and guidance at various stages of this project, and even letting me step into a 'circle of knowledge'. I would like to acknowledge members of the plant eco-physiology research group at Trinity College, especially Dr. Brendan Roth, Niamh Smyth, and Jesko Zimmermann for their stimulating discussions, enthusiasm and encouragement for this project.

I am grateful to Vincent Staples, Brendan Swan, Brendan Connick, and Kevin McNamara for their hard work and lending a helping hand with field work, especially with soil sampling and lysimeters collection. Cathal Somers for constant support in the GC lab, his friendliness and encouragement when things were going wrong and for many inspirational chats. Denis Brennan, not only for analysing water samples, but also for his valuable recommendations on experimental design and sample analysis. Dr. Anna Fenelon for great laboratory support.

I am indebted to everybody in Teagasc, Johnstown Castle for creating such a wonderful atmosphere, for their support and belief in me, for all the cups of tea and warms words, and above all, their friendship. This goes especially to Niamh, Diana, Orlaith, Noeleen, Mrs. Berry, Sophie, Mairead, Emma, Nicola, Sarah, and the WMBSoc. Furthermore to

Dr. Sinead O'Keeffe, Dr. Fiona Brennan, Dr. Maria Ernfors, Dr. Alice Melland, Sarah Mechan, Dr. Dote Stone, Fred Bourdin, Dr. Enda Cahalan, and Dr. Paul Massey.

Finally I would like to thank my parents Aleksandra and Tadeusz and my brother Konrad for their unconditional support throughout these four years and for firmly believing in me, especially when I doubted myself. My heartfelt gratitude goes to Donal Whelan for his support and understanding over the past year. The best is yet to come…

Table of Contents

DECLARATION	**II**
SUMMARY	**III**
ACKNOWLEDGEMENTS	**V**
CHAPTER 1: INTRODUCTION	**1**
1.1 Outline	2
1.2 Political implications of rising N losses to the environment at global, regional and national scale.	2
1.3 Composition of agricultural Greenhouse Gas Emissions	3
1.3.1 Implication of GHG and energy policies in Ireland	*5*
1.4 The Nitrogen cycle	6
1.4.1 Reactive nitrogen in the environment	*6*
1.4.2 Overview of nitrogen cycling	*8*
1.4.3 Nitrogen transformation processes	*9*
1.4.4 Mineralisation and immobilisation	*9*
1.4.5 Nitrification	*10*
1.4.6 Denitrification	*11*
1.5 Drivers of nitrous oxide (N_2O) emissions	13
1.5.1 Soil moisture content and soil oxygen availability	*13*
1.5.2 Soil temperature	*15*
1.5.3 Soil nitrogen	*16*
1.5.4 Soil management	*16*
1.6 N_2O emissions from soils	16
1.7 Agricultural N_2O mitigation options	17
1.8 Energy crops	19
1.8.1 Types of energy crops and emissions associated with their cultivation	*20*
1.8.1.1. Miscanthus	*20*
1.8.1.2 Reed canarygrass	*22*
1.9 Land use change into energy crops cultivation in Ireland	22
1.10 Objectives of the project	23
CHAPTER 2: THE EFFECT OF PLOUGHING ON SHORT-TERM EMISSIONS OF N_2O FROM THREE SOIL TYPES OF VARYING ORGANIC MATTER CONTENT.	**27**
2.1. Introduction	29

2.2 Materials and methods 34
2.2.1. Experimental site *34*
2.2.2. Layout *34*
2.2.3. Experimental treatments and design *35*
2.2.4. Nitrous oxide emissions measurements and analysis *36*
2.2.5. Calculation of N_2O emissions *37*
2.2.6. Leachate collection and analysis *38*
2.2.7. Calculation of leached N *39*
2.2.8. Soil bulk density *39*
2.2.9. Soil mineral nitrogen content *39*
2.2.10. Ancillary measurements *40*
2.2.11. Statistical analysis *40*
2.3. Results 40
2.3.1. Rainfall and temperature distribution patterns *40*
2.3.2. Daily and cumulative nitrous oxide emissions *42*
2.3.3. Relationship between emissions and soil and environmental factors *45*
2.3.3.1. Post-ploughing N_2O emissions vs. clay content *45*
2.3.3.2. Post-fertiliser N_2O emissions vs. soil temperature and moisture *46*
2.3.3.3. Available nitrogen *47*
2.3.3.4. N leaching *47*
2.3.3.5. Overall N losses *49*
2.4. Discussion 50
2.4.1. N_2O emissions *50*
2.4.2. Leached N *52*
2.4.3. Overall N losses *52*
2.5. Conclusions 53
CHAPTER 3: FIELD SCALE STUDY ON THE EFFECT OF LAND USE CHANGE FROM GRASSLAND INTO ENERGY CROPS-*MISCANTHUS* AND REED CANARYGRASS CULTIVATION ON N CYCLING, IN PARTICULAR N_2O EMISSIONS. **55**
3.1 Introduction; objectives and hypotheses of the experiment 57
3.2. Materials and methods 61
3.2.1. Experimental sites *61*
3.2.2. Experiment design and management *61*
3.2.3. N_2O emissions measurements and analysis *65*

3.2.4. Ancillary measurements 66
3.2.4.1. Herbage 66
3.2.4.2. Background soil sampling and analyses of soil physical and chemical properties. 67
3.2.4.3. Soil water content and soil temperature 68
3.2.4.4. Soil mineral N content 69
3.2.5. Statistical analysis 69
3.3. Results 70
3.3.1. Meteorological data 70
3.3.2. Nitrous oxide 71
3.3.3. Herbage 76
3.3.4. Background soil sampling and analysis 79
3.3.5. Soil water content and soil temperature 80
3.3.6. Soil mineral N content 83
3.4. Discussion 85
3.4.1. Nitrous oxide emissions 85
3.4.2. Comparison between treatments and years 86
3.4.3. Other N_2O emissions from LUC into energy crops reported from Ireland and Europe 87
3.4.4. The effect of land use change on soil conditions 88
3.5. Conclusions 89
CHAPTER 4: ^{15}N STABLE ISOTOPE STUDY ON N CYCLING PROCESSES IN PLANT-SOIL SYSTEMS UNDER GRASSLAND AND ENERGY CROP COVERS: *MISCANTHUS* AND REED CANARYGRASS. THE EFFECT OF CROP COVER AND SOIL PROPERTIES ON N PROCESSES. **93**
4.1. Introduction; objectives and hypotheses of the experiment 95
4.2. Materials and methods 98
4.2.1. Sites and plant selection 98
4.2.2. Lysimeters design 99
4.2.3. Lysimeter collection 101
4.2.4. Sealing the lysimeters 104
4.2.5. Growth room set up 106
4.2.6. Treatments 107
4.2.7. Gas sampling and analysis 109

4.2.8.Leachate sampling and analysis *112*
4.2.9. Herbage sampling and analysis *112*
4.2.10. Soil sampling and analysis *113*
4.2.11. Roots sampling and analysis *113*
4.2.12. Diffusion of leachate samples *113*
4.3. Results 114
4.3.1. N_2O emissions in response to a mineral fertilizer application *114*
4.3.2. Nitrification and de-nitrification rates *117*
4.3.3. ^{15}N enrichment of gaseous emissions post-fertilisation *119*
4.3.4. Leached N losses in response to a mineral fertilizer application *121*
4.3.5. The magnitude of reactive N losses following fertiliser application and partitioning between the pathways *123*
4.3.6. ^{15}N fertiliser recovery rates 124
4.4. Discussion 127
4.5. Conclusions 132
CHAPTER 5: GENERAL DISCUSSION **133**
5.1. Overview 135
5.2. Findings of the study 136
5.3. Limitations of the study 140
5.4. Future work 141
5.5 Implications 142
5.6. Conclusions 144
REFERENCES: **147**

List of figures

Figure 1.1 Simplified nitrogen cycle with the emphasis on its terrestrial part, in particular soil processes. 8

Figure 2.1 Location of the experimental site..... 34

Figure 2.2 Daily rainfall (grey bar) and mean temperature (grey line) records for year 2009 collected by a met station in Johnstown Castle. Values observed throughout the experiment are marked in black. 41

Figure 2.3 Temporal pattern of N_2O fluxes throughout the experiment for conventional (a) and minimum (b) tillage treatments from Rathangan (●), Elton (○), and Clonakilty (▼) soils. Arrows indicate application timing of tillage (first arrow) and fertilisation (second arrow). Error bars indicate SEM (n=3)..... 43

Figure 2.4 Relationship between clay content of the soils and cumulative N_2O emissions from CT (●) and MT (○) treatments expressed as percentage of the total emissions over the course of the experiment. Error bars indicate SEM (n=3)..... 46

Figure 2.5 Correlation between WFPS and daily N_2O flux over fertiliser period from CT (●) and MT (○) treatments..... 47

Figure 2.6 Cumulative leached N losses over the whole experiment for (a) CT and (b) MT treatments from Rathangan (●), Elton (○), and Clonakilty (▼) soils. Arrows indicate application of tillage (first arrow) and fertilisation (second arrow). Error bars indicate SEM (n=3)..... 48

Figure 2.7 Total N losses throughout the experiment as a sum of combined gaseous emissions and N leaching. Error bars indicate SEM (n=3). Capital letters indicate statistically significant differences at p<0.05.....49

Figure 3.1 Map of Johnstown Castle Estate, Wexford. Red circle marks the part of the farm where the two experimental sites were chosen.....61

Figure 3.2 Schematics of the treatments applied throughout the land use change experiment. 63

Figure 3.3 Layout of frames positions on the experimental plots recorded with GPS on a well drained site (W/D) (a), and poorly drained site (P/D) (b).....66

Figure 3.4 Daily rainfall and mean temperature records for 1.04.2009 until 15.04.2011 collected by a meteorological station in Johnstown Castle.....70

Figure 3.5 Temporal patterns of N_2O emissions (●) throughout the measurement period of the field study from Miscanthus W/D (a), *Miscanthus* P/D (b), Reed canarygrass W/D (c), and Reed canarygrass P/D (d). *Black arrows indicate planting Miscanthus rhizomes in plots (a) and (b), and sowing Reed canarygrass in plots (c) and (d).* 72-73

Figure 3.6 Differences between Miscanthus and grassland N losses in year one (a), and between Miscanthus and Reed canarygrass in year two (b) expressed in g N_2O-N. Capital letters in brackets indicate statistically significant differences at $p<0.05$.....75

Figure 3.7 Above (positive values) and below (negative values) ground biomass at measurement times from Miscanthus W/D (a), Miscanthus P/D (b), Reed canarygrass W/D (c), and Reed canarygrass P/D (d) . Miscanthus (a, b) above ground biomass was partitioned into stems and leaves, except for Miscanthus P/D (b) in February 2010 where stems and leaves were aggregated into above ground biomass. Miscanthus below ground biomass was assessed on three separate occasions and was partitioned into live and dead rhizomes and roots. In case of Reed canarygrass (c, d) below ground biomass was also assessed on two separate occasions. Sampling in January 2011 took place post-harvesting hence only below ground biomass was assessed.....77-78

Figure 3.8 WFPS temporal pattern in the experimental plots over two years Miscanthus cultivation on two plots (a), over one year on two plots of Reed canarygrass (b), and a comparison between Miscanthus and Reed canarygrass WFPS values during the second year (c).....81

Figure 3.9 Soil temperature temporal patterns in the experimental plots; (a) over two years Miscanthus cultivation on two plots, (b) over one year on two plots of Reed canarygrass, and (c) a comparison between Miscanthus and Reed canarygrass soil temperature values during the second year. Error bars indicate standard error.....82

Figure 3.10 Temporal patterns of the concentrations of available nitrogen in the soils of the experimental plots: grass later converted into Reed canarygrass in the forms of ammonium (a) and nitrate (b), and Miscanthus in the forms of ammonium (c) and nitrate (d). Error bars indicate standard error.....84

Figure 3.11 Miscanthus and Reed canarygrass yields (black bars) and associated cumulative N_2O fluxes (grey bars) from the three years on Miscanthus cultivation and two years of Reed canarygrass cultivation. No N_2O data is available for the last cultivation year. Error bars indicate standard error.....87

Figure 4.1 Design of the lysimeters used in the experiment together with their dimensions.....100

Figure 4.2 The PVC pipes cut to desired length and sharpened at the bottom.....100

Figure 4.3 Excavation set-up consisting of two trenches (a), pushing the cutting ring into the soil by the digger (b), hand digging to take out a full soil core in the PVC pipe enclosed inside the cutting ring (c).....102

Figure 4.4 Assembly of the stoppers (a), and placing the metal mesh inside the stopper (b). Covering the mesh with a layer of gravel (c).....103

Figure 4.5 Lysimeters prepared for sealing and the equipment used for heating the petrolatum (a), trowel used for precise application of liquidised petrolatum (b), and freshly sealed lysimeter with petrolatum solidifying at the edges of the soil core (c). ...105

Figure 4.6 Temperature and humidity sensors in the growthroom (yellow circle) and a humidifier (red circle) (a), and the lysimeters set up with the leachate containers (b)...106

Figure 4.7 Separate sets of colour-coded fertilisers solutions, beakers, 50ml plastic syringes for application and 20ml plastic syringes for subsequent gas sampling (a, b); manual application of fertiliser solutions (c).....108

Figure 4.8 State of the art plastic chambers for gas sampling (c). Each chamber had two rubber septa (b) and was insulated with neoprene gasket to keep it air tight (a).....111

Figure 4.9 Temporal patterns of N_2O emissions throughout the experiment from the studied grassland systems (a), and energy crops systems (b). Black arrow indicates fertiliser application date.....115

Figure 4.10 Emission factors (EFs) for the crop-soil systems for the synthetic N fertiliser application and a comparison with an IPCC default value for this activity. Capital letters indicate statistically significant differences at $p<0.05$. Error bars indicate standard error of the mean.....117

Figure 4.11 Percent share of N_2O emissions created through nitrification reactions in the five studied crop-soil systems. Capital letters indicate statistically significant differences at $p<0.05$. Error bars indicate standard error of the mean.....118

Figure 4.12 Leached N losses of ammonium N (a) and nitrate N (b) from control, ammonium N (c) and nitrate N (d) from $^{15}NH_4NO_3$ treatment, and ammonium N (e) and nitrate N (f) from $^{15}NH_4{}^{15}NO_3$ treatment following fertilisation.....122

Figure 4.13 Combined leached N and gaseous N losses from all the crop-soil systems. 'C' represents control, and 'F' fertiliser treatments. Capital letters indicate statistically significant differences at $p<0.05$. Error bars indicate standard error of the mean.....124

Figure 4.14 Recovery rates of the applied ^{15}N partitioned between soil, biomass, leachate, and gaseous losses following fertilisation with a) $^{15}NH_4NO_3$ and b) $^{15}NH_4{}^{15}NO_3$. Error bars indicate standard error of the mean.....126

List of tables

Table 2.1 Physical and chemical soils characteristics at 0-10 cm depth (n=3). Capital letters in brackets indicate statistically significant differences at p<0.05.....35

Table 2.2 Two alternative tillage types were applied to three soil types in three blocks of six lysimeters resulting in the below layout.....36

Table 2.3 Average rainfall [mm] and temperature [°C] associated with background period, tillage and fertilisation. Capital letters in brackets indicate statistically significant differences at p<0.05.....42

Table 2.4 Cumulative gaseous N losses [kg N_2O-N ha^{-1}] associated with background period, tillage and fertilisation (n=3). Capital letters in brackets indicate statistically significant differences at p<0.05.....44

Table 2.5 Levels of available nitrogen in the lysimeters soils (n=3). Capital letters in brackets indicate statistically significant differences at p<0.05.....47

Table 2.6 Emission factors for the fertilisation of previously ploughed lysimeter soils. Capital letters in brackets indicate statistically significant differences at p<0.05.....51

Table 3.1 Soil characteristics of both sites at soil depth 0-10 cm (March 2009). Capital letters in brackets indicate statistically significant differences at p<0.05.....62

Table 3.2 Detailed information on agricultural management of the experimental sites...64

Table 3.3 Annual N_2O loss from the experimental plots in year one (April 2009-April 2010) and year two (April 2010-April 2011) expressed in kg N_2O-N ha^{-1} measured on 51 occasions from minimum seven chambers per plot. Numbers in brackets indicate standard error of the mean (SE).....74

Table 3.4 Bulk density values [g cm^{-3}] at 0-10 cm obtained prior to land use change, following ploughing and in year three. Capital letters in brackets indicate statistically significant differences at p<0.05.....79

Table 3.5 Soil organic carbon and total nitrogen contents [%] obtained in year three at three depths: 0-10 cm, 10-20 cm, and 20-30 cm at both Miscanthus and Reed canarygrass plots and adjacent grassland that did no undergo land use change. Capital letters in brackets indicate statistically significant differences at p<0.05.....79

Table 4.1 Crop-soil systems utilised in the experiment.....99

Table 4.2 Soil core properties of the lysimeters.....99

Table 4.3 Set up conditions of the programme manipulating environmental parameters inside the growthroom.....106

Table 4.4 Comparison of cumulative N_2O-N losses from control and fertiliser treatments on all crop-soil systems. SE values indicate standard error of the means. Capital letters in brackets indicate statistically significant differences at $p<0.05$.....116

Table 4.5 Daily mean N_2O emissions, corresponding N_2 emissions and N_2:N_2O ratios after application of both fertiliser treatments to the various crop-soil lysimeters based on non gap-filled data (fertiliser period between 06.09.2010 and 20.09.2010). SE values indicate standard error of the mean.....120

Table 4.6 Comparison of cumulative ammonium and nitrate in leachates from control and fertiliser treatments on all crop-soil systems. Capital letters in brackets indicate statistically significant differences at $p<0.05$.....121

Chapter 1: Introduction

1.1 Outline

The latest assessment report of the International Panel on Climate Change (IPCC) (Fourth Assessment Report-AR4) confirmed that the global temperature has increased by 1°C over the last century as a result of increased inputs of greenhouse gases (GHG) from anthropogenic sources. Concentration of the principle greenhouse gas carbon dioxide (CO_2), in the atmosphere has increased by 30% over the last 250 years and reached 391 ppm in October 2012 (NOAA Manua Loa, 2012). This is primarily due to the large scale combustion of fossil fuels that have occurred since the Industrial Revolution as well as large-scale land-use change, particularly deforestation that has accompanied population increases over the last century (Mosier, 1998). The two other main greenhouse gases, methane (CH_4) and nitrous oxide (N_2O) have recorded increases in their atmospheric concentrations of 145% and 15% respectively, over the past 250 years due to increased agricultural production, waste generation and land-use change (Mosier, 1998; Groenigen et al., 2005). Atmospheric N_2O concentration reached 320 ppb in 2007 and is rising by 0.8 ppb annually (IPCC, 2007).

GWP is a metric describing radiative forcing (RF) of one kilogram of a compound relative to that of one kilogram of a reference gas-CO_2, over chosen timeframe (IPCC, 2007). Radiative forcing is the difference between the energy received by the earth's surface and energy radiated back into space. Greenhouse gases such as N_2O absorb infrared radiation therefore they are characterised with positive radiative forcing warming up the earth system. However atmospheric concentration of N_2O is nearly thousand times lower than this of CO_2, N_2O is a potent greenhouse gas with a global warming potential (GWP) of 310 on a 100 year basis (IPCC, 2007).

1.2 Implications of rising N losses to the environment at global, regional and national scale

The Intergovernmental Panel on Climate Change (IPCC) set up by the United Nations Environment Programme (UNEP) and the World Meteorological

Organisation (WMO) in 1988, through their various climate reports, led in 1997 to drawing up the Kyoto Protocol on the reduction of greenhouse gas (GHG) emissions to counteract anthropogenic pressure on climate change. Since its creation, the IPCC has been the main authority and source of information in the matter of climate change (Peake and Smith, 2003) and the Kyoto Protocol the main document regulating GHG emissions and emissions reduction targets. The Kyoto Protocol is an international agreement governed by the United Nations Framework Convention on Climate Change (UNFCCC). The Protocol set binding global GHG reduction targets for industrialised states (termed Annex 1 countries) of five per cent of 1990 emissions reduction to be achieved between years 2008 and 2012. The Kyoto Protocol was ratified in 2002 by Ireland and all other EU Member States and came into force in 2005. It deals with six GHGs, carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), hydrofluorocarbons (HFCs), perfluorocarbons (PFCs), and sulphur hexafluoride (SF_6). Under the articles 3.3 and 3.4 of the Kyoto Protocol Annex 1 Parties are obliged to report their national emissions by sources and removals by sinks of GHGs resulting from land use, land use change, and forestry (LULUCF) activities. Article 3.3. limits direct, human-induced LULUCF activities to afforestation, reforestation and deforestation that took place since 1990. The sinks of GHGs are primarily associated with gains in the carbon stocks in forestry. However, article 3.4 extends the possible sinks by including additional human-induced activities related to LULUCF such as forest management, cropland management, grazing land management and revegetation (UNFCCC, 1997). This opens possibilities of biomass and bioenergy crops cultivation to enhance carbon stocks in soil and therefore remove CO_2 by sinks. Moreover, these crops could provide carbon neutral stocks for electricity and heat generation helping not only to mitigate GHG emissions but also provide a renewable energy source. The GHG balance of these crops depends heavily on the balance between trace gases production and reduction by sinks associated with their cultivation.

1.3 Composition of agricultural Greenhouse Gas Emissions

Agricultural practices contribute to 14% of global anthropogenic greenhouse gases (GHG), with methane (CH_4) from grazing ruminants and nitrous oxide (N_2O) from

organic and mineral N deposition on soils comprising the bulk of these emissions (Bouwmann *et al.* 2001; US-EPA, 2006; Smith *et al.*, 2007). In addition, carbon dioxide (CO_2) emissions associated with land use change (principally from deforestation) account for an additional 15% of anthropogenic CO_2 emissions (US-EPA, 2006).

Ireland is unique among the EU countries for the high proportion of its greenhouse gas (GHG) emissions which originate from agriculture, representing 32% of national and 44% of the non-Emissions Traded Sector (non-ETS) emissions (Duffy *et al.*, 2011). This proportion is high compared to the EU average of 9%, and reflects the relative importance of agriculture, which is predominantly based on production of ruminant livestock products, to the Irish economy (Breen *et al.*, 2010). Amongst the developed economies, only New Zealand has a higher proportion of national GHG emissions associated with agriculture (NZ-MoE, 2010).

The latest reported (2011) emissions from the Irish agricultural sector amount to 17.55 Mt carbon-dioxide equivalents (CO_2eq). Nitrous oxide emissions comprise 38% of this total with emissions arising principally as a result of chemical/organic fertilizer application and animal deposition of N and indirect emissions from leached and volatilised N (Duffy *et al.*, 2011).

In terms of global nitrous oxide emissions, 65% are derived from natural processes, whilst 35% is anthropogenic (IPCC, 2007). In terms of the anthropogenic portion, agriculture and fertiliser manufacture comprises the vast bulk of these emissions. N_2O dominates the greenhouse gas budget of agricultural soils (Rees and Ball, 2010; Thomson *et al.*, 2012) and simple N_2O budgeting based on fertiliser emission factors can underestimate a variety sources or leave sinks unaccounted for. Therefore the IPCC requires methodology for N_2O budgeting including indirect emissions associated with other reactive N losses, such as NO_3^- leaching and NH_3 volatilization (Mosier, 1998). At the European scale five main documents have been drawn up to tackle increasing nitrogen losses to atmosphere, land and water. These are the Nitrates Directive (91/676/EC) and The Water Framework Directive (WFD) (2000/60/EC) both dealing with water quality, The Gothenburg Protocol to abate acidification, eutrophication and ground level ozone, The Convention of Long Range Transboundary

Air Pollution and The National Ceilings Directive (2001/81/EC) applying to gaseous emissions.

1.3.1 Implication of GHG and energy policies in Ireland

The Kyoto Protocol set an ambitious target on Irish GHG emissions of 62.8 Mt CO_2 equivalents annually for years 2008-2012. Currently Ireland is in general compliance with emissions averaging 62.1 Mt CO_2 eq. for the period 2008-11 (EPA, 2012) following two sharp decreases in emissions in 2009 and again in 2011. This has resulted in emissions reductions of 15% from 67.4 Mt CO_2 eq. in 2008 to 57.3 Mt CO_2 in 2011 (Duffy *et al.*, 2011). While the first decrease was largely due to the economic crisis and shrinking economy, the second resulted primarily from a greater share of renewables in the energy mix, combined with lower heat demand from households. Recent reductions in emissions cannot be fully explained by a simple correlation with economic indicators as other variables such as new technologies and policies now also influence the emissions therefore it can be concluded that emission levels are decoupling from economic production. Whilst projections indicate that Ireland will be able to comply with the Kyoto Protocol targets, new, more demanding targets laid down under the EU 2020 'Energy and Climate Package' will be more challenging, with the new commitment period running from 2012-2020. In particular, the Effort-Sharing Decision (Decision No 406/2009/EC) commits Ireland to reduce its GHG emissions from non-emission trading scheme (non-ETS) sectors by 20% for the period 2012-2020. Non-ETS sectors are those branches of economy not covered by the EU Emissions Trading Scheme and these comprise agriculture, transport, residential energy usage and waste. Together, these four sectors produced 64% of total Irish GHG emissions in 2009 with the single biggest contribution of 29.1% from agriculture (EPA, 2011). Agriculture is responsible for 97% of NH_3 emissions, 81% of N_2O, and 82% of N in surface waters (Toner *et al.*, 2005; DAFM, 2007; DEHLG, 2007). Currently agriculture is reducing its annual GHG production. However, the EPA predicts 4.0% increase between years 2009 and 2020 (EPA, 2011) to meet targets from the national 'Food Harvest 2020' policy. As a result, mitigation options will have to be employed to offset this increase.

Moreover, as an EU member state Ireland is also bound by a set of renewable energy targets. Within the directive 2009/28/EC on the promotion of the use of energy from renewable sources at least 20% of energy share in gross final energy consumption will come from renewable sources by 2020. A minimum 10% of the final consumption of energy in transport will come from renewables. Land use change into energy crops cultivation is of increasing importance in meeting the emission cuts and renewable energy targets. Plants used for bioenergy are characterised by high yields, low levels of contaminants and low nutrient requirements (McKendry, 2002). This means that together with providing renewable energy they save GHG emissions associated with fertiliser N application, which is the biggest single source of agriculture-derived N_2O (Storey, 1997). They can also reduce the risk of groundwater nitrate leaching (Borjesson, 1999) and enhance carbon sequestration. However, the establishment phase of these crops involves heavy land preparation like ploughing which is normally associated with SOC loss, and large CO_2 and N_2O emissions (Rowe, 2009). Achieving both economical and environmental targets from agricultural sector will only be possible if knowledge-based GHG mitigation options are employed together with improved management systems.

1.4 The nitrogen cycle

1.4.1 Reactive nitrogen in the environment

Nitrogen is, along with carbon, phosphorus, oxygen and sulphur, a necessary element supporting life on Earth, but despite its abundance in the atmosphere, consisting of 78% N, its availability is very limited to living organisms. This is due to nitrogen's chemical form in the atmosphere as an inert dinitrogen (N_2) gas with two atoms connected by a strong triple bond. Only small a number of specialised N-fixing microbes are able to convert N_2 to reactive nitrogen (Nr) (Galloway *et al.*, 2003; Galloway *et al.*, 2004; Galloway *et al.*, 2008). Reactive nitrogen includes all biologically, photochemically, and radiatively active nitrogen compounds, and encompasses both inorganic (ammonia (NH_3), ammonium (NH_4^+), nitrogen oxide (NO_x), nitric acid (HNO_3), nitrous oxide (N_2O), nitrate (NO_3^-)) and organic forms (urea, amines, nucleic acids, and proteins). Nr can be created from N_2 naturally through

biological nitrogen fixation (BNF) and lightning, and as a product of human activity through energy generation (combustion) and Haber-Bosch process used in fertiliser production. Anthropogenic Nr has shifted the balance between inactive and reactive N, resulting in an accumulation of the latter in the environment. This is of a major concern as Nr accumulation contributes to a) tropospheric ozone and aerosol production linked to respiratory and cardiac diseases, as well as cancer, b) forest and grassland productivity decrease caused by over-fertilisation through atmospheric Nr deposition, c) acidification and loss of biodiversity in surface waters), d) eutrophication, oxygen deficiency, loss of biodiversity, and habitat degradation in coastal ecosystems, e) global climate change and stratospheric ozone depletion (Aber *et al.*, 1995; Vitousek *et al.*, 1997; Wolfe and Patz, 2002; Rabalais, 2002; Townsend *et al.*, 2003; Marshall *et al.*, 2008). Creation of Nr is a critical step setting off the N cascade (Galloway, 1998), the sequential transfer of Nr through environmental systems, where it can be rapidly converted between its various reactive forms. In the effect of the N cascade, each N atom can be responsible for more than one of the numerous negative effects stated above. Nr production rate increases with a demand for food and energy and in 2005 reached 187 Tg N yr^{-1} globally in contrast to a pre-industrial rate of 15 Tg N yr^{-1}. Nr transfer is of a transboundary character making it extremely difficult for policy makers to tackle. In agricultural systems, N fertiliser produced in one country can be used elsewhere, and subsequently generated yield exported further, and additional Nr accumulated in the environment as a result of this process could be attributed to numerous sources. Two main strategies can be employed to reduce Nr and its effects on the environment: these are a) decreasing the rate of Nr creation, and b) increasing the conversion of Nr to N_2 (Galloway, 2003). The removal of Nr from the environment takes place mainly through denitrification, which is the microbial process of reduction of nitrate (NO_3^-) and nitrite (NO_2^-) to nitric oxide (NO), nitrous oxide (N_2O), and finally to dinitrogen (N_2) (Machefert *et al.*, 2002; Baggs, 2011). However the amount of N converted back to N_2 is a large uncertainty in the global N cycle (Galloway *et al.*, 2004).

Rising rates of reactive nitrogen production and a subsequent N cascade is not only responsible for negative environmental effects mentioned above but is also threatening to create what is called 'nitrogen saturation' when increased N availability reduces plant production and carbon storage (Vitousek *et al.*, 1997). Such effects can already

be seen in forests (Aber *et al.*, 1998, Fenn *et al.*, 1998) and marine coastal and estuarial ecosystems (Rabelais, 2002). Such nitrogen saturation can also cause species change in seminatural ecosystems (Vitousek *et al.*, 1997). In terrestrial ecosystems, soils contribute between 63% and 70% of N_2O production (Brown *et al.*, 2001; Johnson *et al.*, 2007) due to high N storage in soil organic matter (SOM) (Humphreys *et al.*, 2008) and through nitrification and denitrification processes (Barnard *et al.*, 2005; Baggs, 2011). Only between 6% and 12% of the N applied to agricultural soils is denitrified to N_2 removing reactive nitrogen from the environment (Smil, 1999).

1.4.2 Overview of nitrogen cycling

Nitrogen has the most complex biogeochemical cycle of all the major elements. Figure 1.1 presents a scheme of a terrestrial part of the nitrogen cycle.

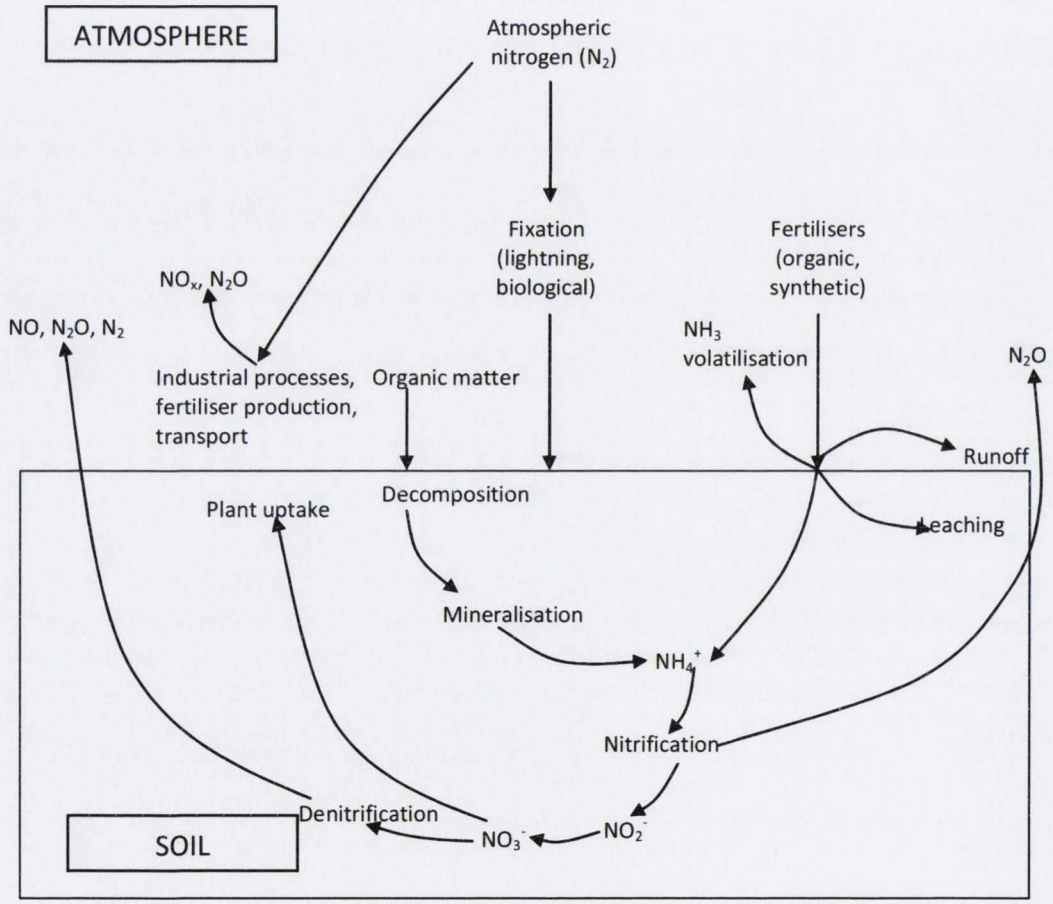


Figure 1.1 Simplified nitrogen cycle with the emphasis on its terrestrial part, in particular soil processes.

Despite its large abundance in the atmosphere, hydrosphere, and biosphere reaching 4 $\times 10^{21}$ g (atmosphere, soils, and waters combined) 99% of all nitrogen is unavailable to living organisms (McLaren and Cameron, 1996; Galloway *et al.*, 2003; Galloway *et al.*, 2004; Galloway *et al.*, 2008). N_2 in the atmosphere can be fixed by leguminous plant species like clover because of their symbiotic relationship with N-fixing micro organisms, or through chemical manufacturing processes. N applied to agricultural systems provides substrate for plant growth through synthesis of amino acids but also induces reactive N loss to the environment. Only between 40% and 60% of applied N is utilised by the plants and the remainder is either immobilised by soil microflora or lost through gaseous emissions and leaching (Parton, 1994). Approximately 1% of the applied inorganic N fertiliser is lost through N_2O shortly after application (IPCC, 2006) through nitrification and denitrification; however the magnitude of losses can vary hugely, between 0.01% and 7.2% (Dobbie, 1999; Hyde, 2006; Flechard, 2007).

Complete denitrification plays a critical role in removing Nr from the environment by creation of N_2 and therefore closing the N cycle started by N_2 fixation (Seitzinger *et al.*, 2006). However, if denitrification is only carried to its penultimate step, N_2O creation and accumulation in the atmosphere occurs. The N cycle is an important concept but the transformations within the cycle do not have to take place sequentially, many of them can occur simultaneously, and they can run in opposite directions at any time mediated by physical, biological and chemical processes (Whitehead, 1995).

1.4.3 Nitrogen transformation processes

1.4.3.1 Mineralisation and immobilisation

Various N transformations take place in soil depending on the size, structure and activity of soil microbial community, climate conditions, and soil characteristics (Bremner, 1997). Over 95% of nitrogen found in soil is in a form of soil organic matter (SOM) unavailable to plants. These complex organic compounds have to be broken down to simple inorganic compounds in the process of mineralisation before they can be utilised by plants (McLaren and Cameron, 1996). High-molecular weight SOM is decomposed to low molecular weight amino acids by microbial extracellular enzymes and the amino compounds are then taken up by the microorganisms and utilised for

energy generation through intercellular enzymatic degradation (Ladd and Jackson, 1982; McLaren and Cameron, 1996). Ammonium is created as a by-product, therefore these series of reactions is called ammonification. If microbial catabolic metabolism outweighs anabolic reactions, excess ammonium is released (Myrold and Bottomley, 2008).

Nitrogen is necessary for microbial growth, the need that is satisfied by assimilation of both the inorganic N forms (ammonium and nitrate), and the organic form (amino acids). The process of N uptake and incorporation into microbial cells is called immobilisation (Myrold and Bottomley, 2008). Mineralisation and immobilisation are concurrent processes taking place simultaneously with either net uptake or release of N. Net mineralisation can be observed when SOM has a high N content and consequently, low C:N ratio, in agricultural soils typically less than 25:1. In contrasting conditions, net N immobilisation will occur (McLaren and Cameron, 1996).

1.4.3.2 Nitrification

Ammonium (NH_4^+) can be oxidised to nitrite (NO_2^-) and nitrate (NO_3^-) in the process of nitrification carried out mainly by autotrophic bacterial activity. *Nitrosomonas spp.* is the main group of bacteria carrying out the first reaction ($NH_4^+ \rightarrow NO_2^-$), however it can also be completed by other groups of bacteria like *Nitrospira, Nitrosolobus, Nitrosovibrios*, and *Nitrosococcus*. The second step of oxidation ($NO_2^- \rightarrow NO_3^-$) is carried out solely by *Nitrobacter spp* (Whitehead, 1995, McLaren and Cameron, 1996, Norton, 2008). There are no known bacteria able to oxidise NH_4^+ to NO_3^- (Hooper *et al.*, 1997).

Ammonium is rapidly converted to nitrate in agricultural soils, therefore the levels of the latter are commonly higher in soil solution; nitrate is also more mobile in comparison to ammonium therefore it is more accessible for plant uptake. However, its mobility in soil makes it more vulnerable to loss from the system through leaching or by denitrification (Norton, 2008). Other processes, such as heterotrophic nitrification catalysed by a variety of microorganisms including fungi can be of importance in forest soils (Killham, 1990, Bateman and Baggs, 2005).

Nitrification rates in soils are controlled by the substrate availability, ammonia sensitivity, oxygen availability, soil moisture content, temperature, and pH. Due to general availability of CO_2, it is the availability of NH_4^+/ NH_3 that limits nitrification. However, in case of some bacteria strains high levels of the substrate can cause substrate inhibition of nitrification (Bollmann and Laanbroek, 2001). Oxygen is crucial for carrying enzymatic reactions at various stages of nitrification. Soil aeration at or below field capacity provides enough oxygen for the process; however, even in well aerated soils microsites of low O_2 availability can be found inside soil aggregates (Norton, 2008). The effect of soil moisture content is complimentary to oxygen availability. Soil water has a direct effect on cell physiology and metabolic activity, and indirectly affects substrate availability (Stark and Firestone, 1995). Nitrification is strongly controlled by soil temperature with a bell-shaped response curve to this factor (Barnard *et al.*, 2005). Optimal temperature for the process is environment specific, generally in between 25 °C and 30 °C. Freezing and thawing cycles, similarly to drying and wetting, stimulate bursts of microbial activity and encourage nitrification (McLaren and Cameron, 1996, Norton, 2008). The optimal soil pH to support nitrification is between 4.5 and 7.5; lower pH can slow the process down due to nutrient deficiencies, whereas high pH might be a result of toxically high NH_3 levels. Additionally, competition for ammonium from plants, poor soil nutrient status, and application of agricultural chemicals such as herbicides, can inhibit nitrification rates (McLaren and Cameron, 1996).

1.4.3.3 Denitrification

Denitrification is a process in which facultative anaerobic bacteria use NO_3^- as an electron acceptor instead of oxygen, in their respiratory metabolism, in poorly drained soils. Nitrate is converted, in a series of reduction reactions to nitrite (NO_2^-), nitric oxide (NO), nitrous oxide (N_2O), and dinitrogen (N_2) (McLaren and Cameron, 1996). Essential to conduct denitrification are anaerobic soil conditions, available organic or inorganic electron donors, and nitrogen oxides as electron acceptors (Coyne, 2008). Denitrification can be carried by not only abundant anaerobic bacteria, including *Pseudomonas, Bacillus, Thiobacillus, Propionipacterium*, and others, but also fungi,

archea, and aerobic bacteria (Firestone, 1982; Hayatsu *et al.*, 2008). The last step of denitrification removes reactive nitrogen from the environment, however if the process terminates earlier, NO and N_2O are created. Numerous variables control the final product of denitrification, the most important being available carbon (C) and soil aeration, as described by Firestone and Davidson (1989) in the 'hole in the pipe' model. Denitrification is characterised by an extreme spatial variability as the process is focused in anaerobic soil microsites (Parkin, 1987). A lot of attention was given to various aspects of denitrification, nevertheless denitrification rates to N_2 and controlling factors across ecosystems remain largely unknown (Davidson and Seitzinger, 2006).

Denitrification is a highly variable process controlled by the following factors: soil oxygen availability which in turn influences water-filled pore space (WFPS) and sreoil; dox potential; temperature, pH, soil organic matter (SOM), organic amendments, and soil management (Stevens *et al.*, 1998; Šimek *et al.*, 2002; Coyne, 2008). Being the main electron acceptor, oxygen regulates denitrification directly with the potential denitrification rate in less aerobic conditions being only 0.3% to 3% of the anaerobic rate (Parkin and Tiedje, 1984). In situation of low oxygen concentration increasing WFPS sees an increase in the denitrification rates, the effect becoming stronger with soil texture becoming finer (Weier *et al*, 1993). Denitrification is hysteretic, meaning that the rate of the process differs in soils becoming wetter or drier (Groffman and Tiedje, 1988). The process and its products are affected by the soil redox potential. Denitrification takes place at a range of redox potentials, from +200 to +400 mV in flooded environments (Bailey and Beauchamp, 1973), however other studies found no denitrification above +300 mV (Letely *et al.*, 1980). Optimum pH for the process varies from 6 to 8, although it was found to occur in conditions between 3.5 and 11 (Prakasam and Loehr, 1972; Klemmedtsson *et al.*, 1978; McLaren and Cameron, 1996). Temperature has a pronounced effect on the denitrification rate described by the Arrhenius equation of biochemical activity as an exponential function of temperature (Coyne, 2008). The process slows down below 10 °C and stops below 2 °C (McLaren and Cameron, 1996). Denitrification is also highly dependent on soil organic matter, in particular soil carbon which is necessary for the microbial activity. Organic carbon is commonly an electron donor in this respiratory process therefore any organic amendments to the soil will influence denitrification through a new C source, however

the relationship is more complex as the amendment C:N ratio is of major importance. Similarly, soil management (tillage, fertilisation, and agricultural chemicals) can indirectly affect denitrification through influencing many of the variables stated above (Coyne, 2008).

1.5 Drivers of nitrous oxide (N_2O) emissions

Nitrous oxide (N_2O) is a long-lived, radiatively active gas, which along with CO_2 and CH_4 is one of the main contributors to the global climate change (Mosier *et al.*, 1991) and also contributes to the destruction of stratospheric ozone (Badr and Probert, 1993, Ravishankara *et al.*, 2009). Over the last 25 years, N_2O concentrations in the atmosphere have been steadily increasing by approximately 0.2-0.3% yr^{-1} (Houghton *et al.*, 2001; Machefert *et al.*, 2002, Thomson *et al.*, 2012). Soils contribute approximately 70% of the global emissions with the agricultural soils being the largest single source of N_2O producing 4.2 Tg N_2O-N yr^{-1} from N fertilisation, 2.1 Tg N_2O-N yr^{-1} from manure management, and 0.5 Tg N_2O-N yr^{-1} from biomass burning (IPCC, 2007). Whilst N_2O is produced in soil through microbial processes, mainly denitrification and nitrification (Firestone and Davidson, 1989; Wrage *et al.*, 2004; Bateman and Baggs, 2005,), other pathways have been discovered like dissimilatory nitrate reduction to ammonium (DNRA) (Silver *et al.* 2001), nitrifier denitrification (Wrage *et al.*, 2001), heterotrophic, mainly fungal nitrification, denitrification, and codenitrification (Laughlin and Stevens, 2002; Su *et al.*, 2004; Laughlin *et al.*, 2008). The processes of N_2O production are controlled by numerous variables and their interactions already mentioned in controllers of nitrification and denitrification. However, here controls of N_2O production and release from soil will be further explained.

1.5.1 Soil moisture content and soil oxygen availability

Soil moisture and aeration status are tightly interconnected, one determining the other as soil pores are always filled with either water or air. Dynamics of these two parameters affect nitrogen cycling in the soil enhancing and suppressing various reactions and their products (Bollmann and Conran, 1998). It is also crucial to

understand how soil moisture is driven by rainfall, and therefore how rainfall is ultimately responsible for the N_2O response to moisture. The effect of soil moisture on N_2O emissions has been extensively investigated over the past few decades. Numerous studies have found a positive relationship between soil moisture and N_2O emissions (e.g. Tortoso and Hutchinson, 1990; Skiba *et al.*, 1993, Bollmann and Conran, 1998; Dobbie and Smith, 2001; Dobbie *et al.*, 2003; Wrage, 2004; Bateman and Baggs, 2005; Ruser *et al.*, 2005; del Prado *et al.*, 2006; Liu *et al.*, 2007; Ciarlo *et al.*, 2008;). Soil water content is often the most important controlling factor of N_2O fluxes (del Prado, 2006). Soil water content is commonly expressed as per cent of water filled pore spaces (% WFPS), which takes soil bulk density into account (Aulakh *et al.*, 1991). At low WFPS, 20% and below, N_2O production is restricted by low microbial activity and substrate availability, the limitation that is removed at 35% WFPS. N_2O efflux increases with WFPS, with nitrification being the dominant process until 60% WFPS. After reaching approximately 60-65% WFPS soil-specific threshold N_2O increases substantially and above 70% WFPS denitrification becomes dominating production process. Only above 90% WFPS is N_2 produced as a result of complete denitrification (Stevens *et al.*, 1997; Bateman and Baggs, 2005; Ruser *et al.*, 2005; Neftel *et al.*, 2006). Rewetting dry soil is associated with high N_2O release due to rapid increase in WFPS and microbial activity (Hutchinson *et al.*, 1993; Ruser *et al.*, 2005). Hsieh *et al.* observed that 60% of annual emissions from humid grassland occurred during eight major driver events like fertiliser applications and rainfall especially after dry period (Hsieh *et al.*, 2005). Soil texture affects N_2O emissions through its drainage properties and subsequent moisture content. Heavy texture soils retain more moisture promoting more optimal conditions for denitrification, which has been shown in many studies (Bollman and Conran, 1998; Dobbie and Smith, 2001; Wrage, 2004; Ruser *et al.*, 2005; Liu *et al.*, 2007). Agricultural soils in temperate, maritime regions such as in Ireland are subjected to high rainfall which can, in turn influence nitrification and denitrification, and subsequent N_2O emissions (Jarvis *et al.*, 1994, Abassi and Adams, 2000).

1.5.2 Soil temperature

Temperature has a positive effect on N_2O as shown in numerous studies (e.g. Keeney *et al.*, 1979; Goodroad and Keeney, 1984; Maag and Vinther, 1996; Clayton *et al.*, 1997; Skiba *et al.*, 1997; Fowler *et al.*, 1997; Smith *et al*, 1998; Dobbie and Smith, 2001; Smith *et al.*, 2003). Dobbie and Smith (2001) found a strong non-linear response to temperature, more so in grassland than in arable land. Skiba *et al.* (1997) also found a strong correlation of N_2O with temperature and the rate of the increase was soil-dependent. The response to temperature is suggested to be of a biochemical nature (Mosier, 1998; Machefert *et al.*, 2002) which sees rising N_2O emissions as a result of increase in the anaerobic fraction of the soil due to increased respiration, decreased oxygen diffusion or both (Dobbie and Smith, 2001). N_2O emissions also increase with increased temperature due to enhanced microbial activity. Elevated temperature results in higher enzymatic activity, which in turn leads to faster biochemical reactions and more N_2O being produced. Combined effect of temperature and soil moisture on N_2O emissions has also been investigated. Flechard *et al.* (2007) in the study on 10 grassland sites across Europe presented limitations of analysing the separate effects of soil temperature and WFPS on N_2O fluxes due to a strong negative relationship between these two variables over seasonal time scales. When analysed separately, N_2O fluxes were positively correlated with soil temperature, however no correlation with WFPS was found. Background N_2O fluxes could be usefully predicted as function of temperature, whereas predicting event-based emission factors (EFs) was less straightforward. EFs peaked in the WFPS range 65-85% but decreased below and above this range. Therefore the relationship was described as a bell membership function B (WFPS). Multiple linear regression of ln(EF) versus soil temperature, B(WFPS), as well as cumulative rainfall and grazing density enabled parameterization of the EF. EF of up to 6.5% was predicted for a soil temperature of 25°C and WFPS 70-80%. Kammann *et al.* (2008) in their long term study on an old, N-limited temperate grassland on a sandy clay loam over a clay layer soil found the largest N_2O losses at soil temperatures above 10 °C and 55-60% volumetric water content.

1.5.3 Soil nitrogen

Nitrogen inputs into soils from organic N deposition (via animal urine or manures) or application of synthetic fertilisers are the largest contributers to global N_2O emissions (Bouwman, 1996; IPCC, 2006; Jones *et al.*, 2007) and has been investigated in numerous studies (e.g. Clayton *et al.*, 1997; Mulvaney *et al.*, 1997; Smith *et al.*, 1998; Hellebrand *et al.*, 2003; Ruser *et al.*, 2005; del Prado *et al.*, 2006; Liu *et al.*, 2007; Ciarlo *et al.*, 2008). Nitrogen applied to soil is only partially taken up by plants, and partially utilised by soil microorganisms, or leached. The fraction utilised by soil micro flora undergo the processes described above such as mineralisation, immobilisation, nitrification, and denitrification. As a result of microbial metabolic activity approximately 1% of the applied N is lost in the form of N_2O (IPCC, 2007). Fertiliser form is an important controller of the emissions (Ciarlo *et al.*, 2008). Ammonium-based fertilisers enhance nitrification, whereas nitrate-based fertilisers enhance denitrification, and subsequently, N_2O emissions from the respective processes (del Prado, 2006). Ammonium fertilisers or ammonium producing fertilisers can promote denitrification in waterlogged conditions and N_2O emissions are found to be larger as a result of alkaline-hydrolysing fertiliser application than from acidic fertiliser (Mulvaney *et al.*, 1997).

1.5.4 Soil management

Soil management is a broad term for carrying out agricultural practice such as ploughing, fertilisation, cover crop, agricultural chemicals application, grazing, grass cutting, and harvesting. All these practices directly or indirectly influence N_2O emission rates, by addition of N to soil, by adding carbon source, and potentially changing soil organic matter C:N ratio, by compaction affecting soil moisture (Dobbie and Smith, 2001; Ruser *et al.*, 2005; Liu *et al.*, 2007). Subsequent paragraphs will provide more information on N_2O from various land uses and soil management options.

1.6 N_2O emissions from soils

Growing human population and its demand for food has led to an increased level of the reactive nitrogen in the environment (Mosier *et al.*, 1998; Thomson *et al.*, 2012).

Nitrogen fertilisation not only enhances N_2O emissions from soils but also decreases soil GHG sink potential (Mosier *et al.*, 1990). To accurately estimate the magnitude of agricultural N_2O emissions all possible sources should be included; native soil nitrogen, nitrogen deposition, previous fertilisation history, crop residues nitrogen, N_2O from subsurface water, and current N fertilisation (Mosier *et al.*, 1994). However not much of the required data is available for this quantification and therefore the concept of the emission factor has been introduced by Bouwman (1996). Based on data from previous studies Bouwman estimated 1.25% of the applied fertiliser N to be released into the atmosphere as N_2O emissions disregarding the form of the fertiliser N, and other controlling factors and background emissions to be at 1 kg N_2O-N ha^{-1} $year^{-1}$. Mosier *et al.* (1998) then developed more detailed methodology for N_2O calculation. This crude approach was employed by the IPCC as the most basic calculation methodology called Tier 1 and the emission factor later revised to 1% (IPCC, 2006).

Numerous studies report N_2O emissions from various natural and agricultural land uses across a gradient of climatic conditions (Schmidt *et al.*, 1988; Mosier *et al.*, 1991; Struwe and Kjoller, 1994; Freney, 1997, Machefert *et al.*, 2002; Smith *et al.*, 2003, Roelandt *et al.*, 2005; Flechard *et al.*, 2007; Neftel *et al.*, 2007; Soussana *et al.*, 2007; Skiba *et al.*, 2009). These can be as little as 0.01 kg N_2O-N ha^{-1} yr^{-1} for temperate forest, or even be a sink of N_2O, and up to 38.3 kg N_2O-N ha^{-1} yr^{-1} for riparian zones. However variable N_2O emissions are, it is commonly accepted that agricultural soils produce more N_2O in comparison to natural land, like forest and unmanaged grassland, due to their N management (Machefert *et al.*, 2002), and that within agricultural soils, intensively managed soils contribute more emissions than extensively managed ones (Flechard *et al.*, 2007). European grasslands can be a sink for CO_2, Soussana *et al.* (2007) in their study on nine grassland sites found the fields to be on average GHG neutral however N_2O and CH_4 emissions offset some of the soil's sink activity affecting the total GHG balance.

Future climate change will have an impact on denitrification rates, and subsequently N_2O produced, through direct and indirect mechanisms (Butterbach-Bahl, 2011). However elevated N_2O production can be offset by enhanced carbon sequestration in

non-agricultural systems, its effects will still pose a threat on biodiversity, eutrophication and human health (de Vries *et al.*, 2011).

1.7 Agricultural N_2O mitigation options

Land use change and land management practices result in GHG emissions (Smith and Conen, 2004), however agriculture has a large potential for GHG mitigation, 10% of which is attributed to reduced N_2O emissions, which can be realised through various mitigation strategies (Cole *et al.*, 1997; Johnson *et al.*, 2007; Smith *et al.*, 2008, Delgado *et al.*, 2010). Nitrogen use efficiency is the first recognised mitigation strategy. Matching nitrogen supply to crop demand, improved animal waste and crop residue management, controlled-release fertilisers, nitrification inhibitors, timing and water management can result in a 0.7 Tg N_2O-N yr^{-1} reduction (Cole *et al.*, 1997; de Klein *et al.*, 2008). Furthermore, improving productivity of existing agricultural lands, lengthening the rotation times, increasing grassland management, crop residues incorporation into the soil, and shift towards perennial rather than annual crops can have a potential mitigation advantage for extensive agricultural systems, whereas effective fertilisation management and the use of cover crops can be beneficial in intensive agricultural systems (Mosier *et al.*, 1998). Although agriculture has significant opportunities for GHG mitigation, policy and technological constraints such as C sequestration saturation, uncertainty, or GHG leakage from production shift to other regions, have to be overcome to fully realise this potential (Smith *et al.*, 2007; Smith *et al.*, 2008).

In addition to the above, bioenergy cultivation, with the emphasis on perennial biomass/ bioenergy systems has been identified as a potential GHG mitigation strategy due to its carbon neutral balance (Johnson *et al.*, 2007; Smith *et al.*, 2008; Bartle and Abadi, 2009; Popp *et al.*, 2001). These crops are characterised by small N input requirements, small pesticides and herbicides needs reducing their impact on the environment (Delgado *et al.*, 2010, Cadoux et al., 2012). Their high above ground litter production paired with extensive root systems as well as no need for annual tillage help increasing C sequestration. Minimising tillage also reduces soil erosion and improves

soil quality, and the perennial character of the crops provides habitats for wildlife (Delgado *et al.*, 2010).

1.8 Energy crops

Bioenergy crops are either conventional annual agricultural crops (first generation) or dedicated lignocellulosic crops (second generation) grown for their biomass used as a renewable energy source (Rowe *et al.*, 2007; Bartle and Abadi, 2009). Biomass utilisation became popular within the last decade due to technological developments, food production surpluses and climate change threat (Sanderson and Adler, 2008). Biomass is most commonly used for combustion, gasification, pyrolysis, fermentation, and mechanical extraction of oils (McKendry, 2002). Bioenergy crops are most commonly divided according to the length of their life cycle (annual and perennial crops) into annual and perennial crops. The attention paid to particular plant species depends on soil and climatic conditions. Known annual crops are maize (*Zea mays*), sugar beet (*Beta vulgaris),* oil seed rape (*Brassica rapa*) and hemp (*Cannabis spp.*), whereas perennial crops are switchgrass (*Panicum virgatum*), reed canarygrass (*Phalaris arundinacea), Miscanthus sp.*, hybrid poplar (Populus spp.), willow (Salix spp.), alder (Alnus spp.), birch (*Betula spp.*), sweetgum (*Liquidambar styraciflua*), sugarcane (*Saccharum)* and sycamore (*Platanus occidentalis*). In Ireland, the most commonly used energy crops are currently willow, poplar, *Miscanthus,* switchgrass and hemp, however other species such as maize, sugar beet, oil seed rape, reed canarygrass, alder, and birch can also be cultivated for energy purposes. Perennial crops have favourable characteristics compared to annual crops as they require less energy and fertiliser inputs. In addition, there is no annual ploughing required, thus preserving soil organic matter and enhancing carbon sequestration. Also cover is provided throughout the year avoiding GHG emissions from fallow period and reducing leaching (Borjesson, 1999; Powlson *et al.*, 2005). The biomass of perennial grasses has also higher lignin and cellulose content than this of annual crops (Lewandowski *et al.*, 2003).

Type of photosynthetic pathway utilised by plants is also an important factor in choosing species suitable for energy purposes. C4 photosynthesis is the most efficient

form of the photosynthesis process with a 40% greater potential of converting intercepted solar energy into biomass in comparison to a C3 pathway (Heaton *et al.*, 2004). This is mainly due to largely eliminating photorespiration. C4 photosynthesis also allows for greater nitrogen and water use efficiency. However, this type of photosynthesis occurs mainly in plants in warm climates. Few plant species in temperate climates utilise this pathway. Some of the temperate climate C4 species are *Miscanthus*, maize, and switchgrass (McKendry, 2002).

Ideal energy crops should have a favourable energy balance, high efficiency use of light, water, nitrogen and nutrient use, low water content of harvestable biomass, C_4 photosynthesis, low cultivation and pest control requirements (McKendry, 2002; Heaton *et al.*, 2004). Cultivation of bioenergy crops can reduce greenhouse gas emissions through improved carbon sequestration and low fertilisation requirements, reduced pesticides use, and reduced leaching. It is also belived that bioenergy crops aid restore degraded land and increase biodiversity (McKendry, 2002; Rowe *et al.*, 2007). However there are voices of concern that their impacts on the environment may be quite opposite (t Mannetje, 2007; Crutzen *et al.*, 2008; Koh and Ghazoul, 2008; Erisman *et al.*, 2010).

1.8.1 Types of energy crops and emissions associated with their cultivation

1.8.1.1. Miscanthus

Miscanthus is a rhizomatous grass originating from Asia first introduced in Europe in 1930s. Varieties used for biomass cultivation are infertile hybrids propagated vegetatively by rhizomes or by micropropagation (Lewandowski *et al.*, 2000). The advantages of *Miscanthus* as a bioenergy crop are high yields, low fertiliser and pesticide requirements, and high radiation and water use efficiencies (Lewandowski and Schmidt, 2006). However, the crop has high establishment costs, a narrow genetic base, and poor over wintering during early seasons (Clifton-Brown and Lewandowski, I. 2000; Lewandowski *et al.*, 2000; Rowe *et al.*, 2007). *Miscanthus* has extremely high solar energy conversion efficiency of 1% owing it to its C4 photosynthesis pathway, making it possible to achieve renewables targets with a lower land requirement

(Heaton, 2008; Hastings *et al.*, 2009). *Miscanthus* yields can be up to 30 t ha^{-1} dry matter in southern Europe, however yields above 10 t DM ha^{-1} are already economically viable (Tampfl, 2007).

The quality of *Miscanthus* biomass used for combustion can be compared with woody biomass, and can be improved by a delayed harvest, beneficial also because of nutrient recycling to rhizomes in low temperatures (Beale and Long, 1997; Lewandowski *et al.*, 2000). Loss of biomass at delayed harvest due to leaves and shoots tips senescence can be compensated by high incorporation of crop residues in soil, and therefore increasing soil organic matter (Kahle *et al.*, 2001). *Miscanthus* is highly N efficient and its fertiliser requirement, which can be affected by soil type and previous land use, is generally low or none (Himken, 1997; Christian *et al.*, 1997). According to the life-cycle analysis (LCA) including both economic and environmental impacts of energy crops fuel chains *Miscanthus* presents an efficient land use option with both financial and GHG benefits (Styles *et al.*, 2007; Styles and Jones, 2007; Styles and Jones, 2008; St. Clair *et al.*, 2008).

Very few studies have investigated N_2O emissions from soils under *Miscanthus* cultivation (Don *et al.*, 2012). Jorgensen *et al.* (1997) found *Miscanthus* emissions to be mostly summer-based with 60% of annual N_2O loss occurring between June and August with approximately 1.7 kg N_2O-N lost between April and October 1995 after fertilisation with 75 kg N ha^{-1} on a well-drained coarse sandy loam soil. Similarly, Drewer *et al.* (2011) noticed peak N_2O emissions during summer months with the total losses of approximately 0.5 kg N_2O-N ha^{-1} yr^{-1} from the unfertilised plots on a fine loam over clay. A study from Ireland on a well-drained sandy loam soil recorded annual N_2O fluxes of approximately 0.4 kg N_2O-N ha^{-1} yr^{-1} from established and 0.6 N_2O-N kg ha^{-1} yr^{-1} from newly planted crop, and 0.8 kg ha^{-1} yr^{-1} following 63 kg N ha^{-1} application, and 1.0 following 125 kg N ha^{-1} application to established crops (Roth *et al.*, 2011).

1.8.1.2 Reed canarygrass

Reed canarygrass is a rhizomatous C3 grass native to Ireland (Sanderson, 2008). It is also one of the best cool season grasses for poorly drained soils and its establishment helps to strengthen the top soil in wet conditions thanks to its extensive root system (Sanderson, 2008). Reed canarygrass normally grows in wet conditions however it is resistant to drought (Lewandowski, 2003). Extensive evaluative work on the Reed canarygrass grown for bioenergy has been done in Sweden where best yields were obtained from soils with lower clay fraction. The yields reached 7.5-9 t DM ha^{-1}, in spring and in autumn harvest, respectively (Lewandowski, 2003). Intensive uptake and fertiliser N remobilisation found in Reed canarygrass proves the importance of rhizome system activity in N cycling within the plant and indicates that the crop is highly N efficient and requires little fertilisation (Partala, 2001). However, little is known about the effect land use change into Reed canarygrass cultivation has on nitrous oxide emissions.

1.9 Land use change into energy crop cultivation in Ireland

Irish agriculture is primarily grass-based with 90% of the 4.3 M ha of agricultural land devoted to grassland, out of which 11% dedicated to rough grazing (Bioenergy Action Plan). Ireland has the third highest fertiliser use per unit land area within the EU countries (CSO, 2009) and the high application rate paired with Ireland's temperate and moist climate can in effect produce large N_2O losses from agricultural soils. Hyde *et al.* (2006) recorded cumulative annual emissions of between 4.21 and 4.66 kg N_2O-N ha^{-1} from unfertilised grazed grassland, between 6.45 and 18.51 kg N_2O-N ha^{-1} from N-fertilised (225 kg N ha^{-1}) grazed grassland, and between 12.55 and 28.93 from N-fertilised (390 kg N ha^{-1}) grazed grassland in the south east of Ireland. Large differences found between the measurement years indicated the importance of weather on the overall losses. To mitigate the emissions and help achieve the renewables targets bioenergy crops might be cultivated at the expense of some grasslands and set-aside areas. Biomass will account for 80% of the increase in the renewables use (Energy White Paper, 2007), and approximately 3.86 Mt CO_2-eq yr^{-1} can be saved through bioenergy crops cultivation (EPA, 2007). According to Bioenergy Action Plan

Ireland's set-aside area comes to 30 000 ha of marginal land, commonly in small parcels therefore it is estimated that approximately 10 000 ha could be available for biomass crops. Following the cease of sugar beet production in 2006 an additional 31 000 ha became available for alternative land uses. This area could be used for bioenergy crops (Teagasc, 2007). However, estimated land area needed for bioenergy crops to meet the Europe 2020 target is 95 000 ha, with another 45 000 ha required to satisfy the co-firing target based on willow and /or *Miscanthus* cultivation (Caslin, 2008).

1.10 Objectives of the project

Ireland is facing changes in agricultural and energy sectors to accommodate emissions reduction targets along with reaching the renewable energy targets. Land use change to bioenergy crops cultivation is a recognised GHG mitigation option (IPCC, 2003) simultaneously contributing to the renewables utilisation. However, currently little is known about the role of energy crops in N_2O emissions and their contribution to the crops' GHG balance. Large scale land use change into bioenergy crops cultivation can have profound implications on the national N_2O losses. Therefore, the objective of this study is to assess the impact of establishing bioenergy crops like *Miscanthus* and Reed canarygrass on N cycling, in particular N_2O emissions. In this project early stages of land use change are investigated such as ploughing and first and second year of the crop growth, and fertilisation, all of which are recognised to contribute substantially to N losses from the agricultural systems. From this perspective, three experiments were included in this study to investigate early stages of LUC into bioenergy crops and its impact on N cycling and N_2O emissions.

Study 1: The effect of ploughing on short-term emissions of N_2O from three soil types of varying organic matter content.

Land use change from grassland to bioenergy crop cultivation requires ploughing the land in preparation for the new crops. This activity enhances N mineralisation, resulting in increased N_2O emissions and N leaching, however the extent of these

effects remains unclear (Kong *et al.*, 2009). Therefore, this experiment has several aims:

To investigate the magnitude of N_2O emissions and N leaching following ploughing to quantify N losses associated with this early stage of land use change.

To compare two ploughing techniques and assess their impact on N cycling to help choose the technique which will minimise the negative impact of land use change on N cycling.

To quantify the scale of N losses from different soil types of contrasting texture and varying drainage properties to verify the most appropriate areas for bioenergy crop cultivation.

Study 2: Field scale study on the effect of land use change from grassland into Miscanthus and Reed canarygrass on N cycling, in particular N_2O emission.

The early stage of conversion to bioenergy crops is characterised by high soil disturbance that can lead to enhanced N_2O emissions (Guo and Gifford, 2002). Management activities such as ploughing and subsequent increased mineralisation, planting or sowing of new plants, can affect microbial community balance and its processes and final products therefore the aims of this experiment are:

To measure N_2O emissions associated with the early stage of bioenergy crop cultivation (first and second year) to identify the impact of these crops on the environment.

To compare N_2O losses associated with production of the biomass yield of two bioenergy crops, *Miscanthus* and Reed canarygrass to assess their sustainability for Irish agriculture.

Study 3: ^{15}N stable isotope study on the effect of crop cover and soil properties on N processes following N fertilisation of grassland and energy crop covers: Miscanthus and Reed canarygrass.

Fertiliser application is the biggest single source of N_2O from agricultural soils (Storey, 1999; Mosier and Kroeze, 2000) therefore low N requirement and high N use efficiency is desirable in plants grown commercially. Bioenergy might in future be fertilised to increase the yields and therefore the fate of the applied N should be assessed (Drewer *et al*, 2012; Don *et al.*, 2012). Currently little is known about the N losses following N fertilisation of bioenergy crops hence the aims of this experiments are:

To investigate the magnitude of plant N uptake and N losses following N fertilisation. In particular, to assess the contribution of various pathways of N losses from the systems, and to quantify N budgets of the two bioenergy crops and grassland based on soils of contrasting drainage.

To identify which microbial processes are the main contributors of N_2O emissions and the structure of gaseous N emissions (contribution of N_2 and N_2O) from various soils and crop covers.

To investigate the differences in N budgets, especially gaseous N emissions, between bioenergy crops and grassland systems based on either well or poorly draining soils.

Chapter 2: The effect of ploughing on short-term emissions of N_2O from three soil types of varying organic matter content.

2.1. Introduction

Land management is an important component in mitigating greenhouse gas emissions from agriculture and in maintaining soil quality (Smith and Conen, 2004; Johnson *et al.*, 2007; Smith *et al.*, 2008). Certain management techniques help conserve soil carbon (C) and encourage C sequestration from the atmosphere. These efforts are part of the national greenhouse gas inventory process (IPCC, 2006). Soil disturbance, such as ploughing, leads to a dramatic loss of soil organic matter (SOM) through break down of soil aggregates (Ogle *et al.*, 2005). In addition enhanced physical and biochemical processes (erosion and microbial activity) can cause significant emissions of trace gases and leaching of nutrients following tillage, however the extent of these effects remains unclear (Kong *et al.*, 2009). The magnitude of gaseous losses can be affected by multiple factors including ploughing technique, timing of cultivation, weather conditions such as temperature and precipitation, soil nutrient status (available nitrogen, organic matter), soil texture (porosity) and soil moisture content (Velthof *et al.*, 2010). Weather and soil conditions also affect fertiliser-derived greenhouse gases emissions. Application of nitrogenous fertiliser to soil is believed to be the main agricultural source of N_2O emissions to the atmosphere. In OECD countries N fertilisation is responsible for 90% of N_2O produced in tillage soils (Storey, 1997). Globally seventy eight million t N is applied yearly as mineral fertiliser to the soils and in return 3.5 million t N_2O-N is produced from fertilised land (International Fertilizer Industry Association Staff, 2001). Bouwman *et al.* (1996) estimated emissions at 1.25% of fertiliser N applied, an approach that was adopted by the IPCC and later this emission factor was revised to 1% (IPCC, 2006). However, numerous studies show that emission factors can vary widely depending on climate, soil type and fertiliser type (Li *et al.*, 1996; Skiba *et al.*, 2002; Dalal *et al.*, 2003; Flynn *et al.*, 2005; Abdalla *et al.*, 2009). Adopting appropriate ploughing and fertilising strategy to agricultural areas is, amongst other management options, crucial to avoid losses and minimise negative environmental impacts.

Europe has been, and may be in the near future, experiencing land use changes that can lead to a shift in soil organic carbon (SOC) levels, influence C sequestration rates, and promote or mitigate greenhouse gases fluxes from the soils (Bouma *et al.*, 1998;

Rounsevell *et al.*, 2006; Verburg *et al.*, 2006). Land use change in general, means either conversion of natural areas for human activity or a change in management practice of land already used for human needs (Foley *et al.*, 2005). Approximately 40% of global land area is already utilised for croplands and pastures (Ramankutty and Foley, 1999; Asner *et al.*, 2004; Ramankutty *et al.*, 2008). The cropland area worldwide doubled in the last 140 years, whereas the inverse trend was found in total forest area which has been decreasing over the last century (Houghton, 1994). Changes in future land use are difficult to predict as they are primarily based on socioeconomic and political conditions. The IPCC drew four possible scenarios based on various development assumptions. These scenarios were a tool to help construct future projections of climate change. Future land use would change adequately to the chosen assumptions (population growth, economic growth, technological advances, emphasis on fossil-fuels, on all energy sources, or on non-fossil fuel sources), which would result in shift in levels of greenhouse gases emissions (IPCC, 2007). However difficult it is to predict future land use change, some of the international and national agreements can indicate its general trends. Under article 3 of Kyoto Protocol land use, land use change, and forestry (LULUCF) activities leading to GHG mitigation can be used to meet countries' emission reductions commitments. These are afforestation, reforestation, deforestation (Article 3.3) as well as forest management, cropland management, grazing land management and revegetation (Article 3.4) (UN, 1997). On the other hand, EU directives on renewables, promotion of electricity produced from renewable energy sources in the internal electricity market and the promotion of the use of biofuels or other renewable fuels for transport (2001/77/EC; 2003/30/EC), set targets on the amounts of bioenergy to be produced. All these activities will enhance land use change to fulfil these commitments. According to Rounsevell *et al.* (2005), cropland and grassland areas in Europe may decline by as much as 50% and some of this surplus land can be used for bioenergy crops production. This prognosis is based on an interpretation of the four storylines of the Special Report on Emission Scenarios (SRES) of the IPCC and the assumption that technology continues to progress at current rates. However this decline will not occur if there is a large increase in the demand for agricultural produce, or for other reasons such as political. Conversion from grassland to crop production can cause large SOC losses (Guo and Gifford, 2002), mainly due to vegetation removal and tillage. Irish agriculture is primarily grass-based with pastures taking up 77% of agricultural land and agricultural land with

significant areas of natural vegetation another 9% (Corine, 2006). This means that land use change will most probably take place at the expense of pasture. Irish soils are, in general, carbon-rich, especially in the west of the country (Xu *et al.*, 2011), hence the risk of unfavourable GHG balance upon LUC. Therefore it is essential to acquire precise knowledge of how LUC will impact SOC of Irish soils and what land managements option are viable for reducing the risk of SOC loss and nutrient loss through greenhouse gasses emissions and leaching.

In order to maintain and enhance soil organic matter (SOM) and reduce gaseous emissions, minimum tillage or no tillage management, is often implemented. Tillage is most often used for seedbed preparation, weed control and fertiliser incorporation. Its effects depend on tillage depth, intensity and frequency. Conventional mouldboard tillage is a widely used technique however it is detrimental to soil structure (Paustian *et al.*, 2000). Soil aggregates are known to stabilise and protect soil organic matter. When macroaggregates are broken down during ploughing, SOM previously enclosed inside the mineral shell becomes readily available as a substrate for soil microbial activity (Beare *et al.*, 1994; Six *et al.*, 2000). Consequently conventional tillage rapidly increases microbial activity and hence heterotrophic respiration leading to enhanced carbon and nitrogen mineralisation (Paustian *et al.*, 2000).

Transition from conventional to minimum or no-till systems not only affects C sequestration but also modifies trace gas emissions and leaching. As these practices conserve moisture and compact soil structure, conditions become more anaerobic therefore stimulating a switch from respiration to denitrification in those microorganisms capable to do so. This normally leads to increased N_2O emissions (Aulakh *et al.*, 1984; Rochette *et al.*, 2008). As soil structure improves in time, conditions become less anaerobic and N_2O losses can be avoided (Arah *et al.*, 1991). Ball *et al.* (1999) investigated the effect of min-till and no-till adaptation on N_2O production. Imperfectly drained soils (loam and clay loam) used in the study were also fertilised and subjected to compaction. Large peaks of N_2O were observed post-fertilisation following heavy rainfall that reduced gas diffusivity from both minimum tilled and no tilled soils. Soil compaction proved to worsen the emissions considerably (Ball *et al.*, 1999; Ball *et al.*, 2008). Results of these experiments indicate the importance of soil type in management practice. For the soils used in the study the

largest emissions were observed when conservation tillage was adopted due to heavy soil structure restricting gas diffusivity. Some short term effects of tillage transition are changes in the nutrient cycling. Nitrogen (N) availability for crops can be altered due to losses (Kong *et al.*, 2009). Some studies suggest that more fertiliser should be applied to offset nutrient losses, preferably in organic form, to allow slow nutrient release synchronised with crop uptake (Kramer *et al.*, 2002).

Ploughing has an impact on water quality through influencing erosion, rain infiltration, runoff and leaching. Water lost in the form of runoff and leaching contains pollutants such as soil particles, pesticides and nutrients. These substances can damage ecosystems when deposited outside their destination. Agriculture-derived nutrients transported to aquatic ecosystems are a source of widespread eutrophication (Carpenter *et al.*, 1998). Conservation tillage is believed to prevent nutrient loss. However if more micropores are created it can lead to more preferential flow and leaching losses. Numerous authors provide contradictory results on the effect of min-till and no-till on leaching. Some record higher NO_3-N concentrations under no-till (Dowdell *et al.*, 1987; Eck and Jones, 1992), whilst others note no difference or lower NO_3-N concentrations (Jordan V.W. *et al.*, 2000). However, according to Fawcett, leachate containing less N can still cause higher losses due to higher total water volume moving through soil in comparison to conventional tillage (Fawcett, 1995). Nitrate leaching is related to soil type, weather conditions and crop management practice (Wang and Alva, 1996; Wu *et al.*, 1996). Well-drained soils in combination with intensive cultivation represent highest risks for nitrate leaching. Consistently higher NO_3^- losses on the loamy sand than on the clay loam were recorded by Sogbedji *et al.* (2000). Increasing N fertiliser use efficiency was concluded to be of major importance in reducing leaching risks (Sogbedji *et al.*, 2000; Beaudoin *et al.*, 2005) and tillage intensity was important in case of poorly draining soils like sandy loam (Hansen and Djurhuus, 1997).

As ploughing and N fertilisation are common agricultural practices in land use change nowadays, the N losses associated with these steps should be quantified in order to predict their impact on GHG budget of the future land use. According to numerous studies mentioned above, the N losses can be substantial therefore shifting the GHG balance significantly. The aim of this study was to quantify the N losses in the forms of

N_2O and leached N following ploughing and fertilisation in various Irish soils, assess the importance of gaseous and leaching pathways and the impact of ploughing technique in the overall losses. To do this, the objectives below were specified:

Objectives and hypotheses of the study:

1) To measure magnitude of N lost as N_2O and leachate following tillage of perennial grassland and nitrogenous fertiliser application to three Irish soils of contrasting drainage. The hypothesis was that ploughing and fertilisation were a source of large N losses both through gaseous emissions and drainage.

2) To assess the effect of soil type on N losses during disturbance period of ploughing, and post-ploughing fertilisation. The hypothesis was that the more free-draining the soil the larger would be the proportion of N loss through leaching. Conversely, the more poorly-draining the soil the higher N loss through gaseous emissions.

3) To investigate the impact of adopting minimum tillage for the LUC on N losses. However minimum tillage can prove beneficial for preserving soil carbon, it can lead to enhanced N_2O emissions, and therefore the hypothesis was that gaseous emissions associated with minimum tillage were larger than those from conventional tillage. The effect of minimum tillage on leached N is unclear. The hypothesis here was that minimum tillage could potentially reduce N leaching from poorly drained soils.

2.2 Materials and methods

2.2.1. Experimental site

The experimental work was carried out between May and October 2009 at the Teagasc Johnstown Castle Environmental Research Centre, Wexford (6° 30' W/ 52° 17'' N) (Figure 2.1). Climate conditions at the site are mild with a mean annual temperature of 10.4°C and a mean annual rainfall of 1036 mm (30-year averages from Johnstown Castle station).

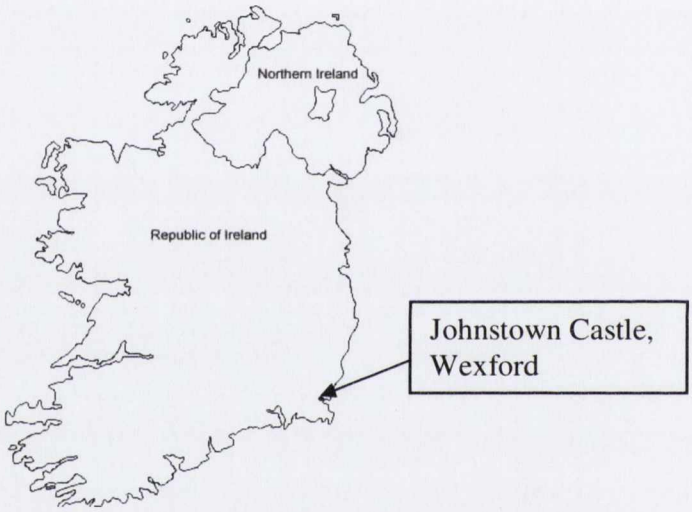


Figure 2.1 Location of the experimental site.

2.2.2. Layout

A set of 18 intact soil monoliths (lysimeters) was used for the experiment. The lysimeters were excavated in 2003 from three grassland sites in Ireland according to the protocol described in Cameron *et al.*, 1992. Lysimeters represented three Irish soils of various drainage properties. These were poorly draining Rathangan gley, moderately draining Elton brown earth and well draining Clonakilty brown podzol. Rathangan soil was sourced in county Wexford, Elton in county Limerick, and Clonakilty in county Cork. Each lysimeter had dimensions of 0.8 m in diameter and 1 m in depth. Each lysimeter was an intact soil core enclosed in an HDPE pipe. To prevent preferential flow liquid petrolatum was injected between the soil and the HPDE pipe. A nylon

tubing was attached to the bottom of the case to enable collection of drainage water. Lysimeters were installed in a field lysimeter facility in the pre-excavated holes that were later backfilled with soil under natural rainfall and meteorological conditions. Nylon tubes from the lysimeters were connected to individual collection containers. Basic physical and chemical soil characteristics were analysed at the time of the monoliths collection in 2003 and are included in Table 2.1.

Table 2.1 Physical and chemical soils characteristics at 0-10 cm depth (n=3) . Capital letters in brackets indicate statistically significant differences at p<0.05.

Soil	Soil group	Drainage	Clay fraction	SOC	Total N	C/N Ratio
			%	%	%	
Rathangan	Gley	Poor	25.2 (A)	4.23 (A)	0.35 (A)	12.5 (A)
Elton	Brown Earth	Moderate	19.2 (B)	5.38 (A)	0.53 (A)	10.9 (A)
Clonakilty	Brown Podzol	Good	12.0 (C)	3.18 (A)	0.30 (A)	10.7 (A)

Plant cover within the lysimeters consisted mostly of perennial ryegrass (*Lolium perenne).* Between 2004 and 2008 the lysimeters were used to simulate typical Irish grazed grassland activities. This required N fertilisation. With the exception of controls, the lysimeter soils received in 2004 and 2005, 291 kg N ha^{-1} yr^{-1} as fertiliser and 310 kg N ha^{-1} yr^{-1} as urine (Stark et al., 2008). In 2006 some lysimeters were switched to receiving 141 kg N ha^{-1} yr^{-1} and nitrification inhibitor dicyandiamide (DCD) was applied. Fertiliser was applied as five or seven split applications, and urea was applied once (Dennis *et al.*, 2012). Study of Byrne *et al.*, 2010 utilised these lysimeters to investigate changes in the hydrophobic acids fraction of drainage water from different land management practices, however existing treatments were used.

2.2.3. Experimental treatments and design

In this experiment three lysimeter soils of contrasting drainage: poorly drained Rathangan, moderately drained Elton and well drained Clonakilty; were ploughed using two different tillage techniques, fertilised and reseeded with perennial ryegrass. Three replicates of each soil-tillage combinations were used in the experiment.Two types of tillage were simulated in this experiment. These were conventional inversion tillage (CT) to a depth of approximately 20 cm applied manually with spade on 16.06.2009 and minimum (conservational) tillage (MT) to a depth of approximately

5 cm applied manually with a trowel and a rake on 18.06.2009. Fertiliser N was applied at 37 kg ha-1 as NPK on 25.06.2009, and at 34 kg N ha-1 as CAN on 24.07.2009. Mid season perennial ryegrass (Portstewart) was sown at 60 kg ha-1. Lysimeters in the field facility are laid out following a randomised block design. The two tillage treatments were applied in a way to compliment this design following the layout in a table below (Table 2.2). Soil samples were collected at the beginning of the experiment to determine available soil nitrogen (ammonium and nitrate) and soil bulk density. Gas samples and leachate samples were collected prior to, and following tillage and fertilisation.

Table 2.2 Two alternative tillage types were applied to three soil types in three blocks of six lysimeters resulting in the below layout.

MT Elton	MT Clonakilty	MT Clonakilty
MT Clonakilty	MT Elton	MT Rathangan
MT Rathangan	MT Rathangan	MT Elton
CT Rathangan	CT Clonakilty	CT Rathangan
CT Elton	CT Elton	CT Elton
CT Clonakilty	CT Rathangan	CT Clonakilty

2.2.4. Nitrous oxide emissions measurements and analysis

Gas samples were collected between 25.05.2009 and 17.09.2009. Samples were collected once a week for three consecutive weeks prior to ploughing to determine background N_2O emissions. Following ploughing on 16th and 18th June for conventional and minimum tillage respectively, gas sampling was performed daily for the first five days, and then once a week. Samples were collected using the static chamber method (Hutchinson and Mosier, 1981; Smith et al., 1995). Lysimeter casings were fitted with troughs around the edge. The troughs were filled with water during gas measurements to ensure the seal of the chamber. Plastic lids 218 l in volume fully enclosed the lysimeters fitting into the water troughs. Chambers were painted reflective white to avoid temperature fluctuation during the measurement in the headspace. Twenty ml samples were drawn 20 and 60 minutes after chamber closure through a rubber septum (Becton Dickinson, UK) using a 20 ml polypropylene syringe (BD Plastipak, Becton Dickinson, UK) with a hypodermic needle (BD Microlance 3, Becton Dickinson, UK) and injected into pre-evacuated 7

ml screw-cap septum glass vials (Labco, UK). The vials were evacuated using an evacuation line in the laboratory in Johnstown Castle. The evacuation line was fitted with BOC Edwards Pirani 501 pressure gauge to ensure all vials were evacuated evenly to 0.13 Millibar. Nitrous oxide concentrations were analysed using a gas chromatograph (Varian CP 3800 GC, Varian, USA) fitted with a ^{63}Ni electron capture detector (ECD) located in the laboratory in Johnstown Castle. During the analysis 700 µl of a sub-sample from each vial was drawn and injected first on to a magnesium perchlorite (14-22 mesh) packed pre-column to remove any moisture followed by a 3m long, 3mm outside diameter stainless steel column packed with Poropak Q (80/100 mesh). The column oven and injector temperature were both 60°C and the detector temperature was 350°C. Argon (BOC Gases, Ireland) flowing at 35 ml min^{-1} was used as a carrier gas. Samples were fed into the system by a Combi-Pal automatic sampler (CTC Analysis, Switzerland) controlled by a computer software. The GC was calibrated daily and a quality control of a known concentration was analysed every eight unknown samples. Replicates of three different concentrations were used for the GC and the evacuation line quality control purposes. Areas under N_2O peaks were integrated using Star Chromatography Workstation (Varian, USA). Six replicates of ambient air were collected together with each gas sampling to be used as a reference for gas fluxes calculations. Minimum detectable flux was 0.45 g N_2O-N ha^{-1} d^{-1}.

2.2.5. Calculation of N_2O emissions

N_2O flux was calculated from the change in headspace concentration over measured period using an equation:

$$Flux = \frac{dGas}{dt} * 10^{x} * \frac{V_{chamber} * p * 100 * MW}{R * T} * 10^{y} * \frac{1}{A}$$

[Equation 1]

where:

dGas- in ppm or ppb to get concentration at certain point in time or ppm h^{-1} or ppb h^{-1} to get concentration change over time

10^{x}- recalculation, put 10^{-6} if start from ppm or 10^{-9} if from ppb

$V_{chamber}$- volume of the chamber used

p- atmospheric pressure

MW- molecular weight, either of N or N_2O depending of which compound you want to express your emissions in

R- gas constant 8,314 J/ mol/ K

T- temperature in Kelvin

10^y- recalculation, put 10^3 if you want your results in mg or 10^6 if in µg

A-area of the chamber

The fluxes were then converted into g N_2O-N ha^{-1} d^{-1}. Mean daily emissions rates were calculated for each replicate by interpolation of values in between the measurement days using arithmetic mean (Velthof and Oenema, 1995; Flechard *et al.*, 2007).

2.2.6. Leachate collection and analysis

Nitrogen losses through leaching were also measured in this experiment. The bottoms of the lysimeters were fitted with nylon tubing that independently collected drainage from every unit through a system of underground pipes. Drainage was collected into plastic containers placed in underground facilities on site. Leachate was regularly sampled throughout the experiment with frequency between once a month to once a week depending on the quantity of rainfall. As leaching is usually a slower release pathway than gaseous emissions samples included in this analysis were collected over a longer period; between 18.05.2009 and 6.11.2009. The start date was chosen as the leachate sampling date closest to first gas sampling date, whereas end date was chosen after plotting leached N load over time to ensure the whole ploughing and fertiliser peak was included in the dataset. Volume of leachates was recorded and sub-samples collected into test tubes on each sampling occasion. The sub-samples were analysed for total nitrogen (TN) concentrations on Shimadzu Total Organic Carbon Analyser using combustion oxidation method with chemiluminescence detection (Ammann *et al.*, 2000).

2.2.7. Calculation of leached N

Total N load was calculated from the concentration and volume of leachate collected in a sampling period using the equation below:

$$TN_l = \frac{TN_c * LV * 20000}{1000000} \qquad \text{[Equation 2]}$$

where:

TN_l- Total N load in kg/ha

TN_c- Total N concentration in mg/l

LV- leachate volume in l

20000- recalculation from lysimeter area ($0.5m^2$) to ha

1000000- recalculation from mg to kg

2.2.8. Soil bulk density

Prior to tillage soil samples were collected at a depth of 0.5 cm from nine randomly chosen lysimeters (three replicates per soil type) using bulk density rings manually driven into the soil (Eijkelkamp, Agrisearch Equipment, the Netherlands). Bulk density was measured according to procedure in British Standard (BSI, 2006). Vegetation was removed from the sampling area and metal ring was driven in the soil with a hammer and a timber block. The ring with encased soil was then carefully recovered from the lysimeter and any excess soil trimmed with a sharp knife. The soil core in the ring was dried at 105°C until constant weight. Correction for stones was applied by recovering any stones from inside the soil core, determining their weight and volume. Dry weight of the soil without the ring and the volume of the ring were used in the dry bulk density calculation. Weight and volume of the stones were subtracted from the weight and volume of the soil core.

2.2.9. Soil mineral nitrogen content

Prior to tillage soil samples were collected at a depth of 0-5 cm from nine randomly chosen lysimeters (three replicates from each soil type) using a 1.5 cm diameter corer.

Three cores were collected from each lysimeter. Soils were sieved through 3.35mm sieve and ammonium (NH_4^+) and total oxidised nitrogen (TON) (NO_2^- and NO_3^-) levels determined by KCl extraction. The method involved shaking 40g of fresh soil sample with 100ml of 2M KCl for 1h following by filtering through Watmann No.2 filter. The filtrates were subsequently analysed by a wet chemistry method using an Aquakem 600 discrete analyser according to Hoekstra *et al.* (2010).

2.2.10. Ancillary measurements

Soil temperature and soil moisture were measured at the time of collecting gas samples using a WET sensor WET-2 attached to a moisture meter –HH2 (Delta-T Devices, Burwell, Cambridge, UK). Weather data such as air and soil temperature, and rainfall were also collected from a meteorological station situated on the adjacent field.

2.2.11. Statistical analysis

Statistical analysis was performed using SAS 9.1.2. The effects of soil drainage and tillage type on cumulative N_2O-N losses and cumulative TN-N losses were analysed as well as on cumulative combined gaseous and leached losses. All data were tested for normal Gaussian distribution and transformed as appropriate. A mixed linear model procedure (PROC MIXED) that permits data to exhibit correlation and nonconstant variability followed by a Tukey's multiple comparison post-test was used. N_2O-N time series data was also analysed in SAS. This dataset was first adjusted to the same starting point as conventional and minimum tillage occurred two days apart.

2.3. Results

2.3.1. Rainfall and temperature distribution patterns

The mean temperature for 2009 was 10.1 °C which was 0.5 °C lower than 30-year average however throughout the experiment temperatures were relatively high (Figure 2.2). Out of 116 days of the experiment only one day was recorded with temperature below 10 °C. Mean daily temperatures ranged from a minimum of 8.3 °C to a

maximum of 18.6 °C, which are typical of a north Atlantic temperate climate. Mean temperature for the experimental period was 14.5 °C. Total annual precipitation was atypical, with a 40% increase relative to the 30-year average, yielding 1452 mm rainfall, of which 36% (526 mm) fell in the experimental period. Out of 116 days of the experiment rainfall was recorded in 74 cases and there were 22 days with rainfall over 10mm.

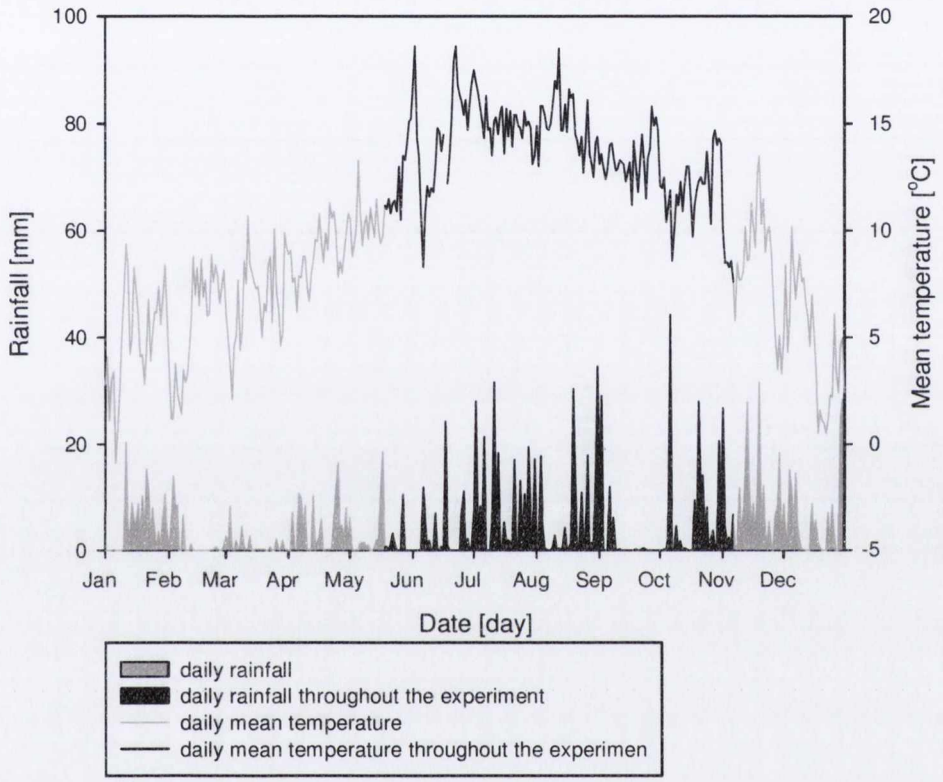


Figure 2.2 Daily rainfall (grey bar) and mean temperature (grey line) records for year 2009 collected by a met station in Johnstown Castle. Values observed throughout the experiment are marked in black.

Table 2.3 shows average rainfall and temperature for the background, tillage and fertilisation period. Additionally, four days pre-ploughing period was extracted from the dataset. This is to analyse the environmental conditions prior to ploughing lysimeters, as conventional and minimum tillage were applied two days apart (on 16.06.2009 and 18.06.2009, respectively). No statistically significant differences were found between corresponding periods for CT and MT treatments ($p>0.05$) therefore recorded fluxes were adjusted to the same starting point to enable statistical analysis of the cumulative N_2O fluxes. This was done by assigning a number to each observation date for both CT and MT treatments, where day of ploughing was zero in both cases,

and observation days prior to that date had negative numbers assigned, and following ploughing positive numbers. Then equal numbers of observations were used to calculate the cumulative fluxes.

Table 2.3 Average rainfall [mm] and temperature [°C] associated with background period, tillage and fertilisation. Capital letters in brackets indicate statistically significant differences at p<0.05.

		Background	Pre-ploughing	Ploughing	Fertilising
Length in days		22	4	4	55
Rainfall	CT	1.8 (A)	2.0 (A)	6.4 (A)	4.9 (A)
Temperature		13.2 (A)	14.1 (AB)	16.8 (AB)	15.4 (B)
Rainfall	MT	2.64 (A)	6.33 (A)	0.23 (A)	5.12 (A)
Temperature		13.30 (A)	14.04 (AB)	14.73 (AB)	15.39 (B)

2.3.2. Daily and cumulative nitrous oxide emissions

Three distinct periods can be observed with regard to N_2O flux, namely background, post-tillage and post-fertiliser application. Day of ploughing is treated as day zero therefore the measurement days prior to ploughing (background) have negative numbers and the measurement days after ploughing (post-tillage and post-fertiliser) have positive numbers. Conventional and minimum tillage were applied to the lysimeters on two separate days due to time constraints and therefore the first gas sampling for the CT treatment occurred on day -22 whereas for the MT treatment on day -24. Prior to ploughing (days 22-6 for conventional tillage or 24-8 for minimum tillage before the application time) emissions were consistently low with mean daily values of -0.0074 ± 0.2 g N_2O-N $ha^{-1}d^{-1}$ for Clonakilty, 1.6170 ± 1.3 g N_2O-N $ha^{-1}d^{-1}$ for Elton and 1.8197 ± 1.3 g N_2O-N $ha^{-1}d^{-1}$ for Rathangan soil (Figure 2.3).

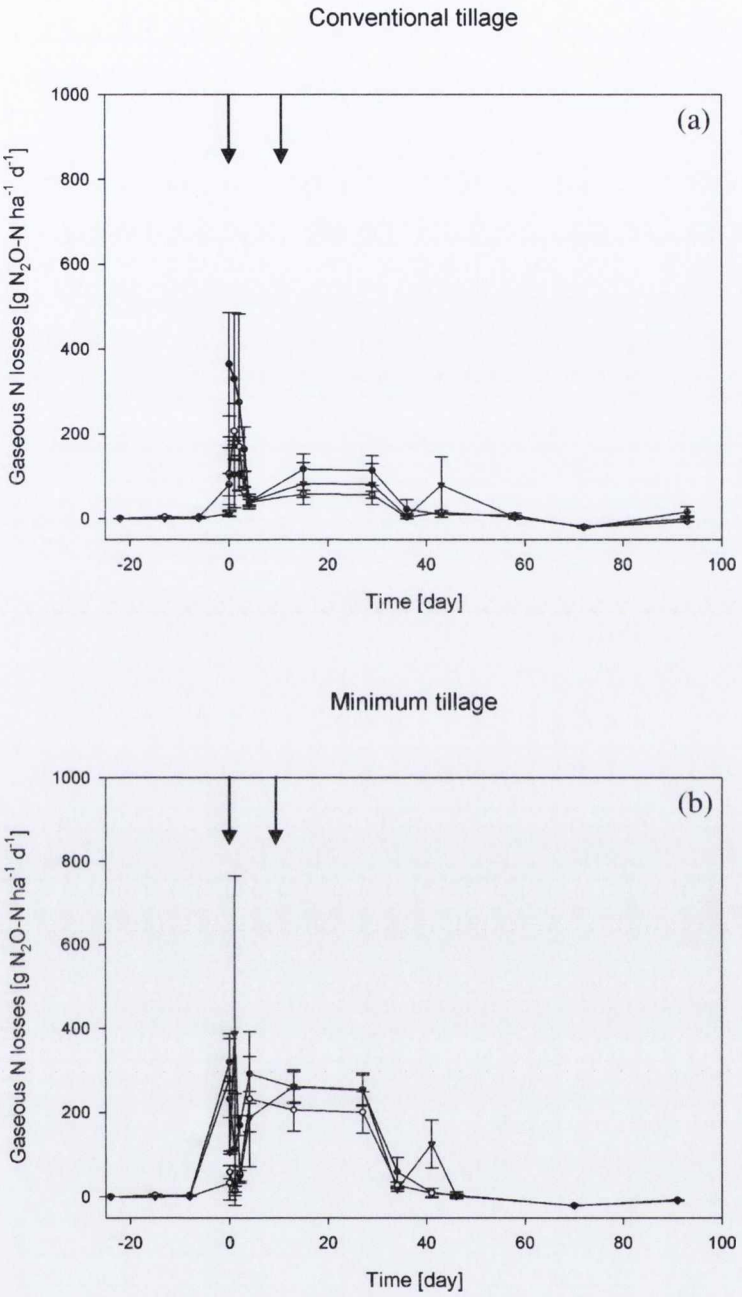


Figure 2.3 Temporal pattern of N_2O fluxes throughout the experiment for conventional (a) and minimum (b) tillage treatments from Rathangan (●), Elton (○), and Clonakilty (▼) soils. Arrows indicate application timing of tillage (first arrow) and fertilisation (second arrow). Error bars indicate SEM (n=3).

The magnitude of cumulative N_2O-N losses from the background, ploughing, and fetilisation periods are presented in Table 2.4 There were no significant differences in cumulative emissions between soils during prior to treatment application ($p>0.05$). Tillage as a source of disturbance to the soil may influence both short and long-term fluxes of greenhouse gases to the atmosphere. N_2O is released into the atmosphere through degassing and also through a rapid turnover of organic matter that becomes available for microbial activity after exposure of soil aggregates. In all treatments noticeable peaks in N_2O emissions were observed following minimum or conventional tillage. However a large variation in between treatments was associated with flux values recorded over this period. Cumulative values ranged between 398.12± 105.3 g N_2O-N ha^{-1} for Clonakilty CT and 1031.25 ± 99.7 g N_2O-N ha^{-1} for Rathangan CT (Table 2.4). According to statistical analysis there was a significant difference between fluxes from Rathangan soil and two other soils ($p>0.05$). However, treatment did not prove significant throughout this period and there was no significant soils*treatment interactions. In the experiment ploughing accounted for between 7.3 ± 1.2% (Clonakilty) and 11.3 ± 2.4% (Rathangan) of total post-treatment (ploughing and fertilising) cumulative emissions in the MT treatments and between 14.6 ± 7.4% (Clonakilty) and 27.8 ± 8.1% (Rathangan) in the CT treatments. Therefore CT treatment resulted in a higher share of the short-term N_2O emissions associated with ploughing than the MT treatment, however the difference was not significant ($p>0.05$).

Table 2.4 Cumulative gaseous N losses [kg N_2O-N ha^{-1}] associated with background period, tillage and fertilisation (n=3). Capital letters in brackets indicate statistically significant differences at $p<0.05$.

		Background	Ploughing	Fertilising	Overall
Length in days		22	4	54	81
Rathangan	CT	0.8678 (CD)	1.0312 (BCD)	3.2219 (ABC)	5.1209 (AB)
	MT		0.9096 (CD)	7.4303 (A)	9.2077 (A)
Elton	CT	0.0959 (CD)	0.5391 (CD)	1.7536 (ABC)	2.3886 (ABC)
	MT		0.4301 (CD)	5.8140 (AB)	6.3400 (A)
Clonakilty	CT	0.3701 (D)	0.3981 (CD)	3.0568 (ABC)	3.8250 (ABC)
	MT		0.7085 (CD)	8.4394 (A)	9.5180 (A)

Fertiliser application provides a readily available source of N for the soil microbial community and therefore enhances denitrification. In this study lower but prolonged peaks in N_2O flux were observed after application of fertiliser for all treatments, with emissions of N_2O not returning to background levels a month after application. The

overall fertiliser period lasted 54 days and produced the majority of the total gaseous N losses (Figure 2.3). Cumulative values ranged between 1753.56 ± 178.5 g N_2O-N ha^{-1} for Elton CT and 8439.40 ± 1940.9 g N_2O-N ha^{-1} for Clonakilty MT (Table 2.4). Treatment was a highly significant source of variation between N_2O fluxes throughout the fertiliser period ($p<0.01$). Soil effect was not significant and there was no significant interaction between soil and treatment. The only difference in post-hoc LSD test was found between Clonakilty MT and Elton CT ($p<0.05$). These were also the two soils that produced the most and the least N_2O, respectively. Fertilisation period was much longer than ploughing period and contributed most of the gaseous N losses producing between 88.7 ± 2.4% (Rathangan) and 92.7 ± 1.2% (Clonakilty) of post-treatment (ploughing and fertilising) emissions for the MT treatment and between 72.2 ± 8.1% (Rathangan) and 85.4 ± 7.4% (Clonakilty) for the CT treatment.

Overall, the gaseous N losses ranged between 2388.5 ± 404.99 g N_2O-N ha^{-1} from Elton CT and 9518.0 ± 2416.95 g N_2O-N ha^{-1} from Clonakilty MT. Most of the gaseous N was lost following fertilisation. Fertiliser-period emissions represented between 63% (Rathangan CT) and 92% (Elton MT) of all the experimental gaseous losses (from background, ploughing, and fertiliser period) (Table 2.4).

2.3.3. Relationship between emissions and soil and environmental factors

2.3.3.1. Post-ploughing N_2O emissions vs. clay content

Clay particles influence both the creation of soil aggregates and the diffusivity of the soil. A strong positive relationship was found between clay content and N_2O flux for both CT and MT treatments (R^2=0.99 and 0.81 respectively) as illustrated in Figure 2.4.

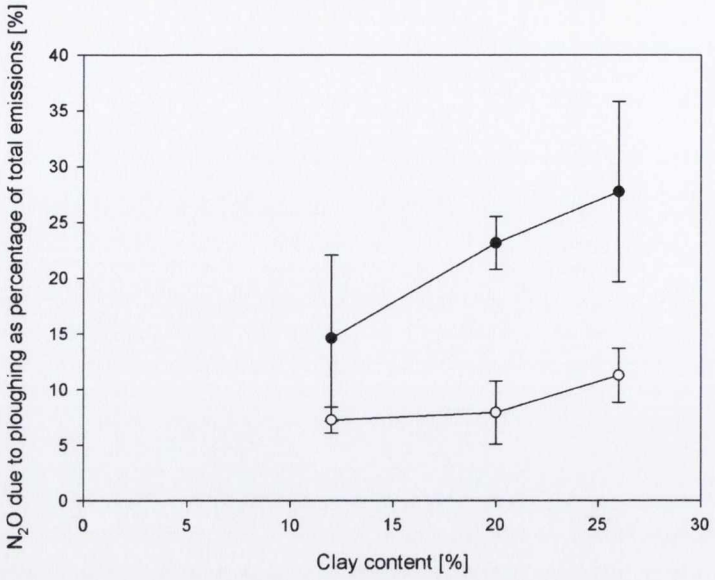


Figure 2.4 Relationship between clay content of the soils and cumulative N_2O emissions from CT (●) and MT (○) treatments expressed as percentage of the total emissions over the course of the experiment. Error bars indicate SEM (n=3).

2.3.3.2. Post-fertiliser N_2O emissions vs. soil temperature and moisture

The relationship between fertiliser-induced emissions and soil moisture was investigated. Minimum tillage was associated with less disturbance and enhanced moisture retention in the soil, providing more anaerobic sites within the soil in comparison to conventional tillage. This was observed as increased water filled pore space (WFPS) in the minimum tillage treatment in comparison with the conventional tillage (Figure 2.5). This resulted in higher N_2O emissions over the fertiliser-induced flux period for the minimum tillage treatment. However the relationship between moisture content and daily N_2O fluxes was not very pronounced (R^2=0.17 for minimum tillage and 0.22 for conventional tillage).

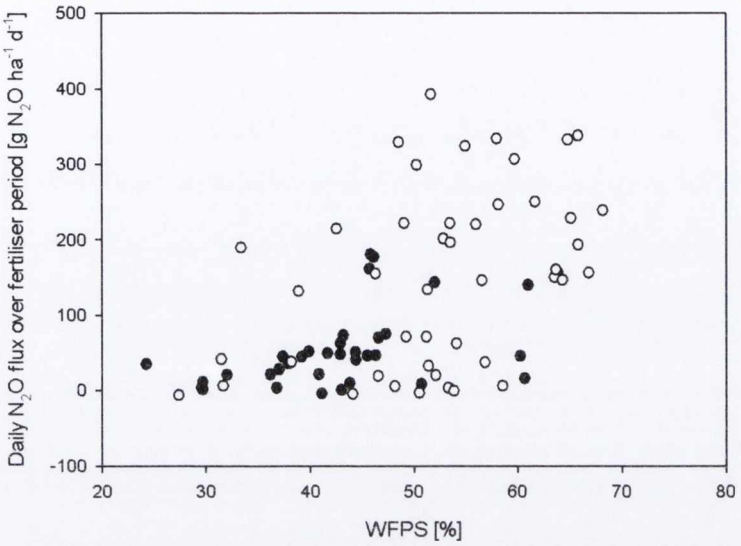


Figure 2.5 Correlation between WFPS and daily N_2O flux over fertiliser period from CT (●) and MT (○) treatments.

2.3.3.3. Available nitrogen

Available nitrogen levels in the lysimeter soils were relatively low in the examined soils (Table 2.5). Rathangan was characterised by the lowest mineral N content, however the differences between soils were not significant ($p<0.05$).

Table 2.5 Levels of available nitrogen in the lysimeters soils (n=3).Capital letters in brackets indicate statistically significant differences at $p<0.05$.

	Soil NH_4^+-N [mg N kg^{-1} soil]	Soil NO_3^--N [mg N kg^{-1} soil]	Soil Total N [%]
Rathangan	2.87 (A)	1.74 (A)	0.35 (A)
Elton	5.37 (A)	2.09 (A)	0.53 (A)
Clonakilty	3.56 (A)	4.14 (A)	0.30 (A)

2.3.3.4. N leaching

The cumulative amount of N measured in the lysimeter leachate over the time period of the experiment was highly influenced by soil type (Figure 2.6). There was approximately a month's lag in response to the ploughing and fertilisation in leached N which was related to leachate transport through the soil cores. Only after this time did N concentration start to rise. For this reason it was impossible to distinguish what

portion of the leached N was lost following ploughing and how much was associated with fertiliser application. N load was therefore integrated over the whole experiment period. Cumulative N losses varied between 1.26 ± 0.5 kg N ha^{-1} for Rathangan CT and 15.38 ± 3.9 kg N ha^{-1} for Elton CT (Figure 2.6 a and b). Soil had a significant effect on leached N losses ($p<0.001$), whilst treatment proved not significant, and there was also no soil*treatment interaction. Rathangan losses were significantly lower than those of the other two soils. Rathangan CT was also significantly different from Clonakilty CT, Clonakilty MT and Elton CT ($p<0.05$).

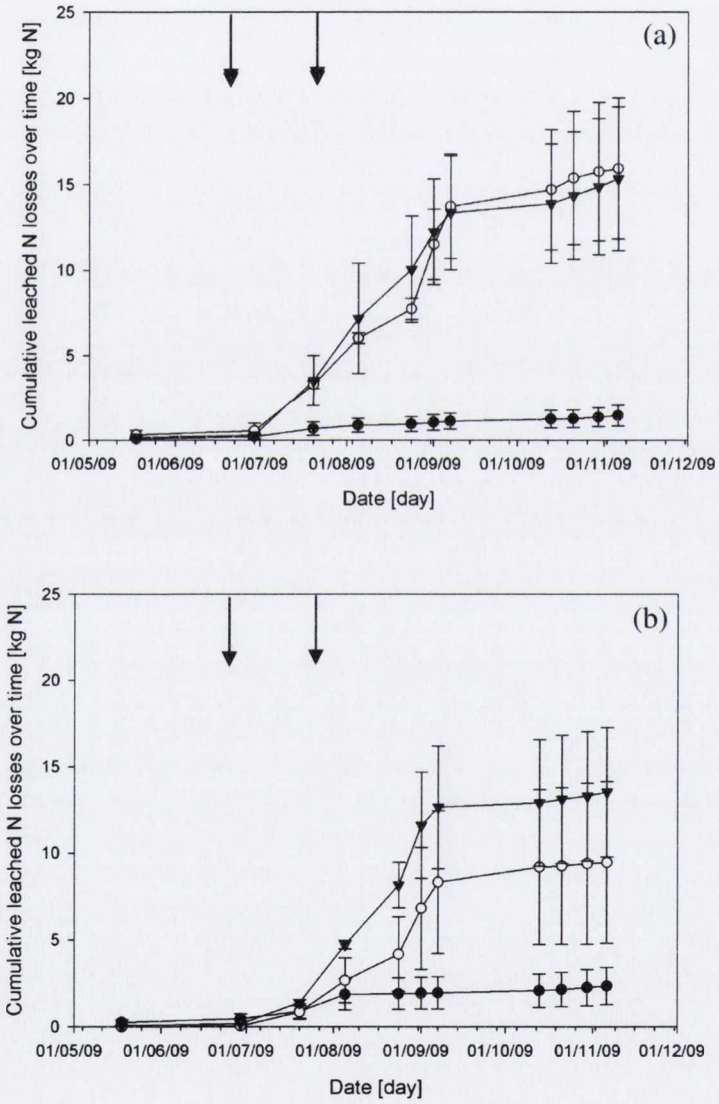


Figure 2.6 Cumulative leached N losses over the whole experiment for (a) CT and (b) MT treatments from Rathangan (●), Elton (○), and Clonakilty (▼) soils. Arrows indicate application of tillage (first arrow) and fertilisation (second arrow). Error bars indicate SEM (n=3).

2.3.3.5. Overall N losses

Gaseous emissions and leached N throughout the experiment were combined together in Figure 2.7 to accurately account for losses resulted from ploughing and fertilisation. Total N losses varied between 6.4 ± 0.7 kg N ha^{-1} for Rathangan CT and 22.6 ± 2.4 kg N ha^{-1} for Clonakilty MT. Here again, Rathangan proved significantly different from the other two soils, however there was no significant difference between CT and MT treatments. The only soil*treatment effect was found between Rathangan CT and Clonakilty MT, the two soils to lose the least and most amount of N, respectively ($p<0.05$). Rathangan lost the smallest share of N through leaching whereas in the other two soils this was the main pathway of losses.

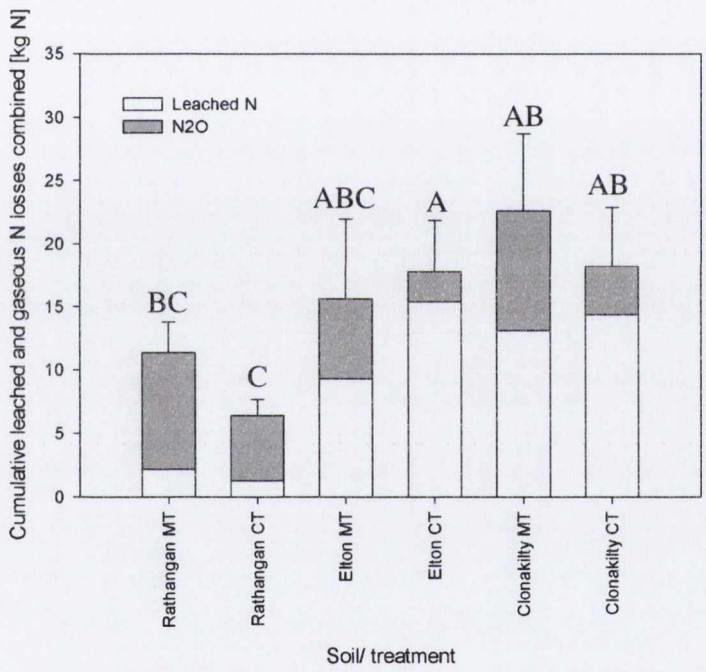


Figure 2.7 Total N losses throughout the experiment as a sum of combined gaseous emissions and N leaching. Error bars indicate SEM (n=3). Capital letters indicate statistically significant differences at $p<0.05$.

2.4. Discussion

2.4.1. N_2O emissions

Similar to previous studies (Mosier *et al.*, 1991; Clayton *et al.*, 1997; Kessavalou *et al.*, 1998; Ball *et al.*, 1999; Choudhary *et al.*, 2002; Estavillo *et al.*, 2002; Vellinga *et al.*, 2004; Ball *et al.*, 2008; Rochette *et al.*, 2008; Velthof *et al.*, 2010) in this experiment ploughing and N fertilisation were sources of large N_2O losses. A trend could be seen where conventional tillage resulted in higher N_2O emissions than minimum tillage in poorly and moderately draining soils solely from the ploughing period, but not in well draining soil (Table 2.4). However these differences were not statistically significant. Ploughing technique did not have a significant effect on the post-ploughing N_2O fluxes. Conversely, soil drainage had a statistically significant effect on the fluxes.

Conventional mouldboard tillage is deeper, turns the soil over and, while doing so, loosens its structure accommodating release of gas trapped in soil air (Ball *et al.*, 1999). N_2O concentration in soil air can be significantly higher from the atmospheric one hence it can be a source of large N_2O emissions through degassing (Heincke and Kaupenjohann, 1999). Poorly draining soils revealed extremely high fluxes of N_2O (over 1kg N ha^{-1} over four days period, Rathangan CT) and the highest share of ploughing-derived N_2O (Table 2.4). Their heavy clay texture can retain more N_2O in soil air that was released upon ploughing. Moreover, poorly draining soils had the highest clay content which is responsible for the quantity and quality of soil aggregates. Once broken down at the time of ploughing, the aggregates cannot protect organic matter, which is then mineralised, nitrified, and denitrified to N_2O (Ball et al., 2008, Drury et al., 2004) (Figure 2.4). There is a potential for reduction of N_2O emissions from poorly and moderately draining soils by minimum tillage as it preserves soil aggregates and restricts the soil air flow. Restricted air flow can lower the emission through physically trapping the gas within the soil whereas moisture and soil aggregates preservation can enhance full denitrification pathway leading to N_2O reduction to N_2.

Whilst ploughing emissions of N_2O were mostly due to degassing followed by rapid mineralisation of exposed organic matter, emissions linked to fertiliser application were predominantly caused by microbial activity acting on this new supply of available N. Emissions following ploughing were high and short-lived (4 days), whereas post-fertiliser emissions remained enhanced over a longer period (54 days). Second fertiliser application a month following tillage can explain the prolonged period of high N_2O flux. However it was also hypothesised that the N_2O flux was influenced by the unusual time of ploughing (summer ploughing) that coincided with very warm and wet weather conditions. This should have affected microbial processes leading to enhanced N_2O production. Post-fertiliser emissions were higher from previously min-tilled soils and ploughing technique had a highly significant effect on the N_2O release. This type of tillage preserved moisture keeping soil conditions more vulnerable to gaseous losses (Clayton *et al.*, 1997). Fertilisation with mineral N supplied microbial community with substrate and resulted in high N_2O emissions. In both conventionally and minimally ploughed soils highest share of N_2O emissions came from well draining soils. Lower fertilisation fluxes from poorly drained soils were most likely due to restricted air flow. However optimal the conditions for denitrification, created N_2O might not be able to escape due to the soil's compacted structure. As N_2O is highly soluble in water the gas remains in balance with soil water solution (Heincke and Kaupenjohann, 1999).

Table 2.6 Emission factors for the fertilisation of previously ploughed lysimeter soils. Capital letters in brackets indicate statistically significant differences at $p<0.05$.

Soil	CT	MT
	EF [%]	EF [%]
Rathangan	1.54 (A)	7.47 (AB)
Elton	2.14 (A)	7.86 (B)
Clonakilty	3.03 (A)	10.61 (B)

All the lysimeters were ploughed and subsequently fertilised therefore emission factors were estimated using comparison between daily mean fluxes from background and post-fertilisation periods against the fertilisation rate. This calculation was possible because environmental factors (temperature, rainfall) did not differ significantly between background and fertiliser period (Table 2.3). Table 2.6 shows that all the emission factors were higher than the IPCC default value of 1% (Ogle *et al.*, 2005). Soils previously min-tilled displayed emission factors between 3.5 to 4.9 times higher

than those conventionally tilled. Here again no significant effect of the tillage treatment was found due to high variation in N_2O fluxes. Poorly draining soil's emission factor was comparable to that found by Hyde *et al.* (2006) from grassland at Johnstown Castle, Ireland, where up to 7.2% of the applied N (urine, dung, and fertiliser) was lost as N_2O.

2.4.2. Leached N

The response of N concentration in drainage to applied tillage and fertilisation was delayed in comparison to gaseous losses by slower transport in soil which is in agreement with the previous study on the same soils (Dennis *et al.*, 2012). Therefore elevated concentration of total N in leachate is directly linked to ploughing and subsequent fertilisation of the lysimeters. Since the lysimeters received no organic fertiliser total N was considered a good indication of nitrate leaching. Similarly to other studies, for free draining soils like Elton and Clonakilty leaching was the main pathway for N losses, whereas for poorly draining Rathangan soil it was mainly gaseous emissions (Sogbedji *et al.* 2000). The study of Sogbedji *et al.* 2000 on maize on two soil types fertilised at three rates found the largest losses to be from a loamy sand soil. These differences between soil drainage were highly significant highlighting the importance of management paired with the soil type.

2.4.3. Overall N losses

N loss associated with soil ploughing and fertilisation was significant. Soil had a significant effect on N_2O emissions post-ploughing, on leached N, and on overall N losses, whereas tillage treatment had significant effect only on post-fertilisation fluxes. This results show the importance of soil type in N loss anticipated with a standard agricultural practice. Leaching was a main loss pathway for well and moderately draining soils, and in poorly draining soils gaseous emissions were responsible for the majority of N loss (Figure 2.7). Due to large variation in collected data, only losses from conventionally tilled poorly draining soil (6.4 ± 0.7 kg N ha^{-1}) and min-tilled well draining soil (22.6 ± 2.4 kg N ha^{-1}) were significantly different. These values, however estimated over short time period, are very low in comparison with these from the study

of Davies *et al.* (2001) which estimated that 140 kg N ha^{-1} was lost through N_2O and leaching from ploughed and reseeded Scottish grassland over 18 months . Overall N losses were in general lowest from poorly draining soil, followed by moderately draining soil, and with well draining soil producing the most losses. Well and moderately draining soils shifted their N loss balance toward N leaching following conventional tillage in comparison with minimum tillage treatment. This relationship was less visible in poorly draining soil where most N left the system in gaseous form. The two applied treatments affected the balance between gaseous and leached N losses however the small differences in overall amounts of N loss did not prove significant.

2.5. Conclusions

Agricultural land is subject to land use changes to accommodate large energy demand through energy crop cultivation. According to the results of this experiment the first steps in land use change (ploughing and fertilisation) will significantly affect the soil GHG budget and N leaching. Losses cannot be entirely avoided however measures can be taken to minimise the negative impact on the environment. Gaseous N lost through ploughing range between 0.4 ± 0.1 and 1.0 ± 0.1 kg N ha^{-1} for well draining and poorly draining conventionally tilled soils, respectively, whereas after fertilisation gaseous N loss range between 1.7 ± 0.2 and 8.4 ± 1.9 kg N ha^{-1} for moderately draining conventionally tilled and well draining min-tilled soils, respectively. Investigated agricultural practices of ploughing and fertilisation enhance N leaching. Leached N losses vary between 1.3 ± 0.5 and 15.4 ± 3.9 kg N ha^{-1} for poorly draining and moderately draining conventionally tilled soils, respectively.

Soil type affects the pathway of N loss. Assuming that N_2O emission and leached N equal to 100% loss following ploughing and fertilisation, poorly draining soil can loose approximately 20% of N through leaching, whereas moderately and free draining soils can be 60 and 80%, regardless of the tillage treatment. Soil type is the most important factor controlling the magnitude of losses strongly suggesting that it should be incorporated in agricultural planning and management. Surprisingly, poorly draining soil expected to produce highest losses, results in the lowest overall N loss (N_2O + leached N). It is believed that the low N loss from this soil could be the effect of

complete denitrification that reduces N_2O to benign N_2. This result could suggest the suitability of the low quality and marginal poorly drained land a viable option for land use change.

Adoption of minimum tillage practice is known to reduce the risk of SOC loss (Paustian *et al.*, 2000) but is also linked to enhanced N_2O, especially in poorly-aerated soils (Rochette *et al.*, 2008). Similarly, in this study, a trend of higher N_2O from min-tilled soils occurred however it was not statistically significant. Adoption of minimum tillage shifts the N losses between N_2O and N leaching, however not significantly. Fertilisation remains a major contributor to N losses therefore LUC to N-efficient crops with low N requirement are desirable. Additionally, employing perennial crops could further reduce the risk of N losses by lower frequency of ploughing and crop cover throughout the year.

Chapter 3: Field scale study on the effect of land use change from grassland into energy crops-Miscanthus and Reed canarygrass cultivation on N cycling, in particular N_2O emissions.

3.1 Introduction; objectives and hypotheses of the experiment

Land use change and forestry are sources of GHG emissions producing 0.2 Gt CO_2 eq of N_2O annually (IPCC, 2007). The process of land use change usually involves soil disturbance and shifts in N fertilisation rates potentially leading to loss of soil organic matter and enhanced GHG emissions (Guo and Gifford, 2002). The extent of these losses depends on the type of anticipated conversion.

The principle driver for enhanced N_2O emissions from land undergoing conversion for agricultural functions is ploughing, and subsequent rapid mineralisation of organic matter. Ploughing inverts the soil breaking down soil aggregates and exposing organic matter to microbial attack and aerobic chemical reactions (Six *et al.*, 1998; Six *et al.*, 2000; Agele *et al.*, 2005). The long term effect of cultivation is a reduction in soil biota diversity which leads to dramatic changes in decomposition and nutrient availability regulation (Matson *et al.*, 1997). The introduction of a new vegetation type or a mixture of species can affect soil microbial community. As the community structure changes, so do microbial reactions and their products. N_2O can be produced in soil through nitrification and denitrification. Denitrification alone can be carried out by over 60 soil microbial species proving the complexity of soil microbial community (Baggs, 2011). It is common for microbial biomass of temperate soils to be dominated by fungi capable of nitrification and denitrification (Ruzicka *et al.*, 2000; Laughlin and Stevens, 2002; Laughlin *et al.*, 2008). Share of N_2O produced by fungal activity can be significant for global N_2O budget. Agricultural management and land use play important role in the balance between fungal and bacterial activity in soil biomass with fungi favouring extensive no tillage systems (Frey *et al.*, 1999). Therefore, fungal/ bacterial ratio can decrease as a result of tillage and N fertilisation and vegetation type (de Vries *et al.*, 2006). Despite the common agreement that soil micro organisms' diversity and activity is affected by the environmental conditions, little is known about the specific responses of the various microbial groups to the land use, land management, and land use change (Jangid *et al.*, 2008; Baggs, 2011).

In various climatic regions, other land management practices such as controlled burning or flooding and drying cycles on rice fields can create large GHG emissions

(Freney, 1997). Land use change through burning in tropical climates can be responsible for substantial N_2O emissions with forest soil converted into pasture releasing for example 429 kg N_2O-N ha^{-1} over 10 years post-burning (Erickson and Keller, 1997). Land use change of forests into arable hugely depletes soil C and N, and even to a larger extent than conversion to pasture. However, some of these losses can be compensated by a higher bulk density after the conversion (Murty *et al.*, 2002). Permanent grassland soils are characterised by high N mineralisation rates and high N supply, therefore cultivation of these soils can produce large emissions of N_2O (Estavillo *et al.*, 2002). Land use change of natural land into agricultural land also increases soil bulk density, which leads to changes in soil aeration status providing conditions more favourable for denitrification and enhanced N_2O emissions (Horn *et al.*, 1995; Parton *et al.*, 2001).

Croplands and pastures occupy nearly 40% of the land surface globally (Ramankutty and Foley, 1999; Asner *et al.*, 2004). This represents a large shift from natural to agricultural land, and is recognised to be one of the major drivers of climate change. N_2O emitted from different land uses are highly variable, with emissions ranging between 0.01 and 2.25 kg N_2O-N ha^{-1} yr^{-1} for temperate forest, 0.16-12.47 kg N_2O-N ha^{-1} yr^{-1} from grasslands with N fertilisation, and 0.27-15 kg N_2O-N ha^{-1} yr^{-1} from arable sites throughout Europe (Schmidt *et al.*, 1988; Mosier *et al.*, 1991; Struwe and Kjoller, 1994; Smith *et al.*, 2003; Flechard *et al.*, 2007; Skiba *et al.*, 2009). Variations in N_2O emissions in humid temperate regions of southern Europe can be up to three fold for cropland and six fold for grassland than these from forest, producing 2.7 kg N_2O-N ha^{-1} yr^{-1}, 4.8 kg N_2O-N ha^{-1} yr^{-1}, and 0.8 kg N_2O-N ha^{-1} yr^{-1} respectively (Merino *et al.*, 2004).

Land use change has commonly been associated with negative environmental impacts like loss of soil C and N, GHG emissions and loss of biodiversity (Pielke, 2005). However, some recent land use changes serve GHG mitigation through both reforestation and bioenergy crops. The cultivation of biomass and biofuel crops has the prospective to save GHG emissions, not only by substituting fossil fuels, but also through their low fertilisation requirements and potential carbon sequestration. The widespread promotion and expansion of dedicated bioenergy crops could have major implications on agriculture-derived GHGs at a field and regional scale. Large areas of

land for cultivation would have to be gained from either set-aside land or through land use change, the latter most probably from grassland and arable land (Rowe *et al.*, 2009). Perennial bioenergy crops, like *Miscanthus* and Reed canarygrass, have been reported to deliver additional environmental benefits in comparison to annual crops by elimination of annual cultivation and reduced N fertilisation (Clifton-Brown *et al.*, 2004; Powlson *et al.*, 2005). When cropland is being displaced for *Miscanthus*, the new crop can display a positive influence on soil condition improving soil texture, soil water retention and fertility. However, this may not be the case for pasture conversion (Rowe *et al.*, 2009). Perennial bioenergy crops have a potential for SOM and SOC sequestration, and thus for reducing the risk of both nitrate leaching and erosion (Reicosky *et al.*, 1995; Christian and Riche, 1998; Borjesson, 1999). They promote aggregate formation leading to improved soil stability (Borjesson, 1999; Kahle *et al.*, 2001).

Currently little is known about the consequences of land use change to energy crops on N cycling and N_2O emissions. Some studies question the feasibility of annual bioenergy crops in reducing GHG emissions because of high N_2O losses associated with agricultural practice, mainly N fertilisation (Crutzen *et al.*, 2008). Indeed the GHG benefits associated with fossil fuels substitution from bioenergy may be significantly reduced due to the high level of trace gas emissions associated with their cultivation (Drewer *et al.*, 2012). However, these assumptions may not be valid for perennial biomass systems, where annual disturbance and fertilisation patterns are very different. Fertiliser applied to various energy crops results in N_2O emissions from annual crops (rape, triticale, rye, and hemp) twofold greater than those of perennial crops (poplar and willow) (Hellebrand *et al.*, 2003; Hellebrand *et al.*, 2008; Kavdir *et al.*, 2008). Nutrient requirements of the perennial grass *Miscanthus* is low. A study of Jorgensen *et al.* (1997) delivers result that fertilisation at a 75 kg N ha^{-1} is sufficient for the crop growth and will only reduce the plant's gross CO_2 displacement potential no more than by 6%.

Therefore, more localised research on the environmental impacts of bioenergy crops is essential to investigate how various bioenergy crops perform under Irish conditions, what is their effect on soil and water conditions, their influence on C and N cycling and consequently CO_2 and N_2O emissions. Bioenergy crop management should

consequently be optimised to local conditions. Here, a field experiment was conducted to study some of these issues. The N dynamics of *Miscanthus* and Reed canarygrass on well and poorly drained soils was investigated through a series of observations to determine trends in N_2O emissions, soil available nitrogen concentrations (NH_4^+ and NO_3^-), soil N content and C: N ratio, and plant N content and N uptake.

Objectives and hypotheses of the study:

1) To measure N_2O emissions from the fields in transition from managed grassland to perennial energy crop cultivation and to assess the extent of gaseous N losses following the conversion. The hypothesis was that early stage of land use change can cause large N_2O fluxes as a result of soil disturbance.

2) To compare gaseous N losses associated with two candidate energy crops for cultivation in Ireland: *Miscanthus* and Reed canarygrass; and assess their impact on agricultural GHG emissions. The hypothesis was that due to their specific plant biology the two species can affect the soil differently and therefore lead to considerably different N_2O emissions.

3) To compare gaseous N losses associated with different drainage regimes within the same crop cover. The hypothesis was that poorly draining site should be characterised by higher soil moisture content therefore providing more optimal conditions for denitrification and denitrification-derived N_2O.

4) To assess the sustainability of LUC into energy crops as a GHG mitigation option through calculating emissions intensity of bioenergy production. The hypothesis was that displacement of fossil fuels by biofuels might be environmentally beneficial in terms of GHG emissions.

3.2. Materials and methods

3.2.1. Experimental sites

The experimental work was carried out between February 2009 and April 2011 on two sites, both located at Teagasc Environmental Research Centre in Johnstown Castle, Co.Wexford, coordinates 6° 30' W/ 52° 17'' N (Figure 3.1).

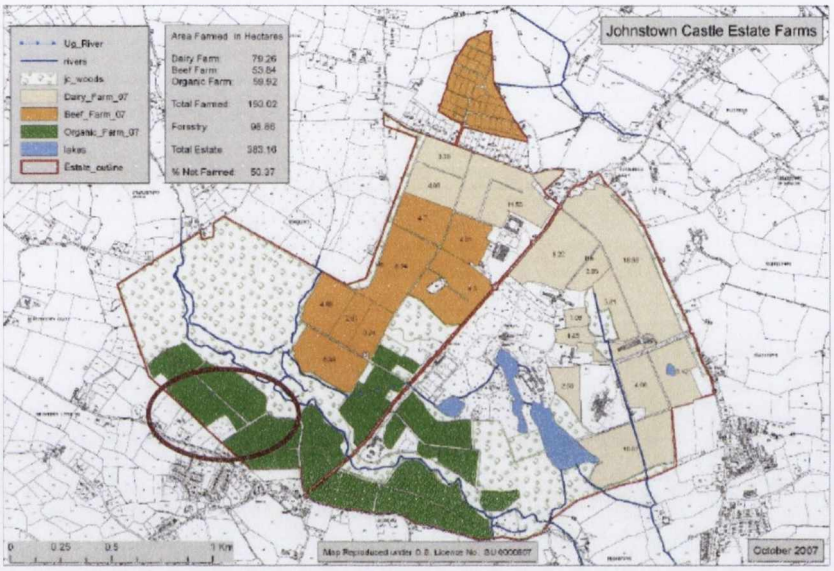


Figure 3.1 Map of Johnstown Castle Estate, Wexford. Red circle marks the part of the farm where the two experimental sites were chosen.

Two sites, approximately 3 ha each, were chosen for the experiment. Soil of the first site was of fine loam texture with poor to imperfect drainage, this site was marked as P/D. Soil on this site is classified mostly as Brown Earth with small area of gley. The topography is reasonably flat. Soil of the second site was of fine loam with well to moderate drainage, this site will be referred to as W/D. Soil on this site is classified as Brown Earth. The topography of this site is gently rolling. Soils characteristics of both sites are outlined in Table 3.1.

Both sites belonged to the farm managed organically prior to the experiment. They both consisted of swards predominantly composed of perennial ryegrass and white clover. Natural fertilisers were applied between 2005 and 2009 in form of farmyard manure, cattle slurry, lime, and rock phosphate. The sites were receiving between 110 and 210 kg N ha^{-1} yr^{-1}. The last farmyard manure application took place in January

2009, three months prior to the start of the experiment. The sites were reseeded between 2005 and 2006 and were grazed every 3-4 weeks until October 2008.

Table 3.1: Soil characteristics of both sites at soil depth 0-10 cm (March 2009). Capital letters in brackets indicate statistically significant differences at p<0.05.

Site	Bulk density		K	Mg	P	pH	Org C	Total N	C : N ratio
	Pre-plough	Post-plough							
I P/D	1.08 (A)	1.11 (A)	61.5 (A)	148.7 (A)	1.8 (A)	6.2 (A)	2.92 (A)	0.29 (A)	10.1 (A)
I W/D	1.21 (A)	1.13 (A)	59.5 (A)	223.2 (A)	1.8 (A)	6.3 (A)	2.71 (A)	0.29 (A)	9.2 (A)

3.2.1. Meteorological data

Rainfall and soil temperature were obtained from Johnstown Castle meteorological station.

3.2.2. Experiment design and management

During this experiment two grassland sites were converted to perennial bioenergy crop cultivation. Each site was divided during the course of the experiment, and planted with *Miscanthus* rhizomes (*Miscanthus* x Giganteus) or sown with Reed canarygrass from seed (*Phalaris arundinacea*). This established four plots in total: poorly drained *Miscanthus*, poorly drained Reed canarygrass, well drained *Miscanthus* and well drained Reed canarygrass (2 ha, 0.8 ha, 2.05 ha, and 0.93 h respectively). *Miscanthus* plots were established in the first year, whereas Reed canarygrass plots in the second year. The plots for Reed canarygrass cultivation were left fallow in the first year. Throughout the experiment N_2O fluxes and ancillary variables were measured from all of them. Later sections of this chapter will describe in detail each type of sample, their collection and analysis. The overview of the applied treatments is shown in Figure 3.2 and the agricultural management practice is described below in Table 3.2.

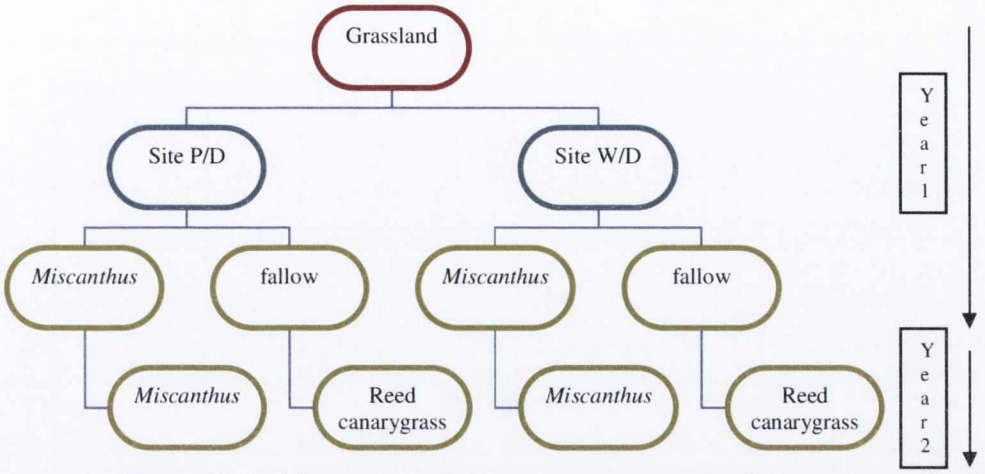


Figure 3.2 Schematics of the treatments applied throughout the land use change experiment.

Table 3.2 Detailed information on agricultural management of the experimental sites.

	Site P/D		Site W/D	
01/04/2009	spraying with Roundup*			
29.04.2009	ploughing with conventional mouldboard and each 3ha site divided into two plots: 1ha and 2ha ; 1ha plots left for the grass to re-grow			
5.06.2009	tillage with power harrow		tillage with power harrow	
9.06.2009	planting *Miscanthus* rhizomes		planting *Miscanthus* rhizomes	
16.06.2009	ring rolling		ring rolling	
13.08.2009	spraying with MPCA**		spraying with MPCA	
18.09.2009		spraying with Roundup		spraying with Roundup
28.09.2009		ploughing with conventional mouldboard		ploughing with conventional mouldboard
4.03.2010	crop topped, biomass left on the field		crop topped, biomass left on the field	
5.03.2010	spraying with Roundup		spraying with Roundup	
15.04.2010		harrowing		harrowing
16.04.2010		sowing with Reed canarygrass at 30kg ha^{-1} followed by rolling		sowing with Reed canarygrass at 30kg ha^{-1} followed by rolling
25.06.2010	spraying with Doxstar***		spraying with Doxstar	
29.06.2010		spraying with MCPA		spraying with MCPA
27.07.2010	spraying with MCPA		spraying with MCPA	
12.10.2010		Harvesting crop		Harvesting crop
4.03.2011	cutting with mower-conditioner set to heavy conditioning; biomass left on the field		cuting with mower-conditioner set to heavy conditioning; biomass left on the field	
7.03.2011	spraying with Roundup		spraying with Roundup	

* N-(phosphonomethyl)glycine

** 2-methyl-4-chlorophenoxyacetic acid

*** triclopyr-2-butoxyethyl ester and fluroxypyr meptyl

3.2.3. N_2O emissions measurements and analysis

N_2O was measured on a weekly basis for two years (April 2009-April 2010 and April 2010-April 2011) with the exception of winter when measurements were taken on a bi-monthly basis. Nitrous oxide fluxes were measured using closed static chambers (Hutchinson and Mosier 1981). These consisted of frames (40 cm x 40 cm, 10 cm deep), evenly distributed on each plot as pictured in Figure 3.3. The frames were manually inserted into the soil, levelled and left to adapt for at least 48 hours prior to the first gas measurement. The frames were left in the soil in the same positions throughout the year with the exception of when plot management required heavy machinery access. The frames were then taken out and put back in place afterwards. To ensure the frames were placed in the same position GPS data was used. At the time of measurement frames were filled with water and covers were placed in the rims to ensure a seal from the atmosphere. Samples were drawn at 0 mins, 15 mins and 30 mins. These sampling times were chosen following a linearity check where samples were drawn every 10 minutes over duration of one hour from five chambers per plot from two plots. Chosen sampling time was shorter than this from chapter 2 due to smaller chamber area. There were between seven and 12 measurements points depending on the plot size: seven in poorly drained Reed canarygrass, eight in well drained Reed canarygrass, 10 in poorly drained *Miscanthus*, and 11 in well drained *Miscanthus*. Samples were collected according to Hutchinson and Mosier (1981) by piercing rubber septum in the top of the chamber with plastic 20 ml syringe with a hypodermic needle (both Becton Dickinson, UK). Samples were then injected into pre-evacuated 7 ml glass vials and analysed by gas chromatography. Detailed information on the method and equipment used in the gas samples analysis can be found in chapter 2, section 2.2.4 and information on N_2O fluxes calculation method in section 2.2.5.

a)

b)

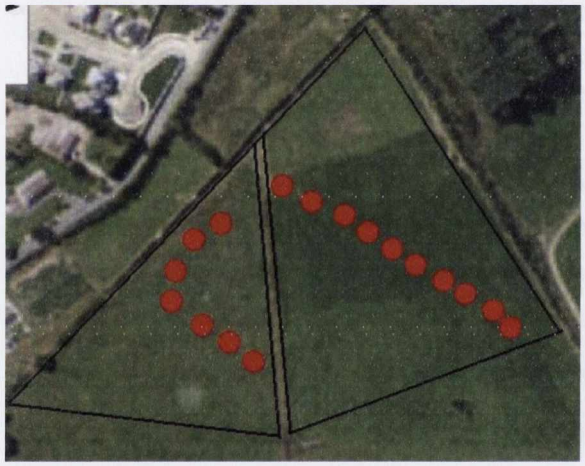


Figure 3.3 Layout of frames positions on the experimental plots recorded with GPS on a well drained site (W/D) (a), and poorly drained site (P/D) (b).

3.2.4. Ancillary measurements

3.2.4.1. Herbage

Herbage samples were taken each winter between January and March and close to the end of the growing season in September. In winter above and below ground biomass was sampled, and in autumn above ground biomass only was sampled in 2010 whereas in 2011 both above and below ground biomass. Above ground biomass was cut with shears from three and five randomly distributed sample points throughout the plots for *Miscanthus* and Reed canarygrass, respectively. Sampling area at the point was marked

with a use of wooden quadrate frame, 2 m x 1.7 m (2.14 m^2) for *Miscanthus* and 0.5 m * 0.5 m for the Reed canarygrass (0.25 m^2). Below ground biomass on Reed canarygrass sites was sampled with a 10 cm diameter corer from five randomly distributed quadrates to a depth of 20 cm. Below ground biomass on *Miscanthus* sites was sampled by excavating the whole rhizome from three randomly chosen locations on throughout the site.

Above ground biomass samples were divided into energy grass, weeds, and in the case of *Miscanthus* also litterfall. Samples were chopped into smaller pieces to facilitate oven drying and dried at 60°C until constant weight. Below ground biomass was washed out of soil, divided between rhizomes and roots, chopped into smaller pieces and oven dried at 60°C until constant weight.

3.2.4.2. Background soil sampling and analyses of soil physical and chemical properties.

Extensive soil sampling was undertaken on the experimental sites between February and March 2009. Sixty six samples were collected from both fields where 40 samples were collected from a poorly drained site and 26 from a well drained site. Samples were collected at two depths: 0-10 cm and 40-50 cm. Parameters measured were: soil bulk density, water holding capacity, texture, pH, P, K, Ca concentrations, C and N contents (Table 3.1).

Soil samples for bulk density and water holding capacity measurements were taken with 251 cm^3 bulk density rings manually driven into the soil and excavated (Eijkelkamp, Agrisearch Equipment, the Netherlands). Water holding capacity was measured on the sand suction table according to standard methodology (BSI, 1999) and the manufacturer's operating procedure and later bulk density was measured using the same samples according to procedure in British Standard (BSI, 2006). Soil texture was analysed in an external laboratory according to its standard procedure (Natural Resource Management Ltd, Bracknell Berkshire, UK). pH was measured in 1:2 suspension of deionised water with a digital pH meter with glass and calomel electrodes. Soil was dried at 40°C before this analysis.

Phosphorus (P), potassium (K) and magnesium (Mg) were analysed after extraction with Morgan's solution (Peech and English, 1944). Top 0-10 cm soil samples were first mixed with Morgan's extracting solution pH 4.8 in 1:5 ratio in round-bottomed shaking flasks, shaken for 30 minutes on a Brunswick gyratory shaker and filtered through Whatman no. 2 filter paper into an Istamec Kasette Track of disposable test tubes. The Kasette Track of extracts was then placed on the automated Istamec transporters for the automated analysis. C and N contents were measured on soils previously dried at 60°C and ground with a ball mill. The analysis was carried out on a TruSpec CN elemental analyser at 950°C where the sample is combusted in oxygen and C converted and analysed as CO_2 and N as N_2.

Another extensive soil sampling was performed in October 2011 where 76 samples were collected at three depths: 0-15 cm, 15-30 cm, and 30-45 cm for texture and C and N analysis. Samples were collected from *Miscanthus* plots (20 sampling points per plot), Reed canarygrass plots (12 sampling points per plot), and adjacent grassland (12 sampling points). C and N contents were measured on a TruSpec CN elemental analyser as described above. Samples from 0-15 cm were used for texture analysis. To reduce the number of samples to analyse adjacent samples were pooled and every second pooled sample was then picked for analysis. Soil texture analysis was performed at the same laboratory as previously.

3.2.4.3. Soil water content and soil temperature

Soil moisture and temperature were measured regularly with WET-2 sensor (Delta-T Devices Ltd, Cambridge, UK). The probe was connected to a HH2 Meter that read and stored measured values. The kit used a standard calibration curve for mineral soil included with the instrument as advised by the manufacturer's manual. Measurements were taken at 0-10 cm depth at the same time as gas samples collection and measurement points corresponded with the chambers positions. Moisture readings were expressed as volumetric water content (%vol) and temperature in Celsius degrees (°C). Soil moisture was then converted into water filled pore space (WFPS%) according to the equation (Aulakh *et al.*, 1991):

$$WFPS\% = 100(\theta v / TPS) \quad \text{and} \quad TPS = 1 - \frac{\rho_b}{\rho_p} \qquad \text{[Equation 3]}$$

Where:

θv – percent volumetric water content

ρ_b – soil bulk density

ρ_p – soil particle density (2.65 g cm^{-3})

3.2.4.4. Soil mineral N content

Soil samples were collected throughout the experiment to monitor available N levels in soil. On seven occasions, a minimum of five randomly distributed replicates were collected from each plot at 0-5 cm after removing vegetation cover. Samples were kept in closed plastic bags in cool conditions and analysed within 24 hours of collection. Ammonium (NH_4^+) and total oxidised nitrogen (TON) (NO_2^- and NO_3^-) concentrations were measured as previously described in section 2.2.8.

3.2.5. Statistical analysis

Statistical analysis was performed in SAS 9.1 statistical package. Cumulative N_2O emissions were calculated by linear interpolation between measured daily fluxes. Skewed data was log transformed prior to analysis. Negative values that were found for N_2O were left in datasets, however if transformation of such datasets was needed, a constant number of a value greater than the lowest value within the dataset was added to each observation to give all data positive values therefore making log transformation possible. PROC MIXED was used to analyse WFPS, soil temperature, mineral N, daily N_2O fluxes time series and cumulative N_2O fluxes. WFPS was analysed separately for each crop to determine effects of drainage and time. WFPS, soil temperature, and daily N_2O fluxes were also analysed to identify significant differences between treatments ($p<0.05$).

3.3. Results

3.3.1. Meteorological data

30-year average mean temperature and rainfall for Johnstown Castle were 10.1 °C and 877.2 mm, respectively. Year 2009 was warmer (+0.5°C) and wetter (142%) than average. More information on summer 2009 can be found in section 2.3.1 of Chapter 2. Year 2010 was colder (-0.2°C) and drier (97% rainfall) than normal. January, February and November were the coldest for at least 25 years, while December was the coldest on record with temperature dropping to -15 °C. May, June and July 2010 were warmer than usual, with end of May and early June being extremely warm whereas August was colder in comparison to 30-year average. May and July were unusually wet months, whereas June and August were relatively dry. Year 2011 was warmer (+0.8 °C) and drier than average (83% of mean rainfall which was the driest in 40 years in Johnstown Castle), however summer months were cooler. In comparison to the mean January was colder and drier, February, March and April were all warmer and drier, to very dry.

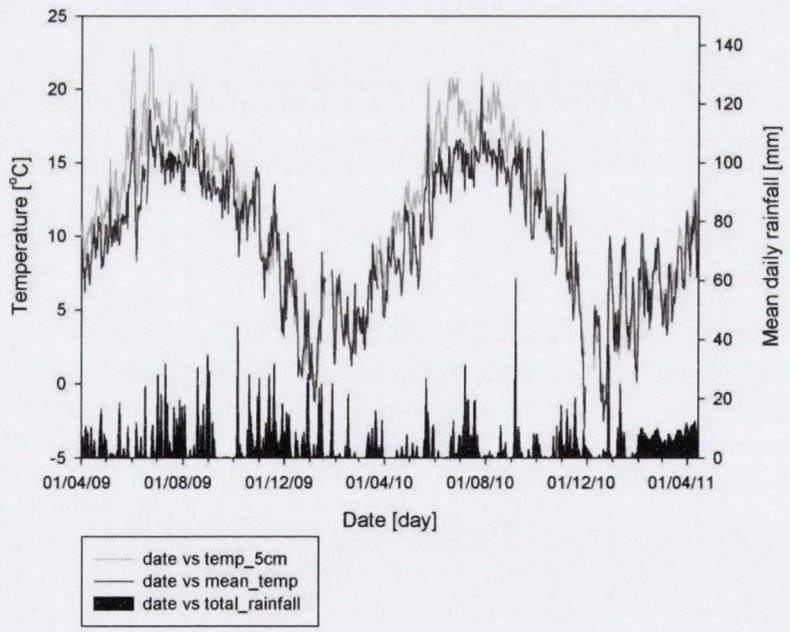
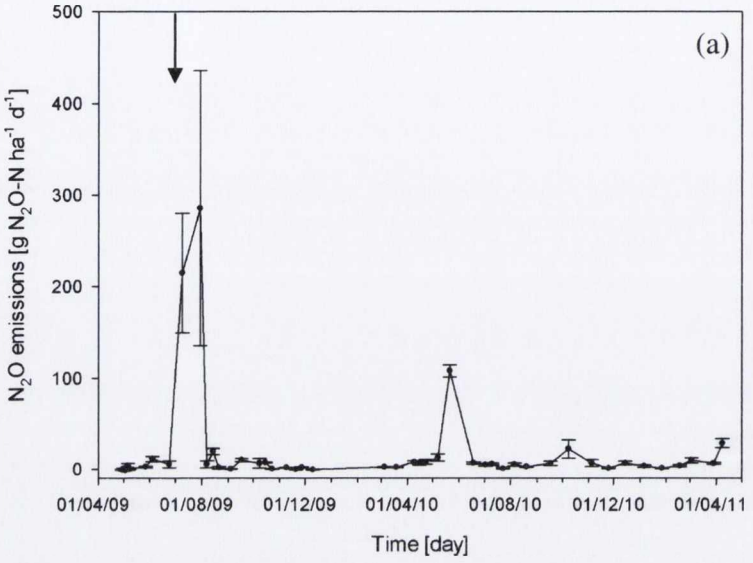
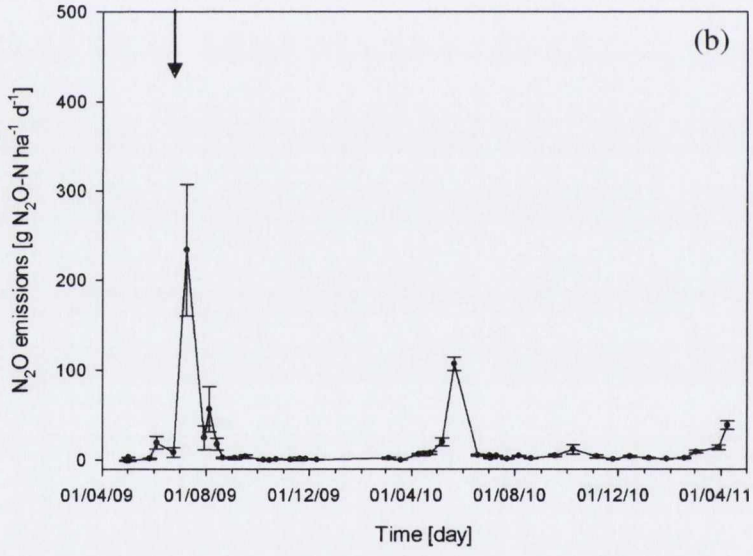


Figure 3.4 Daily rainfall and mean temperature records for 1.04.2009 until 15.04.2011 collected by a meteorological station in Johnstown Castle.

3.3.2. Nitrous oxide

Majority of observed daily N_2O fluxes from the two studied energy crops were low with the daily values not greater than 3 g N_2O-N ha^{-1}, however most of the annual losses were driven by single events as shown in Figure 3.5. In the first year fluxes between 21.06.2009 and 4.08.2009 were responsible for over 85% of the annual N_2O loss from *Miscanthus* W/D plot. The high flux period started 12 days after *Miscanthus* rhizomes were planted therefore they could be linked to that event. Soil disturbance affected mineralisation rate which was additionally enhanced by favourable environmental conditions (mean daily temperature of 15.4 ± 0.18 °C, and mean daily rainfall of 5.9 ± 1.23 mm). In the second year the fluxes between 4.05.2010 and 17.06.2010 contributed over 50% of the yearly N_2O budget.. This distinct peak is clearly visible in Figure 3.5. The peak flux coincided with high temperature and rainfall and the crop's fast growth.


(a)
500
400
300
200
100
0
N_2O emissions [g N_2O-N ha^{-1} d^{-1}]
01/04/09
01/08/09
01/12/09
01/04/10
01/08/10
01/12/10
01/04/11
Time [day]


(b)
500
400
300
200
100
0
N_2O emissions [g N_2O-N ha^{-1} d^{-1}]
01/04/09
01/08/09
01/12/09
01/04/10
01/08/10
01/12/10
01/04/11
Time [day]

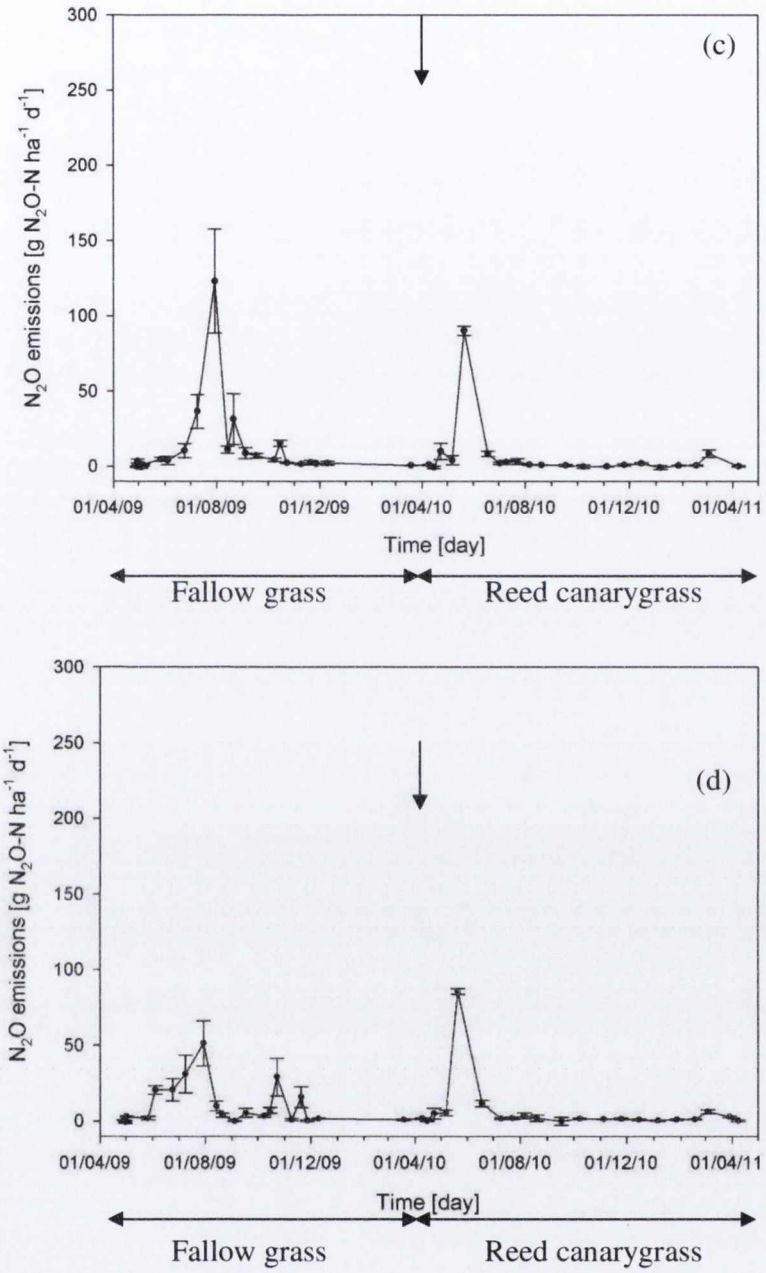


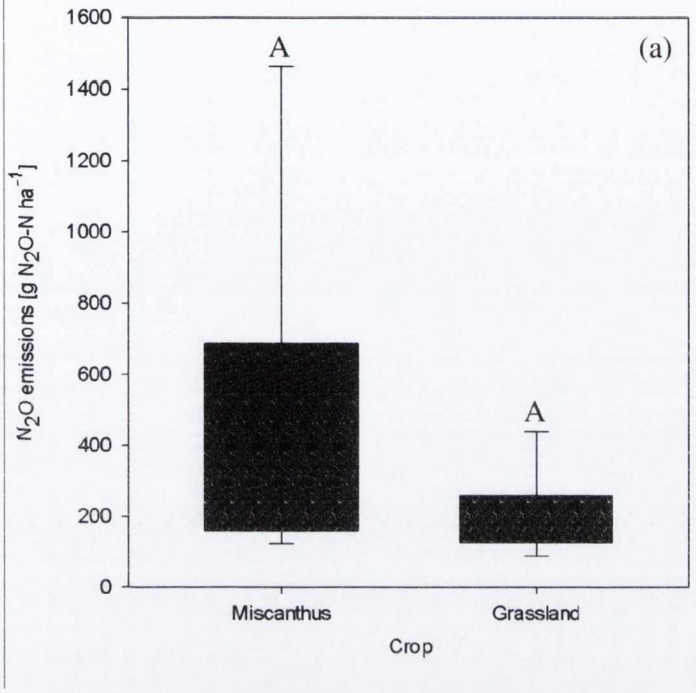
Figure 3.5 Temporal patterns of N_2O emissions (●) throughout the measurement period of the field study from Miscanthus W/D (a), *Miscanthus* P/D (b), Reed canarygrass W/D (c), and Reed canarygrass P/D (d). *Black arrows indicate planting Miscanthus rhizomes in plots (a) and (b), and sowing Reed canarygrass in plots (c) and (d).*

N_2O emissions in the first year of *Miscanthus* cultivation ranged between nearly 6.0 to over 9 kg N $ha^{-1}yr^{-1}$ and in the second year declined to below 5.0 kg N $ha^{-1}yr^{-1}$ as seen in Table 3.3. Fallow grassland produced between approximately 4.0 to over 5.0 kg N $ha^{-1}yr^{-1}$ in the first year, and it was then replaced with Reed canarygrass which losses were approximately 3.0 kg N $ha^{-1}yr^{-1}$ in the second year as shown in Table 3.3.

Table 3.3 Annual N_2O loss from the experimental plots in year one (April 2009-April 2010) and year two (April 2010-April 2011) expressed in kg N_2O-N ha^{-1} measured on 51 occasions from minimum seven chambers per plot. Numbers in brackets indicate standard error of the mean (SE).

	Year 1		Year 2	
	W/D	P/D	W/D	P/D
Miscanthus	9.29 (2.74)	5.77 (1.39)	4.88 (0.40)	4.59 (0.28)
Grassland	5.35 (1.17)	3.83 (0.35)	-	-
Reed canarygrass	-	-	3.10 (0.31)	2.74 (0.09)

Cumulative N_2O-N values in Figure 3.6 are based on measured daily fluxes of N_2O. According to box plots in Figure 3.6 N_2O emissions were not significantly different between *Miscanthus* and grassland treatments in year one ($p>0.05$) however *Miscanthus* and Reed canarygrass treatments differed significantly ($p<0.05$) in year two.



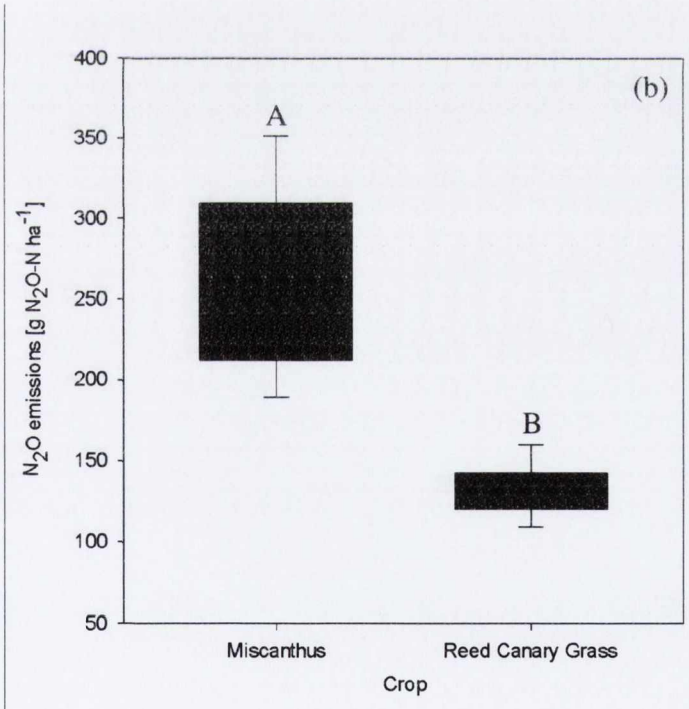
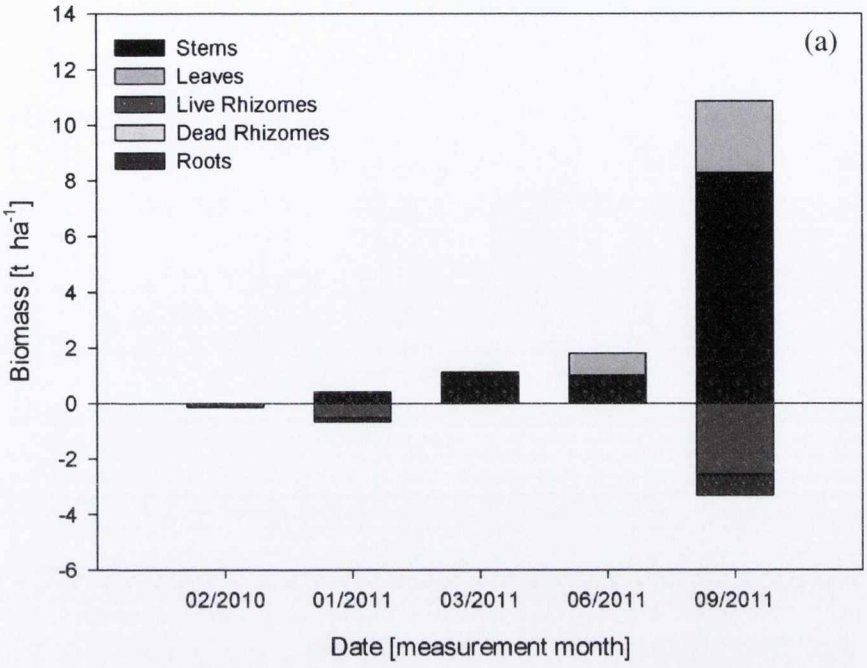


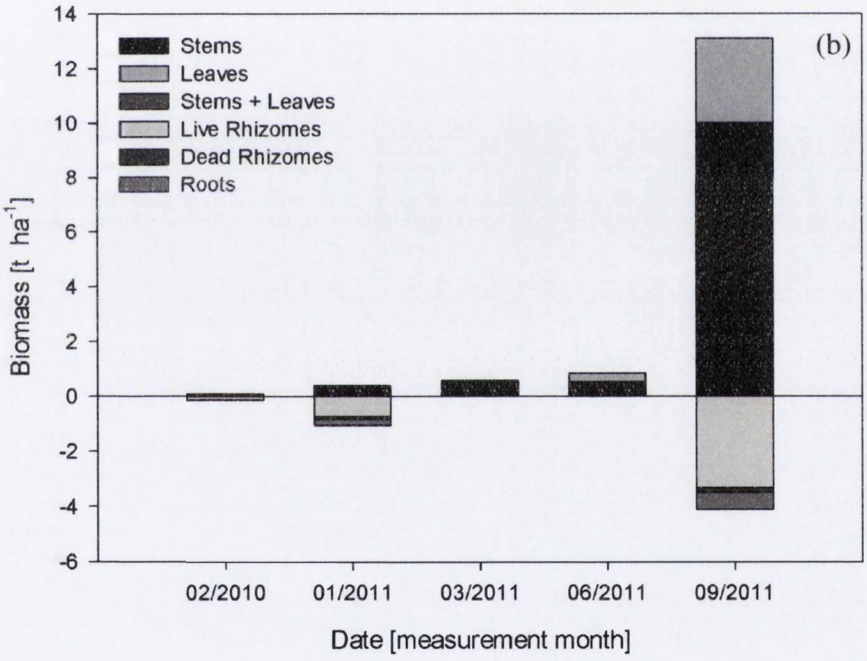
Figure 3.6 Differences between Miscanthus and grassland N losses in year one (a), and between Miscanthus and Reed canarygrass in year two (b) expressed in g N_2O-N. Capital letters in brackets indicate statistically significant differences at $p<0.05$.

3.3.3. Herbage

Figure 3.7 presents details of biomass sampled from both energy crops. *Miscanthus* establishment was slow with the above ground biomass yielding less than 1.0 t DM ha^{-1} on each sampling occasion until September 2011 when it was found to be ten fold higher. At that time *Miscanthus* W/D yielded 10.85 ± 3.88 and *Miscanthus* P/D 13.11 ± 4.65 t DM ha^{-1}. On the first and second sampling date below ground biomass was higher than the above ground in the case of both *Miscanthus* plots however these differences were not significant ($p>0.05$). At the time of final sampling the above ground biomass was higher than the below ground but here again, no significant differences were found between the fractions ($p>0.05$).

Reed canarygrass biomass was measured on five occasions. Unlike *Miscanthus*, this species is ready for harvest every year after the establishment. In the first year it yielded between 6.27 ± 0.63 (P/D site) and 6.53 ± 0.76 t DM ha^{-1} and in the second between 7.06 ± 0.61 and 8.64 ± 0.60 t DM ha^{-1} based on September measurements. At the final measurement both above and below ground biomass was taken. Below ground fraction was somewhat larger however not significantly ($p>0.05$).


(a)
Stems
Leaves
Live Rhizomes
Dead Rhizomes
Roots
Biomass [t ha-1]
14
12
10
8
6
4
2
0
-2
-4
-6
02/2010
01/2011
03/2011
06/2011
09/2011
Date [measurement month]


(b)
Stems
Leaves
Stems + Leaves
Live Rhizomes
Dead Rhizomes
Roots
Biomass [t ha-1]
14
12
10
8
6
4
2
0
-2
-4
-6
02/2010
01/2011
03/2011
06/2011
09/2011
Date [measurement month]

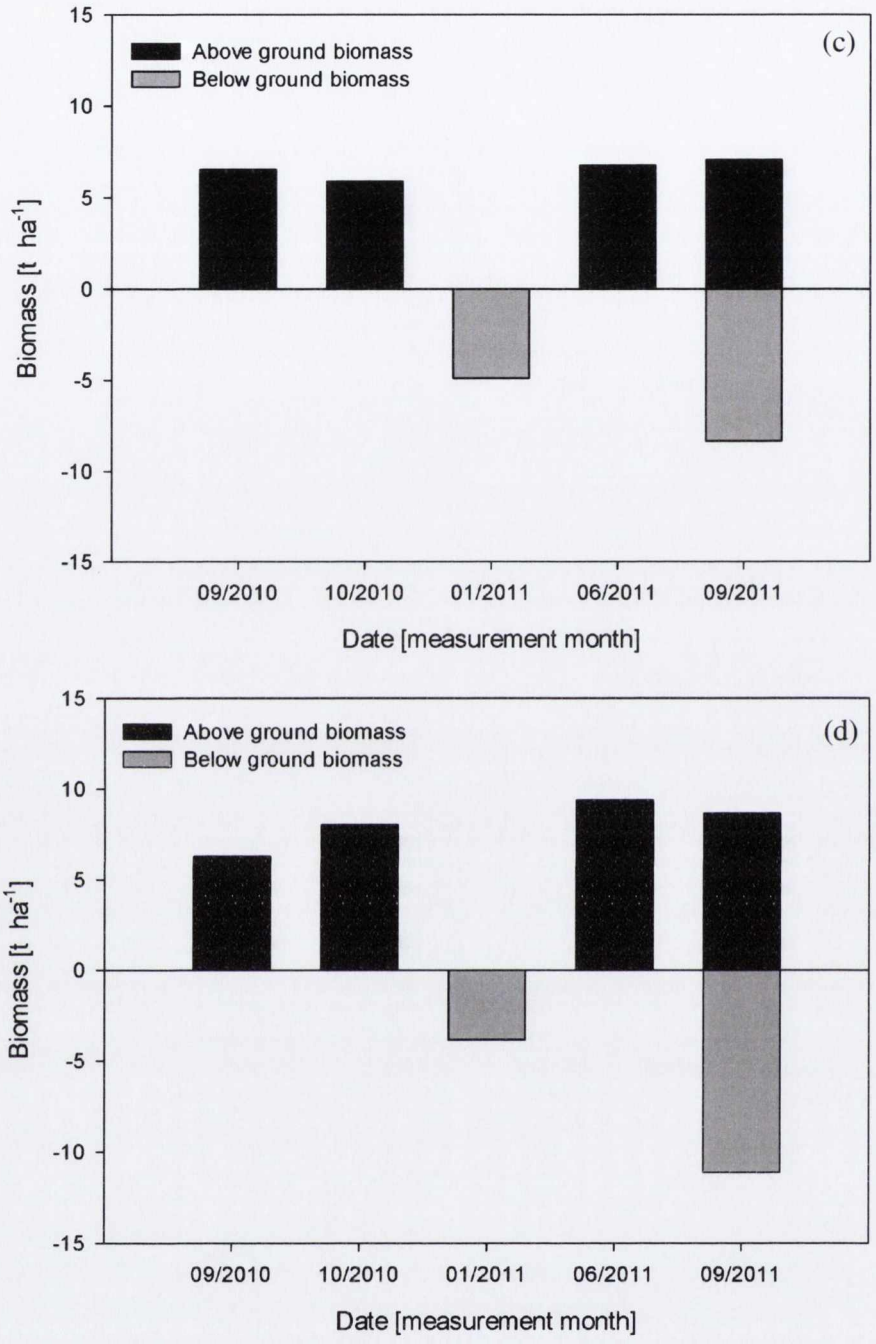


Figure 3.7 Above (positive values) and below (negative values) ground biomass at measurement times from Miscanthus W/D (a), Miscanthus P/D (b), Reed canarygrass W/D (c), and Reed canarygrass P/D (d) . Miscanthus (a, b) above ground biomass was partitioned into stems and leaves, except for Miscanthus P/D (b) in February 2010 where stems and leaves were aggregated into above ground biomass. Miscanthus below ground biomass was assessed on three separate occasions and was partitioned into live and dead rhizomes and roots. In case of Reed canarygrass (c, d) below ground biomass was also assessed on two separate occasions. Sampling in January 2011 took place post-harvesting hence only below ground biomass was assessed.

3.3.4. Background soil sampling and analysis

All the soils were classified as loam according to the USDA classification. No significant differences were found between the sites ($p>0.05$).

Bulk density values across the experimental sites decreased over time however these changes were not significant, there were also no significant differences found between sites ($p>0.05$) as seen in Table 3.4. Decrease of bulk density over time was most likely caused by the management methods, such as the rare use of heavy machinery which minimised compaction associated with its use.

Table 3.4 Bulk density values [g cm^{-3}] at 0-10 cm obtained prior to land use change, following ploughing and in year three. Capital letters in brackets indicate statistically significant differences at $p<0.05$.

	Pre-ploughing	Post-ploughing	Final
W/D *Miscanthus*	1.21 (A)	1.13 (A)	0.98 (A)
W/D Reed canarygrass	1.21 (A)	1.13 (A)	0.97 (A)
W/D Grass	1.21 (A)	1.13 (A)	0.88 (A)
P/D *Miscanthus*	1.08 (A)	1.11 (A)	0.98 (A)
P/D Reed canarygrass	1.08 (A)	1.11 (A)	1.02 (A)
P/D Grass	1.08 (A)	1.11 (A)	0.99 (A)

C and N levels in the examined soils undergoing conversion into energy crops are shown in Table 3.5. Adjacent grassland is an example of the previous land use. No significant differences were found between sites, treatments, and soil depths ($p>0.05$).

Table 3.5 Soil organic carbon and total nitrogen contents [%] obtained in year three at three depths: 0-10 cm, 10-20 cm, and 20-30 cm at both Miscanthus and Reed canarygrass plots and adjacent grassland that did no undergo land use change. Capital letters in brackets indicate statistically significant differences at $p<0.05$.

	Organic carbon (SOC)			Total nitrogen (TN)		
	0-10	10-20	20-30	0-10	10-20	20-30
W/D *Miscanthus*	2.86 (A)	2.58 (A)	1.41 (A)	0.31 (A)	0.27 (A)	0.17 (A)
W/D Reed canarygrass	2.75 (A)	2.47 (A)	1.94 (A)	0.28 (A)	0.25 (A)	0.20 (A)
W/D Grass	2.71 (A)	2.50 (A)	2.05 (A)	0.29 (A)	0.28 (A)	0.24 (A)
P/D *Miscanthus*	3.21 (A)	2.87 (A)	1.52 (A)	0.31 (A)	0.28 (A)	0.17 (A)
P/D Reed canarygrass	2.89 (A)	2.74 (A)	2.24 (A)	0.29 (A)	0.28 (A)	0.23 (A)
P/D Grass	2.92 (A)	2.87 (A)	2.05 (A)	0.29 (A)	0.29(A)	0.21 (A)

3.3.5. Soil water content and soil temperature

Temporal patterns of WFPS in *Miscanthus* and Reed canarygrass plots and the difference between these two crops are shown in Figure 3.8. No significant differences were found between plots with the same crop cover, however WFPS values in the second year attributed to *Miscanthus* were significantly higher that those of Reed canarygrass plots ($p<0.05$) as shown in Figure 3.8 c. According to Figure 3.8 a *Miscanthus* WFPS ranged between 24.30 ± 1.06 and 73.63 ± 1.11% in year one, and between 36.06 ± 2.76 and 61.47 ± 1.54% in year two. According to Figure 3.8 b Reed canarygrass WFPS ranged between 21.61 ± 1.75 and 55.68 ± 2.51%.

Temporal patterns of soil temperature in *Miscanthus* and Reed canarygrass plots and the difference between these two crops is shown in Figure 3.9. No significant differences were found between plots with the same crop cover and between different crop covers as shown in Figure 3.9. *Miscanthus* soil temperature ranged between 3.3 ± 1.1 and 26.6 ± 0.3 °C in year one, and between 11.0 ± 0.1 and 22.2 ± 0.2 °C in year two. It should be noted that the significant difference ($p< 0.05$) in minimum soil temperatures recorded in the first and second year was caused by missing measurements during winter period. Reed canarygrass soil temperature ranged between 10.6 ± 0.1 and 21.0 ± 0.4°C.

The effect of soil moisture (WFPS) and temperature on N_2O emissions was investigated. The large variation in N_2O emission rates due to their nature and associated with disturbed system made the analysis extremely difficult. These difficulties are not unusual in determining the relationship between N_2O and the responsible factors (Flechard *et al.*, 2007). A regression analysis performed in SAS found a weak ($R^2=0.07$) however significant ($p<0.05$) correlation between N_2O emission levels from the experimental plots and the soil temperature.

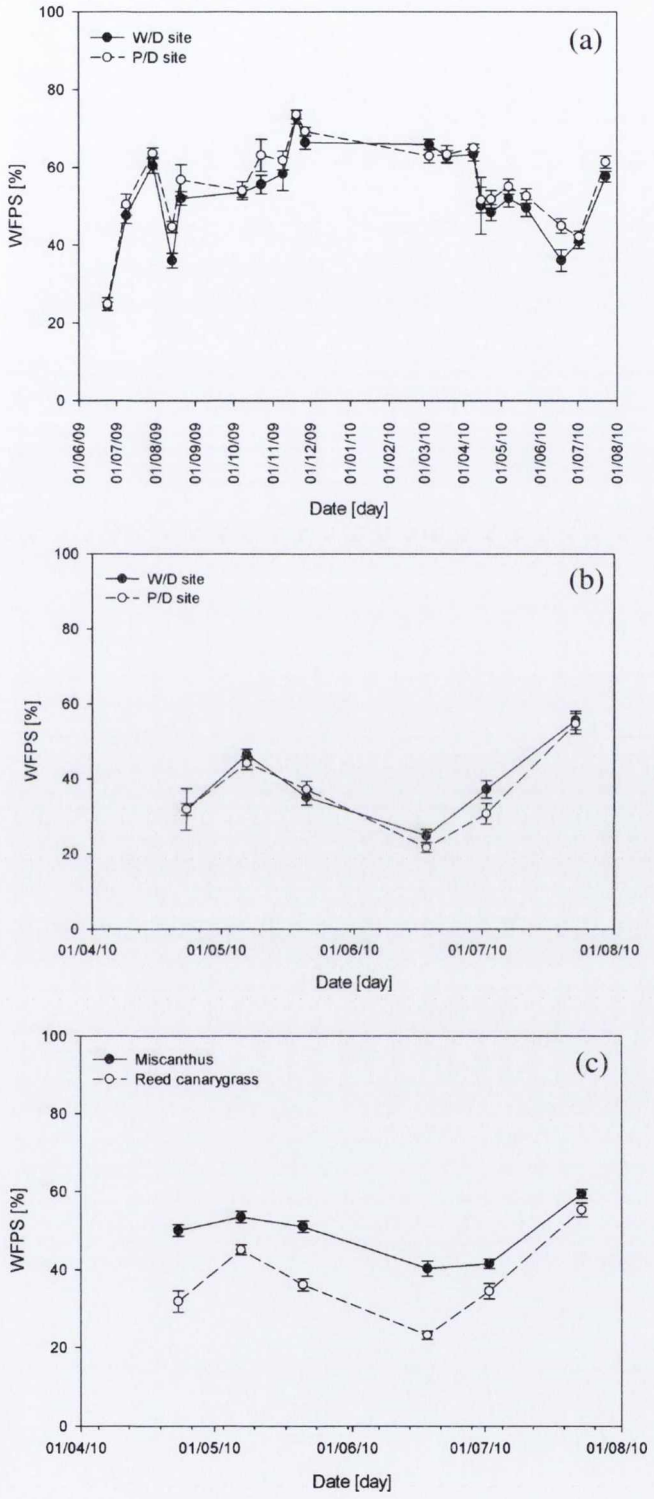


Figure 3.8 WFPS temporal pattern in the experimental plots over two years Miscanthus cultivation on two plots (a), over one year on two plots of Reed canarygrass (b), and a comparison between Miscanthus and Reed canarygrass WFPS values during the second year (c).

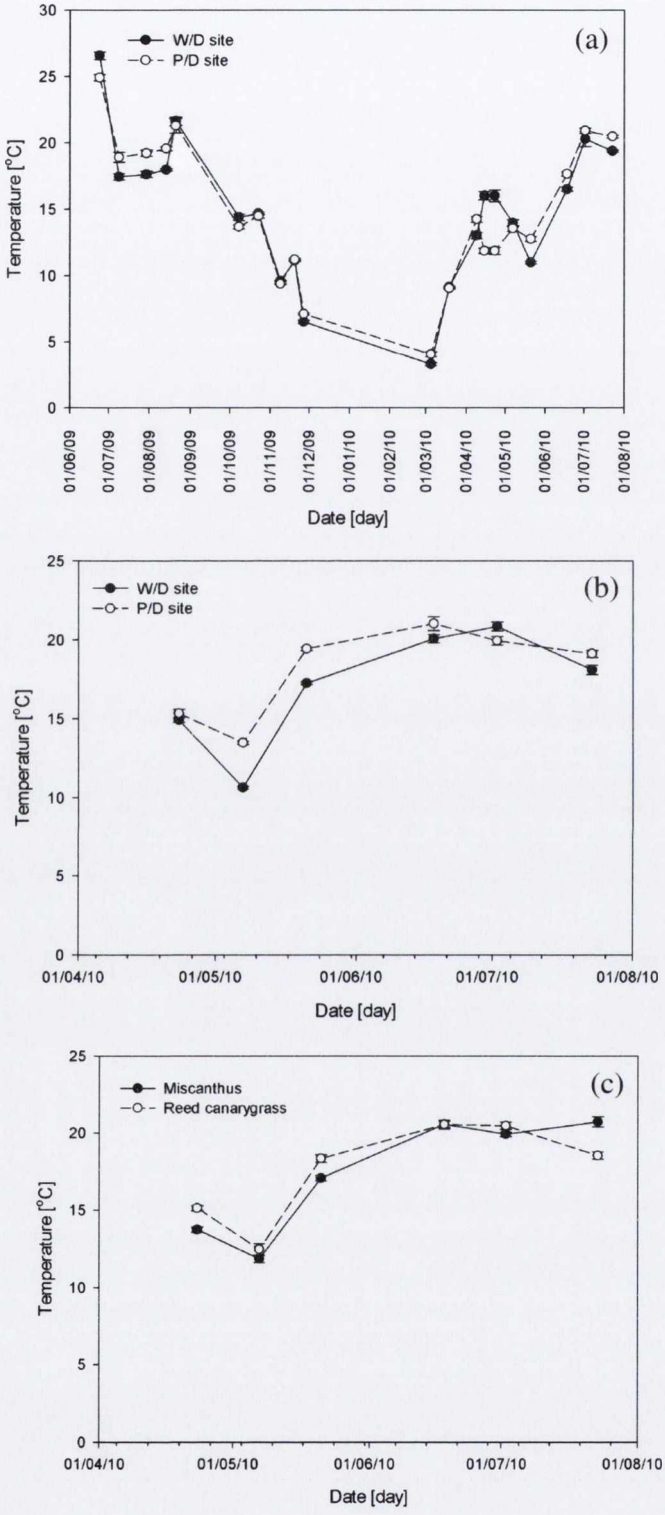


Figure 3.9 Soil temperature temporal patterns in the experimental plots; (a) over two years Miscanthus cultivation on two plots, (b) over one year on two plots of Reed canarygrass, and (c) a comparison between Miscanthus and Reed canarygrass soil temperature values during the second year. Error bars indicate standard error.

3.3.6. Soil mineral N content

Sites used for this experiment were previously a part of the organic farm therefore did not receive any inorganic fertiliser prior to the commencement of the experiment and inorganic nitrogen levels were low, with the majority of the concentrations close to zero. Temporal patterns of ammonium (NH_4^+) and nitrate (NO_3^-) in soils under *Miscanthus* and Reed canarygrass cultivation are shown in Figure 3.10. Nitrate levels in *Miscanthus* soils were both 0.00 mg N kg^{-1} of soil at the first sampling time between the end of February and the beginning of March 2009. Post-ploughing (late April and beginning of May 2009) nitrate levels increased up to 4.34 ± 0.7, and again decreased to 2.38 ± 0.5 mg N kg^{-1} of soil in early August 2009. Nitrate remained at similar levels (between 1.5 and 3.0 mg N kg^{-1} of soil) at the three consecutive sampling occasions until late October 2011 when the levels rose significantly up to between 24.71 ± 5.3 (*Miscanthus* P/D) and 29.04 ± 3.4 (*Miscanthus* W/D). A similar pattern, however not so pronounced, was observed in grassland soils. No apparent pattern was visible in ammonium concentrations. These were low not exceeding 4.0 mg N kg^{-1} of soil until the last measurement date where the maximum found was 5.4 ± 0.3 (Reed canarygrass P/D).

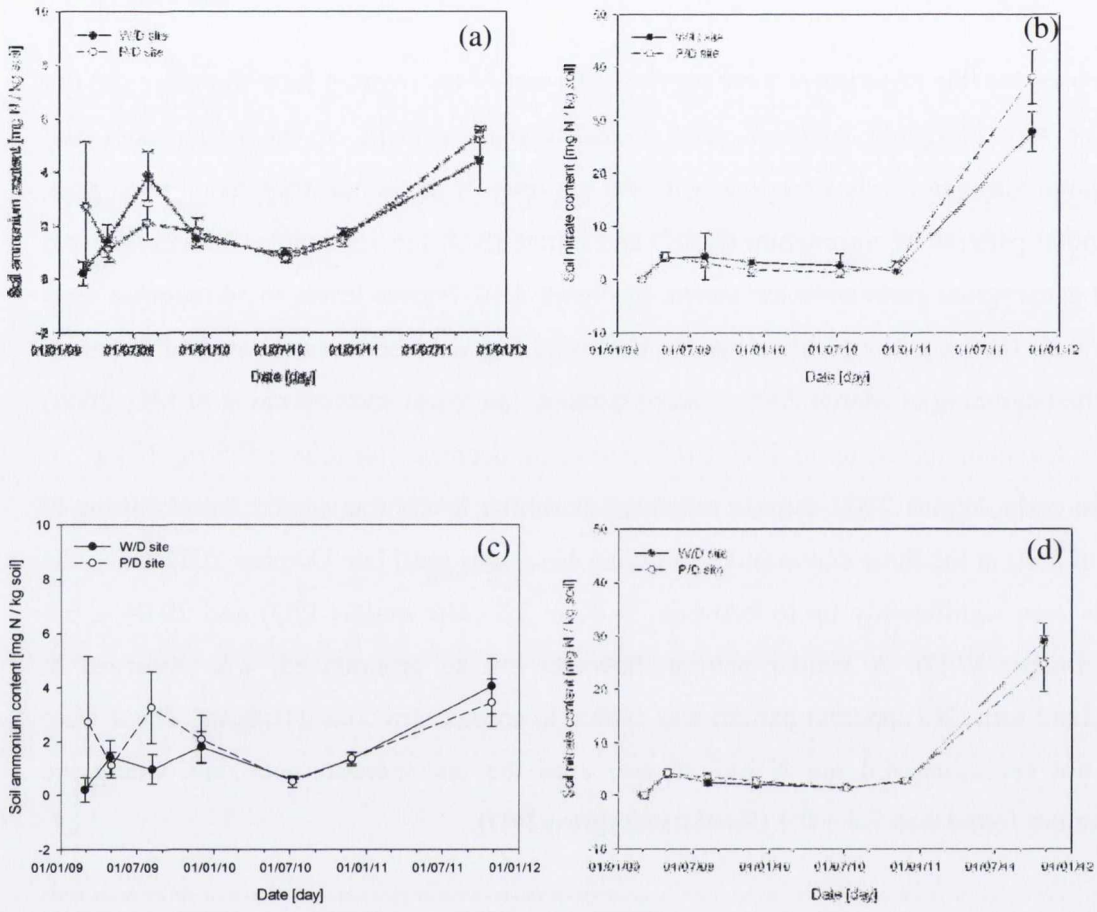


Figure 3.10 Temporal patterns of the concentrations of available nitrogen in the soils of the experimental plots: grass later converted into Reed canarygrass in the forms of ammonium (a) and nitrate (b), and Miscanthus in the forms of ammonium (c) and nitrate (d). Error bars indicate standard error.

3.4. Discussion

3.4.1. Nitrous oxide emissions

Highest N_2O fluxes in this experiment were associated with specific events, mostly soil disturbance and meteorological conditions which is in agreement with Hsieh *et al.*, 2005. In the first year (April 2009-April 2010) of *Miscanthus* cultivation highest N_2O fluxes were observed shortly after planting the rhizomes. This operation disturbed previously ploughed soil providing the microbial community with an opportunity to mineralise large amounts of organic N present in soil from plant residues incorporated by inversion tillage and due to past management. The planting of rhizomes coincided with a period of high temperature (0.6°C higher than the 30-year average), and unusually high rainfall (230% of the 30-year average) which in turn provided microbial community with optimal conditions for utilisation of freshly mineralised nitrogen and explained large N_2O losses. Similar loss, however to a smaller extent, was observed in previously ploughed and left fallow grassland soils.

In the second year (April 2010-April 2011) a similar period of high N_2O fluxes was observed in the case of all the experimental plots, however it took place earlier that year, between May and July. Again, these months were warmer, and May and July also wetter than usual. Fallow grassland plots were sown with Reed canarygrass in mid April and *Miscanthus* was starting to grow vigorously.

N_2O measurements were typically taken on a weekly basis unless sampling could not be performed, therefore commonly used linear interpolation was employed to account for N_2O emissions in between the measurement days and enable calculation of annual losses (Smith and Dobbie 2001; Pennock *et al*, 2006; Petersen *et al.* 2006). Linear interpolation results are usually in good agreement with intensive automated systems measurements however when high fluxes are observed, low temporal sampling resolution can cause over estimation of the actual loss (Smith and Dobbie 2001). It is therefore possible that annual N_2O emissions might be over estimated due to the frequency of measurements at the time

of high fluxes. As these fluxes were not expected (no fertiliser N applied) no intensive sampling campaign was planned.

3.4.2. Comparison between treatments and years

In the first experimental year cumulative N_2O losses from *Miscanthus* and fallow grassland treatments were not significantly different when ploughing both sites was taken into account. N_2O followed a similar temporal pattern in both crops, however more pronounced in *Miscanthus* than in fallow grassland. In the second year fallow grassland was converted into another energy crop-Reed canarygrass. Reed canarygrass exhibited N_2O emissions significantly lower than *Miscanthus* ($p<0.05$).

Throughout the first two years of cultivation *Miscanthus* does not produce enough yield for commercial use. This is due to slow establishment and investing the plant's resources into extensive rhizomatic system. Therefore N_2O emitted per tonne of *Miscanthus* biomass is high. According to Figure 3.11 when N_2O emissions in the first year reached 7.5 kg N_2O-N ha^{-1} (± 1.8 kg N_2O-N ha^{-1}) with average yields of 0.1 t ha^{-1} (± 0.02 t ha^{-1}), the average N_2O emitted per tonne of *Miscanthus* biomass was 106.3 kg N_2O-N (± 48.6 kg N_2O-N). In the second year this measure decreased ten fold to 11.2 kg N_2O-N (± 1.6 kg N_2O-N) with the emissions of 4.7 kg N_2O-N ha^{-1} (± 0.15 kg N_2O-N ha^{-1}) and the yield of 0.4 t ha^{-1} (± 0.01 t ha^{-1}). In the third year the yield was on average 12.0 t ha^{-1} (± 1.1 t ha^{-1}). Assuming decrease of N_2O emissions over time, N_2O flux per tonne of biomass should be substantially lower than in the previous years. In comparison N_2O flux per tonne of the Reed canarygrass biomass was 0.4 kg N_2O-N t^{-1} ha^{-1} (± 0.1 kg N_2O-N t^{-1} ha^{-1}) with 2.9 kg N_2O-N ha^{-1} (± 0.2 kg N_2O-N ha^{-1}) of nitrous oxide and yield at 7.0 t ha^{-1} (± 1.1 t ha^{-1}).

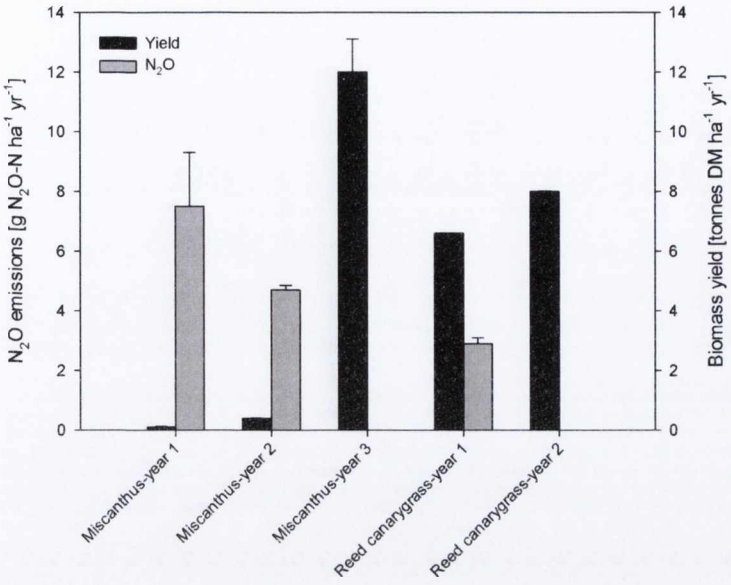


Figure 3.11 Miscanthus and Reed canarygrass yields (black bars) and associated cumulative N_2O fluxes (grey bars) from the three years on Miscanthus cultivation and two years of Reed canarygrass cultivation. No N_2O data is available for the last cultivation year. Error bars indicate standard error.

3.4.3. Other N_2O emissions from LUC into energy crops reported from Ireland and Europe

Article 3 of the Kyoto Protocol drew researchers' attention to energy crops as a greenhouse gas mitigation option. Since then many studies have been carried out on various crops to investigate their biology, climatic adaptation, yields, and processing for energy generation (Beale, 1997; Clifton-Brown *et al.*, 2000; Lewandowski, 2000; Lewandowski, 2003; Clifton-Brown, 2004; Heaton, 2004; Tuck, 2006; Adler, 2007; Styles, 2007; Heaton, 2008; Karp, 2008) however little is known about greenhouse gas emissions related to the cultivation of these crops, especially trace gas fluxes like N_2O. Results of numerous reviews and life cycle analyses (LCA) are available however these approaches do not use measured gas data, but rather modelled values or IPCC emission factors methodology to estimate soil emissions (Schlamandinger, 1997; Adler, 2007; Styles, 2007a; Styles, 2007b; Styles, 2007c; Styles, 2008; St. Clair, 2008). This creates

high uncertainty of the LCA results associated with growing feedstock (Styles, 2007; St. Clair, 2008).

This experiment's results can be usefully compared to those of Roth *et al.* (2011), and Drewer *et al.* (2012) although soil type and climatic conditions during study years varied. Roth *et al.* (2011) at a research site in Carlow (sandy loam soil), Ireland, recorded cumulative N_2O fluxes of 0.6 ± 0.3 kg N_2O-N ha^{-1} yr^{-1} and 0.34 ± 0.1 kg N_2O-N ha^{-1} yr^{-1} from newly established, and mature *Miscanthus*, respectively. Drewer *et al.* (2012), in Lincolnshire (fine loam over clay soil), England, recorded total loss of 152 kg CO_{2eq} ha^{-1} yr^{-1} which corresponds to approximately 0.3 kg N_2O-N ha^{-1} yr^{-1}. Results from the current study are substantially higher than any of the above experiments. This suggests that more measurements covering the main climate and soil variables are needed. However, it should be noted that the Carlow site is characterised by lighter, sandy soils and which could explain low N_2O emissions in comparison to the results in this thesis. In the Lincolnshire study *Miscanthus* was established in 2005 and the gas measurements commenced in mid 2008 possibly missing high N_2O fluxes associated with land preparation and plant establishment phases.

3.4.4. The effect of land use change on soil conditions

Nitrogen and carbon soil contents were, together with bulk density, used as indicators of soil quality following grassland conversion into bioenergy crops cultivation. Land use change is often associated with soil disturbance and is recognised as responsible for soil organic matter losses and greenhouse gases emissions (Guo and Gifford, 2002). However, in this case the above indicator did not prove negative changes in soil conditions following the conversion. None of the parameters changed significantly during the two years of the experiment. However small changes in bulk density can be explained by the management with the use of heavy machinery reduced to a minimum. There were also no significant changes between soil carbon and nitrogen content after the LUC. Both C and N contents were the highest in the top 0-10 cm soil layer and decreased with depth, more so in *Miscanthus* than in Reed canarygrass and grassland, however these differences were not statistically significant ($p>0.05$).

Soil available nitrogen levels were low in the examined soils throughout the experiment, except for the last sampling occasion when the ammonium and nitrate concentrations rose significantly in all the plots. Low mineral nitrogen levels combined with high N_2O emissions following land use change in year one can be explained by high mineralisation rates (fast turnover) and rapid utilisation of mineralised N. However, on an experiment time scale, only labile N was changing while more stable N fractions were retained in the soil as suggested by the soil N content described above.

3.5. Conclusions

Very few studies have measured nitrous oxide fluxes from energy crops, especially throughout their early establishment phase and therefore data available for comparison is scarce. In this experiment large, however not significantly larger, N_2O fluxes were associated with land use change from grassland to *Miscanthus* in the first year of the conversion with emissions decreasing in the second year of cultivation. N_2O emissions after conversion into Reed canarygrass were lower than these of the previous year's fallow grassland.

Cumulative N_2O emissions were similar for *Miscanthus* and fallow grassland (first year), however the two examined dedicated bioenergy crops differed significantly with *Miscanthus* emissions larger than those of Reed canarygrass (second year), which most likely was the result of different plant biology. These crops utilises different photosynthesis pathways with *Miscanthus* being a C4 grass and perennial ryegrass and Reed canarygrass C3 grasses which can cause differences in water and N use efficiencies and therefore affect the plant N uptake, N_2O fluxes and leaching. The two crops have contrasting plant morphology and growth strategies, and very different rooting systems, with *Miscanthus* characterised by a longer establishment phase and investment in below ground biomass in the first three years of cultivation, which may affect soil conditions and the soil microbial community in various ways. Therefore the known factors influencing N_2O such as meteorological and soil physical and chemical properties which were similar for both treatments can produce different response in these two species. These conditions however, together with soil disturbance due to management operations carried out in the field and land management history facilitated N mineralisation leading to high gaseous

losses. These losses were high in comparison to unfertilised fallow grassland from this study as well as in Hyde *et al.*, (2006) who recorded 4.21 kg N ha^{-1} yr^{-1} from the same soil type at a site in Wexford but much lower in comparison with fertilised grassland (11.6 kg N ha^{-1}) from the study of Hsieh *et al.*, 2005. Typically, Irish grasslands receive substantial amounts of fertiliser N and are major source of N_2O (Mishurov, 2010) therefore highly N efficient energy crops that require little or no fertilisation might prove beneficial in terms of future GHG emissions. Cumulative annual N_2O emissions from *Miscanthus* decreased by approximately 40% between the first and second cultivation year therefore further decreases can be expected in future, possibly to levels quoted by Roth *et al.*, 2011, and Drewer *et al.*, 2012. However newly established crops are characterised by high N_2O fluxes, on mature short rotation coppice (SRC) plantations for bioenergy production N_2O emissions are, on average, reduced by 64% in comparison with conventional annual crops (Don *et al.*, 2012). Extensive no-till systems like those of perennial energy crops can reduce N losses by avoidance of annual ploughing and subsequent tillage-induced N mineralisation and through low fertilisation rates as well as through their effect on soil microbial community structure.

Miscanthus is slow in establishment and only produces enough harvestable bioenergy from the third year onwards. N_2O emissions from soil during the establishment of the crop are comparable to those of intensive grassland. However mature crop produces consistently high yields associated with low N_2O fluxes per unit bioenergy produced (Roth *et al.*, 2011). Reed canarygrass on the other hand produces viable yields of bioenergy in its first growth season while emitting significantly less N_2O than *Miscanthus* or intensive grassland. However its life cycle is shorter than that of *Miscanthus* therefore more frequent tillage is required for another crop establishment. Additionally, mature *Miscanthus* produces more biomass than mature Reed canarygrass. It is impossible to simply conclude which candidate crop for bioenergy production is preferable based solely on two years of measurements. As the two crops differ significantly, their yields and the costs of production of the bioenergy should be assessed throughout their life cycle.

Former land use is of critical importance in energy crop cultivation to ensure that the conversion does not enhance GHG emissions (St. Clair, 2008). The most probable land use for conversion into energy crops in Ireland would be at the expense of grassland since 90% of agricultural land area is utilised for grass (EPA, 2011). Conversion of grassland

may lead to high N mineralisation and subsequent N_2O emissions as well as to C loss from soil making such land use change undesirable (Estavillo *et al.*, 2002; Rowe *et al.*, 2009). Ideally, only land under cultivation or that has been cultivated before should be used for energy crops since any conversion of native vegetation or perennial grassland will not be GHG neutral (Styles, 2008; Don *et al.*, 2012).

Energy crops are a viable option for climate change mitigation however their GHG balance depends on many factors such as crop type, climatic and soil conditions, soil management and previous land use. Therefore policy constraints should be employed to regulate future land use change for bioenergy.

Chapter 4: ^{15}N stable isotope study on N cycling processes in plant-soil systems under grassland and energy crop covers: Miscanthus and Reed canarygrass. The effect of crop cover and soil properties on N processes.

4.1. Introduction; objectives and hypotheses of the experiment

Perennial bioenergy crops are believed to have a high biomass potential production with low N inputs (Lewandowski and Schmidt, 2006). Indeed, *Miscanthus* may require little or no fertilisation at all (Himken *et al.*, 1997; Boehmel *et al.*, 2008). *Miscanthus* is said to respond to N fertiliser application only after the establishment stage (Migues *et al.*, 2008). However it is very probable that energy crops will be fertilised in future to maximise yields and that this can significantly affect their GHG balance (Teagasc, 2008; Drewer *et al*, 2012; Don *et al.*, 2012).

The fate of fertiliser N applied to energy crops is influenced by numerous environmental factors, soil characteristics and plant biology. *Miscanthus* fertilised with 60 kg N ha^{-1} has been shown to utilise between 38% (1yr old plants) and 65% (3yr old plants) of applied N (Christian *et al.*, 2006). The remainder is either sequestered in the soil (between 19% and 26%) or lost via various pathways (Christian *et al.*, 2006). On the other hand, the N recovery rates in Reed canarygrass are between 42% and 68% in shoots, and between 2% and 6% in roots, which is comparable with grassland and crops like winter wheat, oil seed rape, and sugar beet (Partala *et al.*, 2001). Fertiliser, not immediately utilised by plants or immobilised by soil microbial community, may be lost from the system either through nitrate leaching or gaseous emissions of which nitrous oxide is of major interest due to its role as a greenhouse gas. The amount of N_2O and particularly the proportion of N_2O to N_2 emitted as a result of fertiliser application varies significantly between crops, climatic and soil conditions (Li *et al.*, 1996; Dalal *et al.*, 2003; Flynn *et al.*, 2005; Abdalla *et al.*, 2009). The IPCC default emission factor of 1% can be extremely inaccurate therefore research is needed to better predict fertiliser responses specific to crop, soil type, and climate (IPCC, 2006). Low emission factors, together with low fertilisation rates, are desirable characteristics in commercially farmed crops because they lower both the economic and environmental costs of yields.

N_2O is produced in soil mainly through nitrification and partial denitrification. Both these processes are largely controlled by soil water content and soil texture with denitrification-derived N_2O increasing with increasing WFPS and soil texture becoming finer (Weier *et al.*, 1993; Parton *et al.*, 1996; Bollman and Conrad, 1998; Bateman and Baggs, 2005). The

final product of denitrification is the benign atmospheric gas N_2, however the reduction of N_2O to N_2 requires certain conditions of soil moisture, pH, temperature, soil redox conditions and C supply, and hence more often both N_2 and N_2O are produced simultaneously at a specific N_2:N_2O ratio (Firestone and Tiedje, 1979; Ruser *et al.*, 2006; Liu *et al.*, 2007; Cuhel *et al.*, 2010). The denitrification of nitrate from applied fertiliser thus consequently increase both N_2 and N_2O emissions with the balance between partial and complete denitrification dependent principally on soil redox potential (Smith *et al.*, 1998; Dobbie and Smith, 2003; McSwiney and Robertson, 2005).

Soil type, drainage, climate and crop management influence the main pathways of N losses with nitrate leaching becoming more significant in free draining soils (Wang and Alva, 1996; Wu *et al.*, 1996). Intensively cultivated free draining soils are vulnerable to nitrate leaching, however these losses can be reduced with improved agricultural practices (Gheysari *et al.* 2009; Constantin *et al.*, 2010). Fertiliser introduces available N to the soil which can easily be lost from the system through nitrate leaching (Bergstrom and Johansson, 1991; de Vries *et al*, 2006) therefore fertiliser management, in particular weather conditions during fertilisation, and matching demand with supply, are of great importance in minimising these losses. Some studies provide evidence that perennial energy crops like *Miscanthus* can significantly reduce nitrate leaching from soil hence improving water quality (Christian and Riche, 1998).

In order to elucidate N cycling in biomass crops, a ^{15}N tracing study was carried out. ^{15}N stable isotope is a useful tool to obtain information about the fate of the applied N with regard to what fraction is taken up by plant, and how much is lost via which processes depending on factors like plant biology, soil type, soil water filled pore space, and soil temperature (Baggs, 2008; Rosenkraz *et al.*, 2005). In this experiment two differently labelled ammonium nitrate fertilisers were used; ^{15}N-ammonium with unlabelled nitrate, and ^{15}N-ammonium with ^{15}N-nitrate. $^{15}N_2O$ is created in soil through both nitrification and denitrification of labelled ammonium and nitrate whereas $^{15}N_2$ emitted after fertilisation comes from denitrification after either labelled ammonium or nitrate application. N uptake in plants, leaching and N retained in the soil are assessed along with N_2O and N_2 emissions (Arah *et al.*, 1991; Arah, 1997; Stevens *et al.*, 1997).

The experiment was carried out in controlled environmental conditions in order that plant and soil type were solely responsible for responses to treatments. Grassland, *Miscanthus*, and Reed canarygrass on poorly or well drained soils were fertilised with ^{15}N labelled fertiliser and the dilution technique employed to follow all the main pathways of N movement through crop-soil systems.

The aim of this part of the study was to investigate N processes under energy crops using ^{15}N stable isotope methodologies. Six objectives of the study are listed below:

Objectives and hypotheses of the study:

1) To quantify the fate of applied N in biomass cropping systems and specifically to characterise the size and extent of the various N loss pathways (N_2O and N_2 emissions and N leaching) following N fertilisation. The hypothesis was that application of fertiliser N is likely to enhance N_2O emissions and concentration of N in drainage; and affect rye grass and energy grass systems to a different extent. Commonly only 40-60% of applied N is utilised by plant and the remaining N is lost from the system through various pathways.

2) To assess the effect of plant and soil type on N cycling. The hypothesis was that poorly drained soils are expected to produce more gaseous losses complimented by leachate losses whereas well drained soils are bound to remove more N from the system through leaching, and only a small portion through gases.

3) To identify the main processes responsible for N_2O emissions in the studied systems; whether it is nitrification or denitrification. The hypothesis was that poorly drained soils are more likely to create N_2O denitrification whereas well drained soils might do so through nitrification.

4) To measure N_2 emissions to assess the N_2:N_2O ratio from soils of contrasting drainage properties. The hypothesis was that poorly drained, wetter soils are expected to be characterised by higher N_2:N_2O ratio than drier, well drained soils and remove N through inert N_2 rather than through potent GHG- N_2O.

5) To assess plants N uptake rates by measuring ^{15}N content in above and below ground plant material. The hypothesis was that different plant cover is expected to affect N uptake and N losses, with fast growing energy grasses using supplied N more efficiently.

6) To measure the fraction of applied N that remains in soil after fertiliser application. The hypothesis is that inorganic N, especially nitrate is mobile and cannot be stored in soil. It is either leached from the system or utilised by soil microflora therefore the majority of ^{15}N found is soil should be bound into microbial biomass.

4.2. Materials and methods

The experiment was conducted in the Teagasc Johnstown Castle Research Centre between August 2010 and March 2011. Soil lysimeters containing various crop-soil systems were used in this experiment. Two different ^{15}N fertiliser treatments were applied to the lysimeters. Post-fertilisation gas, above and below ground biomass, soil and leachate samples were collected in order to measure the uptake and losses of the applied ^{15}N and quantify their magnitude in each of these sampled fraction.

4.2.1. Sites and plant selection

Three grass types and two soil drainage types were chosen for the experiment. Grasses included two energy crops: *Miscanthus* and Reed canarygrass, and rye grass, whereas soils included poorly and well draining soils. A combination of the three grasses and two soil drainage types resulted in six different crop-soil systems. However, *Miscanthus* based on well drained soils did not survive the excavation of the soil cores and treatments based on this crop-soil system had to be excluded from the experiment. Lysimeters were prepared with nine of each of the following crop-soil systems resulting in the total of 45 lysimeters. Three replicates of each crop-soil system were used for control, $NH_4{}^{15}NO_3$ fertiliser treatment, and $^{15}NH_4{}^{15}NO_3$ fertiliser treatment; this can be seen in Table 4.1.

Table 4.1 Crop-soil systems utilised in the experiment.

Treatment	Plant / Soil drainage					
	Poor	Poor	Good	Poor	Good	
Control	*Miscanthus*	Perennial grassland		Reed canarygrass		
$NH_4{}^{15}NO_3$	*Miscanthus*	Perennial grassland		Reed canarygrass		
$^{15}NH_4{}^{15}NO_3$	*Miscanthus*	Perennial grassland		Reed canarygrass		

Soils were chosen according to the plant cover and drainage properties from the soils available in Teagasc Johnstown Castle, Wexford and Teagasc Oak Park, Carlow, properties of the soils used in the experiment are shown in Table 4.2 below. Comparison of the grasses used for bioenergy with rye grass in terms of N cycling was of interest and possible differences in N cycling due to soil drainage properties.

The collection and preparation of the soil lysimeters for the experiment is described in detail in sections 4.2.2-4.2.4. Additionally, bulk density, and available nitrogen were measured in top soil (0-5cm). More information on bulk density and available mineral nitrogen analysis can be found in sections 3.2.4.1., and 2.2.8., respectively. Literature values of the soil porosity from Rowell, 1994 were used to describe well and poorly drained soils used in the experiment.

Table 4.2 Soil core properties of the lysimeters.

Soil drainage	Plant	Origin	Bulk density	Porosity	NH_4^+	NO_3^-
Poor	Rye grass	Wexford	1.18	55.5%	58.92 (11.72)	-
Good	Rye grass	Wexford	1.31	50.5%	58.92 (11.72)	-
Poor	Reed canarygrass	Wexford	1.18	55.5%	2.51 (0.62)	-
Good	Reed canarygrass	Carlow	1.06	60.0%	2.06 (0.26)	4.91 (0.54)
Poor	*Miscanthus*	Wexford	1.18	55.5%	2.51 (0.62)	-

4.2.2. Design of lysimeters

Lysimeters used in this experiment were designed according to Figure 4.1. A lysimeter consisted of a PVC tube approximately 43cm long and 15cm diameter (Figure 4.2), and a matching stopper at the bottom. A 7mm hole was drilled in each lysimeter bottom and plastic drainage tubing fitted and sealed with technical silicone. The hole was covered by a square piece of metal mesh glued to the inside of the bottom and then 2-3cm gravel layered on top of it. A special heavy-duty metal cutting ring was designed in order to

remove intact soil monoliths. The ring was sharpened at one end so that it could be pushed to the ground with a digger and its diameter was big enough to contain a PVC casing for the lysimeter inside the ring.

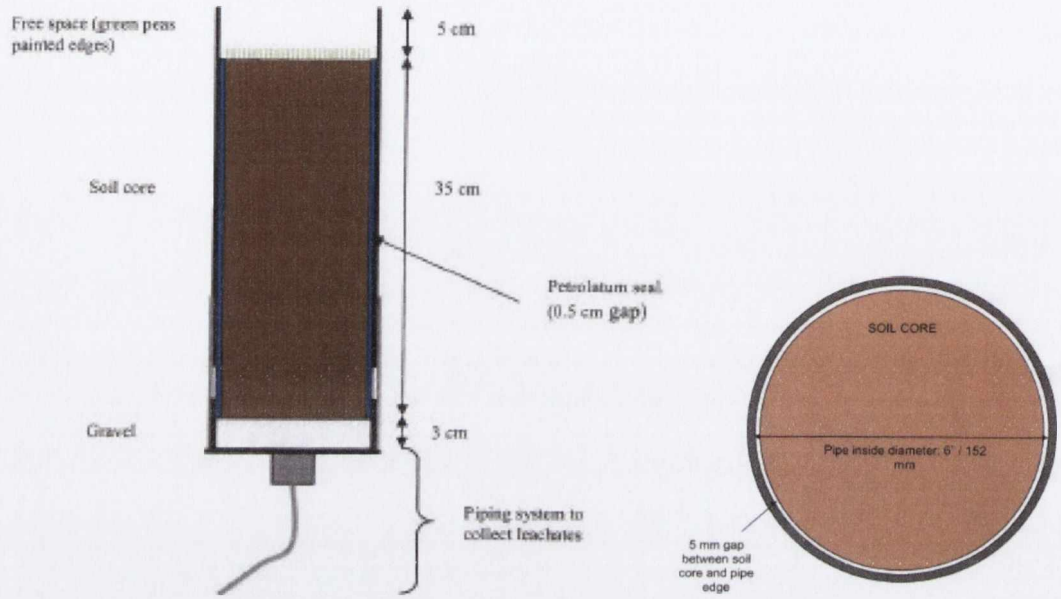


Figure 4.1 Design of the lysimeters used in the experiment together with their dimensions.

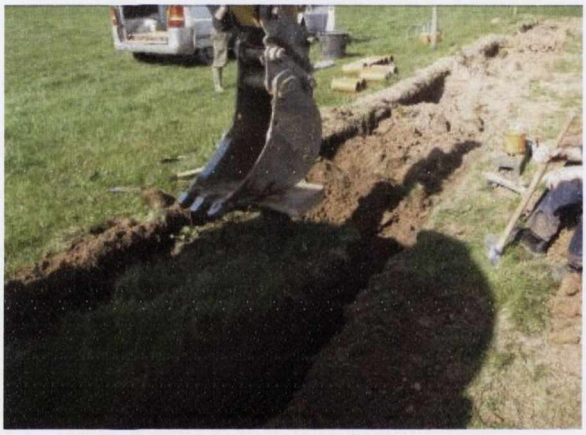
Figure 4.2 The PVC pipes cut to desired length and sharpened at the bottom.

4.2.3. Lysimeter collection

Lysimeters were collected by encasing intact soil cores. Firstly, two trenches were prepared approximately 1-1.5m away from each other using a digger and later the soil cores harvested from the soil between the trenches using the metal cutting ring (Figure 4.3). The metal cutting ring was designed in a way to contain the PVC pipe inside so that when the ring was pushed into the ground a soil core was encased by the pipe. The pipe with the soil inside was then carefully excavated, taken out and trimmed of excess soil. A plastic stopper was prepared by placing a piece of metal mesh with a small amount of silicon sealant over the tubing outlet and covering the bottom of the stopper with a layer of gravel (Figure 4.4). The pipe was then carefully placed over the stopper and when in the exact vertical position, pushed tightly inside to the bottom of the stopper.

a)

b)

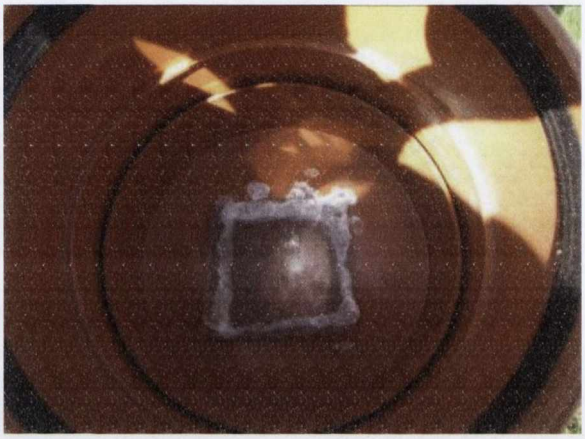


c)

Figure 4.3 Excavation set-up consisting of two trenches (a), pushing the cutting ring into the soil by the digger (b), hand digging to take out a full soil core in the PVC pipe enclosed inside the cutting ring (c)

a)

b)



c)

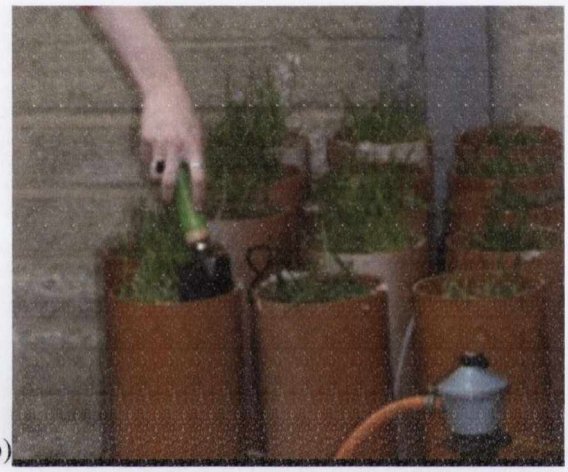
Figure 4.4 Assembly of the stoppers (a), and placing the metal mesh inside the stopper (b). Covering the mesh with a layer of gravel (c).

4.2.4. Sealing the lysimeters

The soil cores diameter was approximately 1cm smaller than the inside diameter of its plastic casing creating a small gap. After collection these gaps were sealed with white petrolatum in order to prevent preferential flow of water down the sides of the lysimeter casing (Figure 4.5). The white petrolatum was slowly heated until melted and then slowly poured into the lysimeters around the soil core edge to fill the gap between the soil and the pipe wall (Figure 4.5 b). Sealed lysimeters were left to adapt while the petrolatum cooled down and solidified (Figure 4.5 c). After a few days the lysimeters were ready to transport and be set up in the controlled environment chamber when the experiment was conducted.

a)

b)



c)

Figure 4.5 Lysimeters prepared for sealing and the equipment used for heating the petrolatum (a), trowel used for precise application of liquidised petrolatum (b), and freshly sealed lysimeter with petrolatum solidifying at the edges of the soil core (c).

4.2.5. Growth room set up

The experiment was conducted in a controlled environment growth room (TAS, Temperature Applied Sciences Ltd., Goring-by-Sea, UK). The programmed growth conditions are presented in Table 4.3 The lysimeters were set up inside the chambers on 6.07.2010 on three shelves according to the treatment to be applied: control, $^{15}NH_4NO_3$ fertiliser, and $^{15}NH_4{}^{15}NO_3$ fertiliser. Each lysimeter had an individual marked leachate container placed under the shelf with the appropriate tubing put inside (Figure 4.6).

Table 4.3 Set up conditions of the programme manipulating environmental parameters inside the growthroom.

Phase	Phase length	Temperature	PAR (light intensity)	Humidity
Night	8h	14°C	0%	50%
Day	16h	20°C	100%	50%

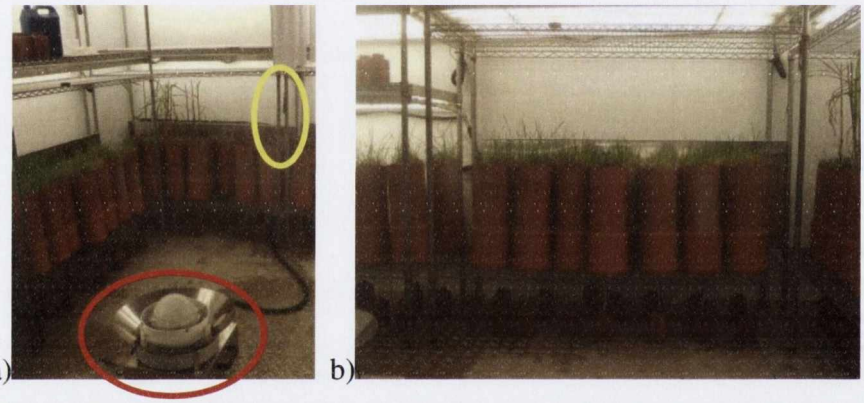


Figure 4.6 Temperature and humidity sensors in the growthroom (yellow circle) and a humidifier (red circle) (a), and the lysimeters set up with the leachate containers (b).

The water content of the lysimeters was maintained above field capacity throughout the experiment. In order to ensure the appropriate soil moisture levels, the cores were first saturated with water and left to adapt overnight with the drainage tubing closed prior to the commencement of the experiment. After that the tubing was re-opened and excess water collected. The lysimeters were left to freely drain for the next few hours and the intensity of leaching was being observed. When the leaching stopped the lysimeters were assumed to be at field capacity. To retain soil moisture above field capacity the cores were regularly watered with the equivalent of the average daily rainfall for Wexford and Rosslare. Average daily rainfall value for these stations was 2.4mm. Assuming that

2.4mm rainfall was equivalent to 2400ml of precipitation per m^2 and that each soil core area was 0.015m^2 the volume of water used was 36ml water per core per day.

4.2.6. Treatments

Two fertiliser treatments were applied in the experiment on 6.09.2010. Three replicates of the cores were fertilised with ammonium nitrate at 50 kg N/ha, either single (Treatment 1) or double labelled (Treatment 2).

- In treatment 1 $^{15}NH_4NO_3$ at 98 atom% was used. Only the NH_4^+ fraction of the fertiliser was labelled hence the dilution to 50 atom% resulted in 25% of the applied N to be ^{15}N.
- In treatment 2 $^{15}NH_4{}^{15}NO_3$ at 98 atom% was used. Both forms of mineral N in the compound were labelled hence the dilution to 50 atom% resulted in the same enrichment in the final fertiliser N.

Fertilisers were dissolved in water ensuring that the 50 kg N/ha could be applied in the daily watering rate of 36ml per core (40ml per core for more accurate application). Fertilisers solutions were manually applied on 6th September 2010 with 50ml plastic syringes, separate and colour coded for each treatment; control: green, $^{15}NH_4NO_3$: yellow, and $^{15}NH_4{}^{15}NO_3$: red (Figure 4.7 a, b, c).

a)

b)

c)

Figure 4.7 Separate sets of colour-coded fertilisers solutions, beakers, 50ml plastic syringes for application and 20ml plastic syringes for subsequent gas sampling (a, b); manual application of fertiliser solutions (c).

4.2.7 Gas sampling and analysis

Static chambers for headspace gas sampling from the lysimeters were constructed from PVC and were 7.1 cm diameter and 20cm height (Figure 4.8 c). The chambers were insulated with neoprene gasket to ensure airtight headspaces at the time of measurement (Figure 4.8 a). Chambers were tightly fitted over the lysimeters for the measurement and gas samples were deployed with a 20ml plastic syringe with a hypodermic needle (both Becton Dickinson, UK) through the rubber septum (Figure 4.8 b) at time 0 min, 15 min, and 30 min. Gas samples were collected following a standard procedure as in chapter 2, section 2.2.4. 20ml gas samples were injected into pre-evacuated 7ml glass vials (Labco, UK) for gas chromatography analysis. Over pressurising of vials was performed in order to prevent inward leaks to vials.

Gas sampling was performed between 13.08.2010 and 9.11.2010. Gas samples were collected once a week prior to fertilisation. After fertiliser application samples were collected daily for the first nine days, then every second day for a week, and every third day for another nine days before going back to sampling once a week until the end of the sampling period. Nitrous oxide concentrations were determined by gas chromatography and fluxes calculated using the same method as in section 2.2.5. Additionally, at 30 min duplicate gas samples for isotopic analysis were collected into pre-evacuated 11ml glass vials (Labco, UK). 11ml of sample was deployed from the chamber and injected into the vial. Gas samples for the isotopic analysis were not over-pressurised as this was a requirement of the UC Davis Stable Isotope Facility. Only one of the duplicates was analysed. The second sample acted as a backup. Trace gas isotope ratios in the sample were measured using a ThermoFinnigan GasBench + PreCon trace gas concentration system interfaced to a ThermoScientific Delta V Plus isotope-ratio mass spectrometer (IRMS)(Bremen, Germany). Gas samples were purged into a helium carrier stream (20 ml/min), and then N_2 and N_2O were isolated and concentrated for the analysis. First N_2 was passed to the IRMS through a molecular sieve 5A GC column (15m x 0.53mm ID, 25°C, 3 ml/min) to capture the peak N_2 concentration. A reference N_2 peak was used to calculate provisional isotope ratios of the sample N_2 peak. While N_2 was being analysed, the rest of the sample was passed through a CO_2 scrubber (Ascarite) and N_2O was trapped and concentrated in two liquid nitrogen cryotraps. The concentrated N_2O was then passed to the IRMS through a Poroplot Q GC column (25m x 0.53mm, 25°C, 1.8ml/min) to

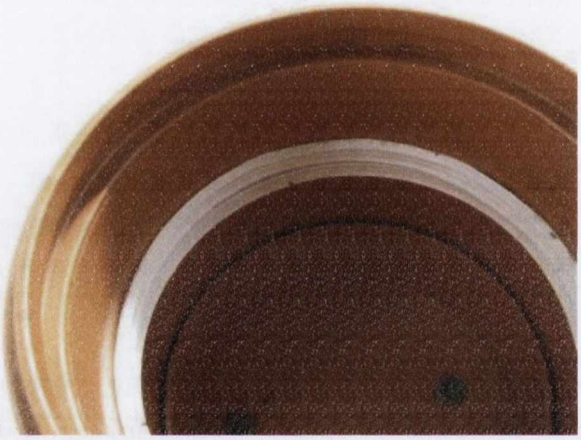
measure the peak N_2O concentration. A reference N_2O peak was used to calculate provisional isotope ratios of the sample N_2O peak.

The ion currents m/z 44, 45, and 46 were used in N_2O and $^{15}N_2O$ flux calculations. Values for ^{15}R ($^{15}N/^{14}N$) in N_2O were calculated based on ^{45}R ($^{45}N_2O/^{44}N_2O$) and ^{46}R ($^{46}N_2O/^{44}N_2O$) (Stevens and Laughlin, 1998). The ^{15}N enrichment of N_2O (a_D) was estimated using ^{45}R and ^{46}R (Stevens and Laughlin, 1994). Based on ^{45}R and ^{46}R ratios the portion of N_2O derived from the denitrifying pool of enrichment a_D was calculated ($d_{'D}$) (Arah, 1997).

N_2 and $^{15}N_2$ fluxes were calculated based on the enrichment of the source of N_2 ($^{15}X_N$), which is the difference between natural abundance and enriched atmospheres for $\Delta^{29}R$ ($^{29}N_2/^{28}N_2$) and $\Delta^{30}R$ ($^{30}N_2/^{28}N_2$). Then, the fraction d of N_2 derived from the labelled NO_3^- pool was calculated from $^{15}X_N$ and $\Delta^{30}R$ (Mulvaney, 1984). The method of Stevens and Laughlin (2001) was used, which determined $^{15}X_N$ from N_2O a_D to improve the precision of the N_2 flux and to lower the detection limit of N_2 d.

a)

b)



c)

Figure 4.8 State of the art plastic chambers for gas sampling (c). Each chamber had two rubber septa (b) and was insulated with neoprene gasket to keep it air tight (a).

4.2.8. Leachate sampling and analysis

Each lysimeter was equipped with tubing ending inside an individual 500ml drainage container. Drainage was sampled from every two to three weeks from all the lysimeters at the same date. Sampling intervals were not equal. Leachate samples were collected each time when at least one of the leaching containers was full. Volume of the leachate was recorded and sub-sample taken into 50ml plastic test tube (Sarstedt Ltd., Ireland) for NH_4^+ and NO_3^- analysis on the Aquakem 600 discreet analyser as described by Hoekstra *et al.* (Hoekstra *et al.*, 2010).

4.2.9. Herbage sampling and analysis

Above ground biomass was cut from all the lysimeters on three occasions, at two week time intervals. The grass was dried at 60°C in perforated plastic bags and stored in airtight plastic bags. Due to small sample sizes biomass from the three sampling times from lysimeters with the same treatments was pooled for further analysis. Biomass was ground using grass grinder.In case of smaller samples a ball mill grinder,was used as an appropriate method of generating uniform samples. C and N contents of the sample were measured on a sub sample on a TruSpec CN elemental analyser at 950°C where the samples were combusted in oxygen and C converted and analysed as CO_2 and N as N_2. Another sub sample was weighed out and encapsulated in tin capsule for ^{15}N content analysis. Encapsulated samples were placed in a 96 well micro tray and analysed at UC Davis Stable Isotope Facility. The samples were analysed on PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). The samples were combusted at 1000°C in a reactor packed with chromium oxide and silvered copper oxide. After combustion, oxides were removed in a reduction reactor. The helium carrier then flew through a water trap (magnesium perchlorate) and a CO_2 trap. N_2 and CO_2 were separated on a Carbosieve GC column (65°C, 65 ml/min) and N_2 samples entered IRMS for the peak detection.

4.2.10. Soil sampling and analysis

Destructive sampling was carried out the end of the experiment. Soils from within all the lysimeters were sampled with an auger at 0-10cm and 10-30cm. Soils were sieved with 3.35mm sieve, roots picked out and left aside, and soils dried at 60°C. When dried, the soils were sub-sampled and powdered using a ball mill for C and N analysis. Small amounts of powdered samples were prepared for ^{15}N analysis. C and N, and ^{15}N were analysed using the same methods as for herbage samples above (section 4.2.8)..

4.2.11. Roots sampling and analysis

Roots that were removed from all of the soil samples as described above (section 4.2.9) were later washed and prepared for further analysis in the same way as soil samples. The whole *Miscanthus* rhizomes and root systems were excavated from the lysimeter soils; they were washed and dried at 60°C. Roots were separated from rhizomes, sub-sampled and prepared for C and N, and ^{15}N analysis as previously (section 4.2.8).

4.2.12. Diffusion of leachate samples

A diffusion method was used to recover inorganic nitrogen from water or soil extract samples by converting it to NH_3. The evolved gas was trapped on acidified filter discs suspended above the solution in a closed container. To recover NH_4-N from the solution a redox reaction with MgO was used in which NH_3 is created and captured. To recover NO_3-N Devarda's alloy and MgO were added, however this reaction recovers N from both inorganic forms so the amount of NH_4-N had to be subtracted from the total recovered N. The diffusion procedure was adopted from Brooks *et al* (1989), and Stark and Hart (1996). Due to low N concentrations in the drainage water, it was decided to pool all the leachates collected after fertilisation and perform diffusion on sub-samples. Leachates were pooled for each lysimeter in a way that a proportion of each leachate sampling was sub-sampled to create a composite sample containing 40 µg of N. Samples were transferred into 500ml acid-washed glass laboratory bottles with plastic screw caps with 22.37g KCl. De-ionised water added to make up 150ml of solution. Screw caps had two acidified filter discs each hung from a bent needle attached to the bottom of the lid.

0.4g of Devarda's alloy, and 0.25g of MgO were added into solutions, lids screwed on tightly and bottles gently swirled for a few seconds and left to incubate at 20°C for three days. The bottles were swirled gently twice a day to improve gas diffusion. At the end of the three days the lids were unscrewed and placed in an oven at 40°C for an hour for the filter discs to dry out. When dried, duplicate discs were separately encapsulated using forceps and placed in two 96-well micro trays creating two identical sample sets. One set of samples was sent to UC Davis Stable Isotope Facility for ^{15}N analysis and the second set was kept as a spare one. Two blanks and a standard (10 atom% ^{15}N at 75 μg of N) were also diffused.

4.3. Results

4.3.1. N_2O emissions in response to a mineral fertilizer application

The temporal profile of daily N_2O flux from each crop-soil systems for the duration of the experiment is shown in Figure 4.9. Prior to treatments application, background N_2O emissions were consistently low ranging between -0.09 ± 0.36 g N_2O-N ha^{-1} d^{-1} (well drained grass, 31.08.2010) and 4.47 ± 3.41 g N_2O-N ha^{-1} d^{-1} (well drained Reed canarygrass, 18.08.2010).

Increased N_2O emissions were observed following fertilisation across all treatments with largest daily fluxes observed between one and two days after fertilisation. The period of high N_2O period lasted for approximately 14 days after which emissions returned to background level. The largest daily flux was observed from grass on poorly drained soil with a maximum of 708.34 ± 213.67 g N_2O-N ha^{-1} d^{-1} one day after fertilisation (Figure 4.9 a). The lowest emission peaks were observed two days after fertilisation from Reed canarygrass on well drained soil; these were 42.74 ± 14.77 g N_2O-N ha^{-1} d^{-1} (Figure 4.9 b). In general, higher emissions were observed on poorly drained soils. The exception was *Miscanthus* where peak emissions were only 120.91 ± 67.26 g N_2O-N ha^{-1} d^{-1} one day after fertilisation.

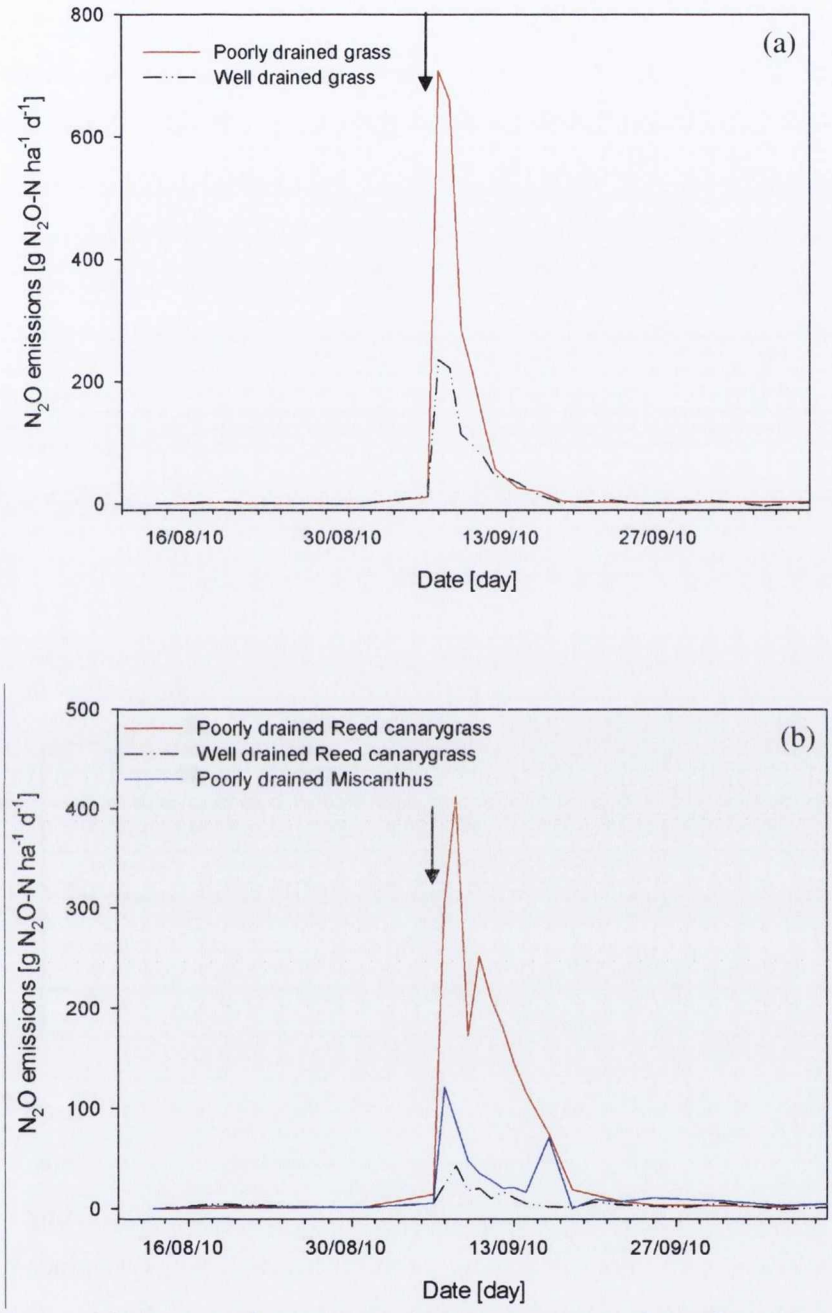


Figure 4.9 Temporal patterns of N_2O emissions throughout the experiment from the studied grassland systems (a), and energy crops systems (b). Black arrow indicates fertiliser application date.

Cumulative emissions from control soils were very low varying between 0.005 ± 0.028 kg N_2O-N ha^{-1} from poorly drained grass and 0.052 ± 0.01 kg N_2O-N ha^{-1} from poorly drained Reed canarygrass (average of between 0.36 and 3.7 g N_2O-N ha^{-1} d^{-1},

respectively, see Table 4.4). There were no significant differences between the controls ($p>0.05$).

Cumulative emissions from fertilised soils followed the same pattern as daily emissions with the highest N_2O emissions associated with ryegrass and Reed Canarygrass cultivated on poorly drained soil (2.21 ± 0.36 kg N_2O-N ha^{-1} and 2.076 ± 0.44 kg N_2O-N ha^{-1} respectively, Table 4.2). Conversely, well drained soils had the lowest emissions (0.17 ± 0.05 and 0.94 ± 0.15 kg N_2O-N ha^{-1} for RCG and grass respectively). *Miscanthus* was the exception to the pattern of high emissions on poorly drained soil, with cumulative N_2O loss of 0.54 ± 0.15 kg N_2O-N ha^{-1} (Table 4.4) Emissions from fertilised soils were significantly larger from those from controls ($p<0.05$). Soil drainage (soil) and fertilisation (treatment) had significant effect on the N_2O emissions, and significant interactions were found for crop*soil, crop*treatment, and soil*treatment ($p<0.05$)

Table 4.4 Comparison of cumulative N_2O-N losses from control and fertiliser treatments on all crop-soil systems. SE values indicate standard error of the means. Capital letters in brackets indicate statistically significant differences at $p<0.05$.

Crop-soil system	kg N_2O-N ha^{-1} from control treatment	SE	kg N_2O-N ha^{-1} from fertiliser treatments	SE
RCG poorly drained	0.052 (CD)	0.010	2.076 (A)	0.441
RCG well drained	0.020 (D)	0.015	0.175 (C)	0.047
Miscanthus poorly drained	0.030 (D)	0.014	0.548 (B)	0.152
Grass well drained	0.011 (D)	0.007	0.941 (B)	0.207
Grass poorly drained	0.005 (D)	0.028	2.210 (A)	0.359

The large variation in cumulative emissions across treatments resulted in a large variation in emission factors for inorganic N fertiliser application of the studied crop-soil systems. These were calculated as the percentage of applied N emitted as N_2O after subtracting control emissions from post-fertilisation emissions, and adjusted to a reference fertilisation rate of 100 kg N ha^{-1} (Figure 4.10). The IPCC default emission factor is indicated by a horizontal line in Figure 4.10. Of the five studied crop-soil systems, only one displayed emission factor lower than the 1% default value, (well drained Reed canarygrass) which was the lowest of all cases ($p<0.05$). Poorly drained *Miscanthus* was very close to the default emission factor (1.04 ± 0.30) whilst the other three systems were all above the IPCC default value with poorly drained grass highest (4.41 ± 0.72), closely followed by poorly drained Reed canarygrass (4.05 ± 0.88), and well drained grass (1.86

± 0.42). However well drained grass emission factor was over the default IPCC value it was similar to that of poorly drained *Miscanthus*. It was significantly higher to that of well drained Reed canarygrass, but was only approximately half of that of the two highest values. Both crop type and soil drainage had a significant effect on emission factors ($p<0.05$).

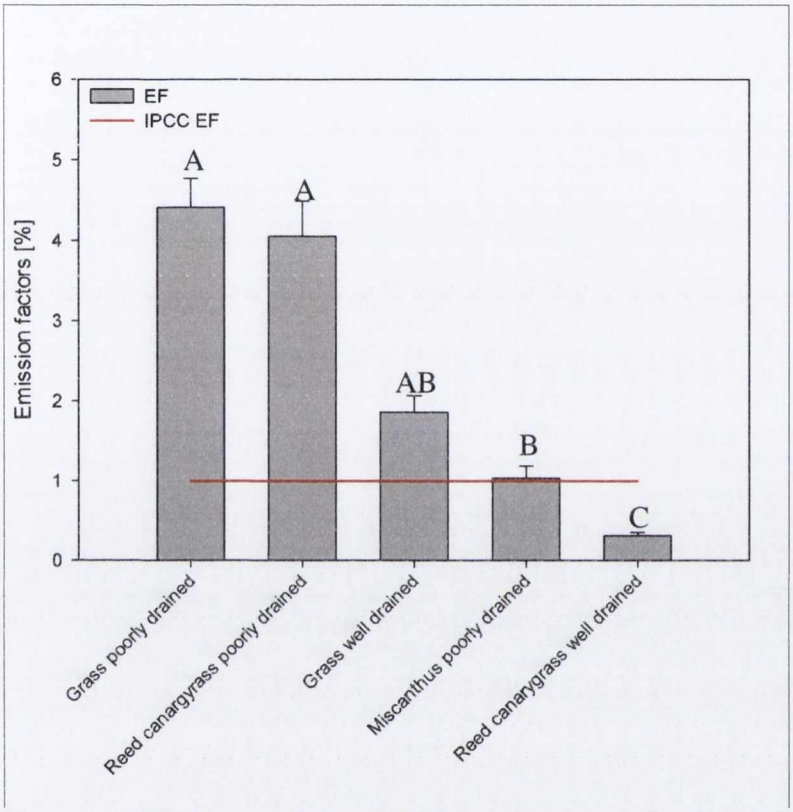


Figure 4.10 Emission factors (EFs) for the crop-soil systems for the synthetic N fertiliser application and a comparison with an IPCC default value for this activity. Capital letters indicate statistically significant differences at $p<0.05$. Error bars indicate standard error of the mean.

4.3.2. Nitrification and denitrification rates

^{15}N tracing was used in order to assess the contribution of nitrification and de-nitrification to N_2O emissions. Generation of N_2 was indicated by data for $\Delta^{29}R$ and $\Delta^{30}R$. For the IRMS system, the between-batch limit of detection at the 95% confidence interval is 8.0×10^{-6} for $\Delta^{29}R$ and 3.2×10^{-7} for $\Delta^{30}R$ (Stevens and Laughlin, 2001). Detectable values for $\Delta^{29}R$ and $\Delta^{30}R$ were therefore observed when the NO_3^- pool was labelled, so the labelled N_2 was associated with NO_3^- reduction.

In case of fertiliser with labelled ammonium fraction $^{15}N_2O$ was coming from nitrification and nitrifier denitrification, and when both ammonium and nitrate fractions of the fertiliser were labelled emissions could be linked to both these processes and denitrification. Figure 4.11 indicates that between 20% to 85% of produced N_2O came from nitrification and nitrifier denitrification; that share depended on the crop and soil type. In general, a larger proportion of N_2O emissions from well drained soils was due to nitrification and nitrifier denitrification processes. Well drained Reed canarygrass exhibited the highest share of nitrification-derived N_2O with 85.5 ± 14.41% and it was also significantly different from the other four crop-soil systems ($p<0.05$). The second largest share of nitrification-derived N_2O with a value of 44.9 ± 3.23% was found in well drained grassland soil. Poorly drained grass had the second lowest share of N_2O coming from nitrification and nitrifier denitrification at 25.0 ± 3.36%. However, *Miscanthus* was the lowest of all treatments with 19.3 ± 3.69% of its N_2O emissions derived from nitrification.

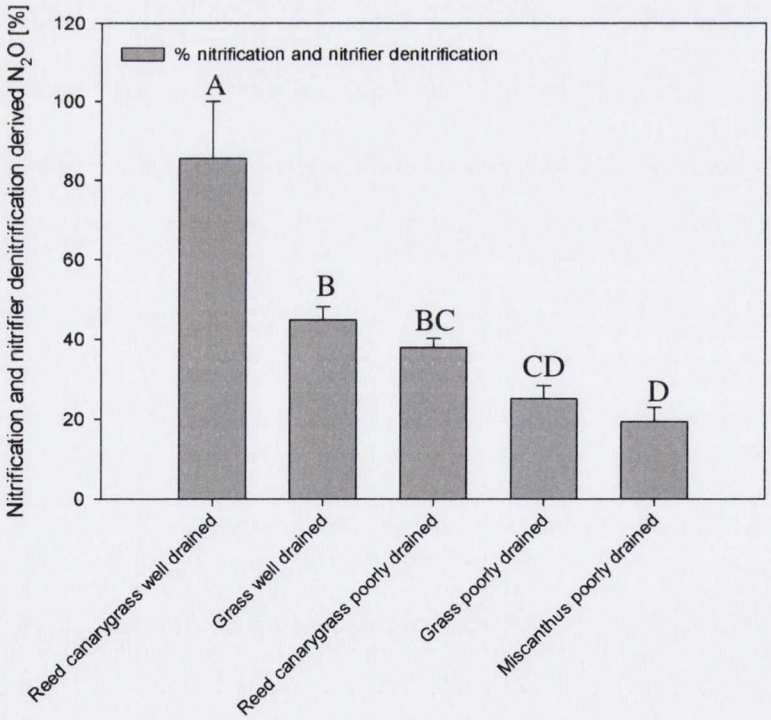


Figure 4.11 Percent share of N_2O emissions created through nitrification reactions in the five studied crop-soil systems. Capital letters indicate statistically significant differences at $p<0.05$. Error bars indicate standard error of the mean.

4.3.3. ^{15}N enrichment of gaseous emissions post-fertilisation

Cumulative emissions of gaseous N products after fertilisation are shown in Table 4.5. N_2 flux data was filtered to incorporate limits of detection of $\Delta^{29}R$ ($^{29}N_2/^{28}N_2$) and $\Delta^{30}R$ ($^{30}N_2/^{28}N_2$) prior to further calculations. Missing values were then generated by linear extrapolation and used to calculate cumulative N_2 losses. In all cases N_2 fluxes were significantly higher to N_2O fluxes and remained the major component of the N emissions. The highest N_2 fluxes were recorded from poorly draining soils with *Miscanthus* and perennial ryegrass cover. This was in agreement with figure 4.11 where these two plant-soil systems had the highest share of denitrification. Following temporal patterns of gaseous emissions two distinguishable peaks were visible over time in some cases (for example *Miscanthus*, Figure 4.9), these could be attributed to denitrification of the NO_3^- pool (first peak) and denitrification of NO_3^- previously converted from NH_4^+ (second peak). Portion of the gases attributed to the ^{15}N was low and most of the enhanced $^{15}N_2$ and $^{15}N_2O$ corresponded to the enhanced N_2 and N_2O fluxes. However, discrepancies between gaseous fluxes and their enrichment levels could be explained by the calculation method using linear extrapolation.

Table 4.5 summarises N_2:N_2O ratios calculated for the mean daily losses of N_2 and N_2O over the first two weeks post fertilisation based on the non gap-filled data. This cut-off point was chosen due to the temporal response of N_2O where enhanced emissions were observed during that period and later returned to background levels and therefore could no longer be associated with the applied fertiliser. N_2:N_2O ratios were characterised by large temporal variability and did not show any clear patterns. No significant differences were found between various plant-soil systems. Mean daily N_2:N_2O ratios were in the range of 18.26-56.28 for $^{15}NH_4NO_3$ treatment and 2.77-18.12 for $^{15}NH_4{}^{15}NO_3$ treatment.

Table 4.5 Daily mean N_2O emissions, corresponding N_2 emissions and N_2:N_2O ratios after application of both fertiliser treatments to the various crop-soil lysimeters based on non gap-filled data (fertiliser period between 06.09.2010 and 20.09.2010). SE values indicate standard error of the mean.

Crop-soil system	$^{15}NH_4NO_3$ fertiliser		$^{15}NH_4NO_3$ fertiliser		$^{15}NH_4NO_3$ fertiliser		$^{15}NH_4{}^{15}NO_3$ fertiliser		$^{15}NH_4{}^{15}NO_3$ fertiliser		$^{15}NH_4{}^{15}NO_3$ fertiliser	
	kg N_2O d^{-1} ha^{-1}		kg N_2 d^{-1} ha^{-1}		N_2:N_2O		kg N_2O d^{-1} ha^{-1}		kg N_2 d^{-1} ha^{-1}		N_2:N_2O	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Reed canarygrass poorly drained	0.14 (BC)	0.03	1.31 (ABC)	0.07	18.26 (BC)	1.53	0.10 (BC)	0.02	0.18 (BC)	0.01	2.77 (C)	0.66
Reed canarygrass well drained	0.03 (C)	0.001	0.62 (BC)	0.08	19.26 (BC)	2.44	0.04 (C)	0.003	0.18 (BC)	0.06	4.48 (C)	1.56
Miscanthus poorly drained	0.04 (C)	0.002	1.99 (ABC)	0.19	56.28 (A)	9.79	0.07 (C)	0.02	1.28 (ABC)	0.38	18.12 (BC)	4.34
Grass well drained	0.09 (BC)	0.01	3.62 (AB)	1.11	28.45 (AB)	8.45	0.09 (BC)	0.03	0.30 (BC)	0.03	5.10 (C)	1.41
Grass poorly drained	0.21 (BC)	0.10	5.30 (A)	2.61	21.28 (BC)	8.56	0.17 (BC)	0.01	0.86 (BC)	0.21	11.01 (BC)	3.00

4.3.4. Leached N losses in response to a mineral fertilizer application

There was no significant effect of fertilisation or soil drainage found in leached mineral N load (Table 4.6). Leached ammonium and nitrate followed similar pattern in all cases regardless of fertilisation (Figure 4.12). Soil and treatment had a significant effect on cumulative ammonium load in leachate collected throughout the experiment ($p<0.05$), with lighter soils exhibiting the highest leaching. Such an effect was not found for nitrate load. However, as the majority of leached N was found in nitrate form (i.e. 2.3 g ammonium-N ha^{-1} and 906.2 g nitrate-N ha^{-1} in RCG well drained) there was no clear effect of either crop or soil type on the cumulative leached mineral N.

Table 4.6 Comparison of cumulative ammonium and nitrate in leachates from control and fertiliser treatments on all crop-soil systems. Capital letters in brackets indicate statistically significant differences at $p<0.05$.

Crop-soil system	Ammonium control [g N ha^{-1}]	Ammonium fertiliser treatments [g N ha^{-1}]	Nitrate control [g N ha^{-1}]	Nitrate fertiliser treatments [g N ha^{-1}]
Reed canarygrass poorly drained	17.1 (AB)	6.4 (AB)	575.5 (C)	833.0 (C)
Reed canarygrass well drained	2.3 (A)	3.5 (A)	906.2 (CA)	578.0 (C)
Miscanthus poorly drained	5.9 (A)	2.4 (A)	864.0 (C)	740.4 (C)
Grass well drained	6.9 (A)	5.0 (A)	1304.1 (C)	833.6 (C)
Grass poorly drained	9.1 (A)	10.9 (A)	512.2 (CA)	297.6 (C)

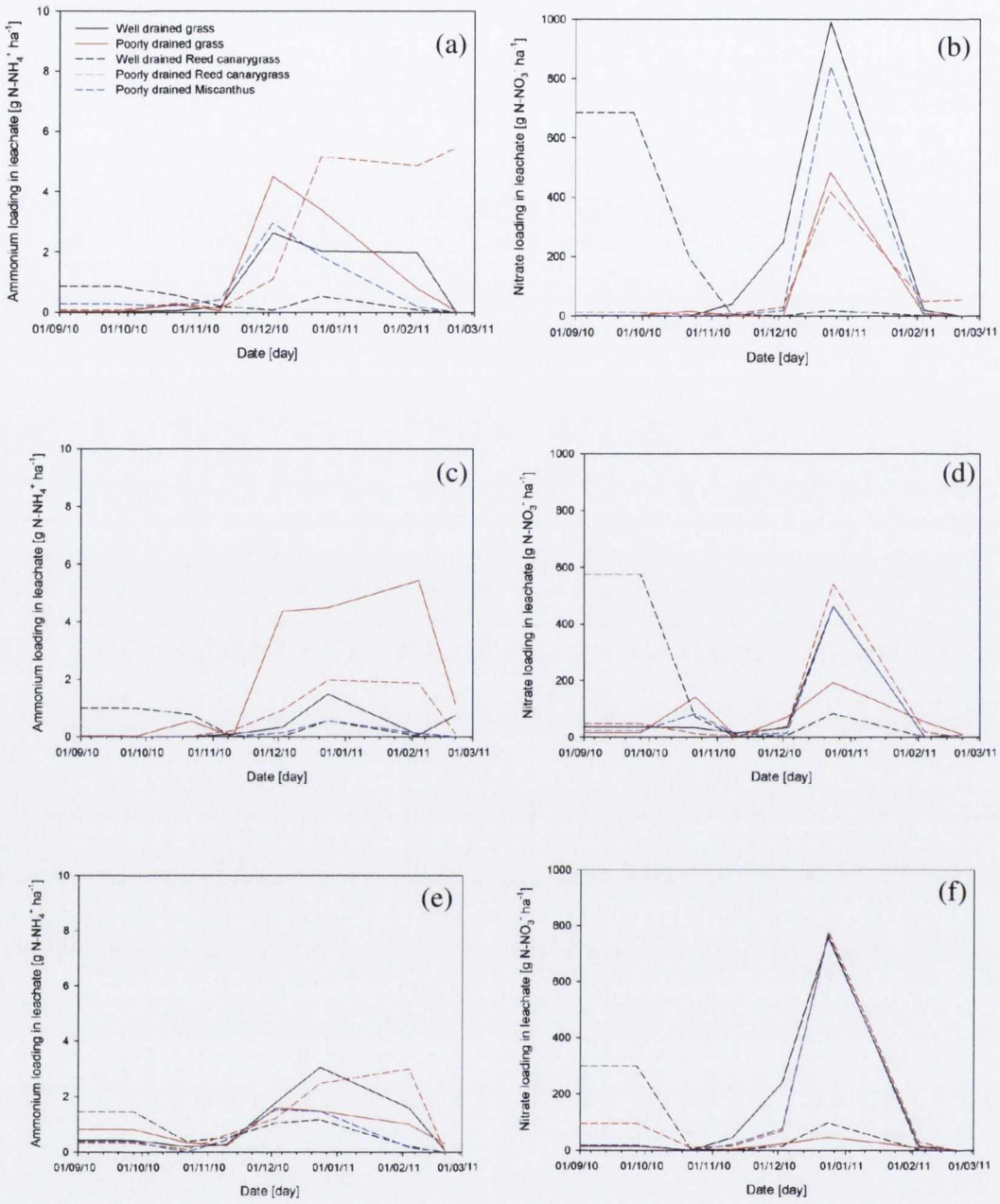


Figure 4.12 Leached N losses of ammonium N (a) and nitrate N (b) from control, ammonium N (c) and nitrate N (d) from $^{15}NH_4NO_3$ treatment, and ammonium N (e) and nitrate N (f) from $^{15}NH_4{}^{15}NO_3$ treatment following fertilisation.

4.3.5. The magnitude of reactive N losses following fertiliser application and partitioning between the pathways.

Loss pathways investigated in this experiment were gaseous emissions (N_2 and N_2O) and leaching losses (NH_4^+ and NO_3^-). N_2 as a benign, non-reactive gas is not responsible for any adverse environmental effects; however its emissions remove nutrients from agricultural systems. Therefore N_2 emissions should be taken into account when considering nutrient balance and loss. Over the whole experimental period cumulative N_2 loss varied between 11.9 ± 0.3 and 33.1 ± 5.9 kg $^{15}N_2$ $ha^{-1}d^{-1}$ from single labelled grassland over well drained soil and double labelled RCG over poorly drained soil, respectively (Figure 4.13). The remaining loss pathways (N_2O emissions and leaching combined) totalled between 0.5 ± 0.34 and 1.3 ± 0.18 kg N ha^{-1} from control poorly drained and well drained grassland, respectively, and between 0.8 ± 0.22 and 2.9 ± 0.46 kg N ha^{-1} from fertilised well drained and poorly drained RCG, respectively. There was a significant treatment effect ($p<0.05$) on the overall leached N and N_2O losses, and a significant soil*treatment interaction was discovered. The majority of reactive N loss were incurred via leaching in all the controls, with poorly draining soils losing marginally less N from the crop-soil systems than well draining soils (not statistically significant). However post-fertilisation, this proportion changed and only well draining Reed canarygrass and poorly draining *Miscanthus* derived most of their N losses from leaching. Well draining grass lost almost an equal share of N through both pathways, and poorly draining grass and Reed canarygrass were characterised by highest gaseous emissions share. These were also two crop-soil systems with the lowest losses amongst all the controls and the largest losses in the post-fertilisation group. Their losses were also significantly larger in comparison to all the controls.

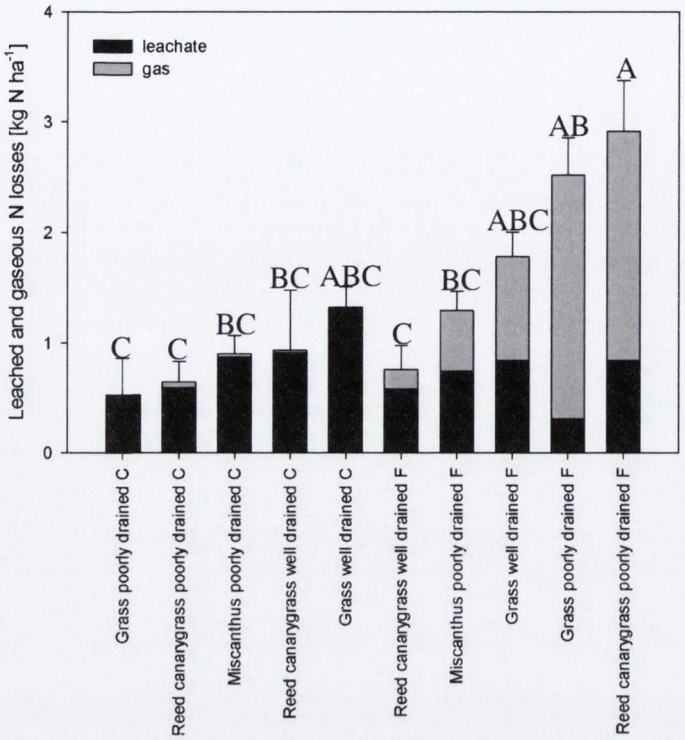


Figure 4.13 Combined leached N and gaseous N losses from all the crop-soil systems. 'C' represents control, and 'F' fertiliser treatments. Capital letters indicate statistically significant differences at $p<0.05$. Error bars indicate standard error of the mean.

4.3.6. ^{15}N fertiliser recovery rates

Recovery of the applied ^{15}N and the apportioning of the recovered tracer within the fractions: soil, herbage, leachate, N_2, and N_2O is presented in Figure 4.14. Standard error was estimated assuming independent errors across fractions as an indicative estimate of the reliability of these figures. In general, higher recovery rates were found in the $^{15}NH_4NO_3$ treatment, with values between 42.6% and 136.8%. Values above 100% are attributed to the sampling errors and calculation method. In the case of all lysimeters the majority of recovered ^{15}N was found in soil, between 12.9% (poorly draining Reed canarygrass fertilised with $^{15}NH_4{}^{15}NO_3$ and 86.2% (well draining grass fertilised with $^{15}NH_4NO_3$). Although ^{15}N in soil was analysed in the form of total N it is believed that the majority of the applied tracer was bound in the soil organic N

fraction, as ^{15}N was still present in soil at the time of measurement seven months following its application. Soil sampling was delayed by this time to account for long travel time of leachates in poorly draining soils and allow collecting leachate samples prior to destructive soil sampling. The second highest recovery rate varied between plant-soil systems between herbage and N_2 fractions. Within the $^{15}NH_4NO_3$ treatment herbage ^{15}N recovery rate was the second largest fraction in well draining grass only, in the other plant-soil systems N_2 emissions had the second largest fraction of ^{15}N recovery. Within $^{15}NH_4{}^{15}NO_3$ treatment recovery rates in herbage in N_2 are equal in well draining grass and both Reed canarygrass systems, and in poorly draining grass and *Miscanthus* N_2 is the more important pathway. Across both treatments and all plant-soil systems the recovery rates in leachates and in N_2O emissions were less than 2%. There was a significant difference between ^{15}N levels in control and fertilised lysimeters soils (except for poorly draining Reed canarygrass associated with low soil ^{15}N recovery rates). However herbage ^{15}N concentration in fertilised soils only differed from controls in both grass lysimeters in $^{15}NH_4NO_3$ treatment, and in both grass, and well draining Reed canarygrass in $^{15}NH_4{}^{15}NO_3$ treatment. There was no significant response in herbage ^{15}N in the rest of the energy crops.

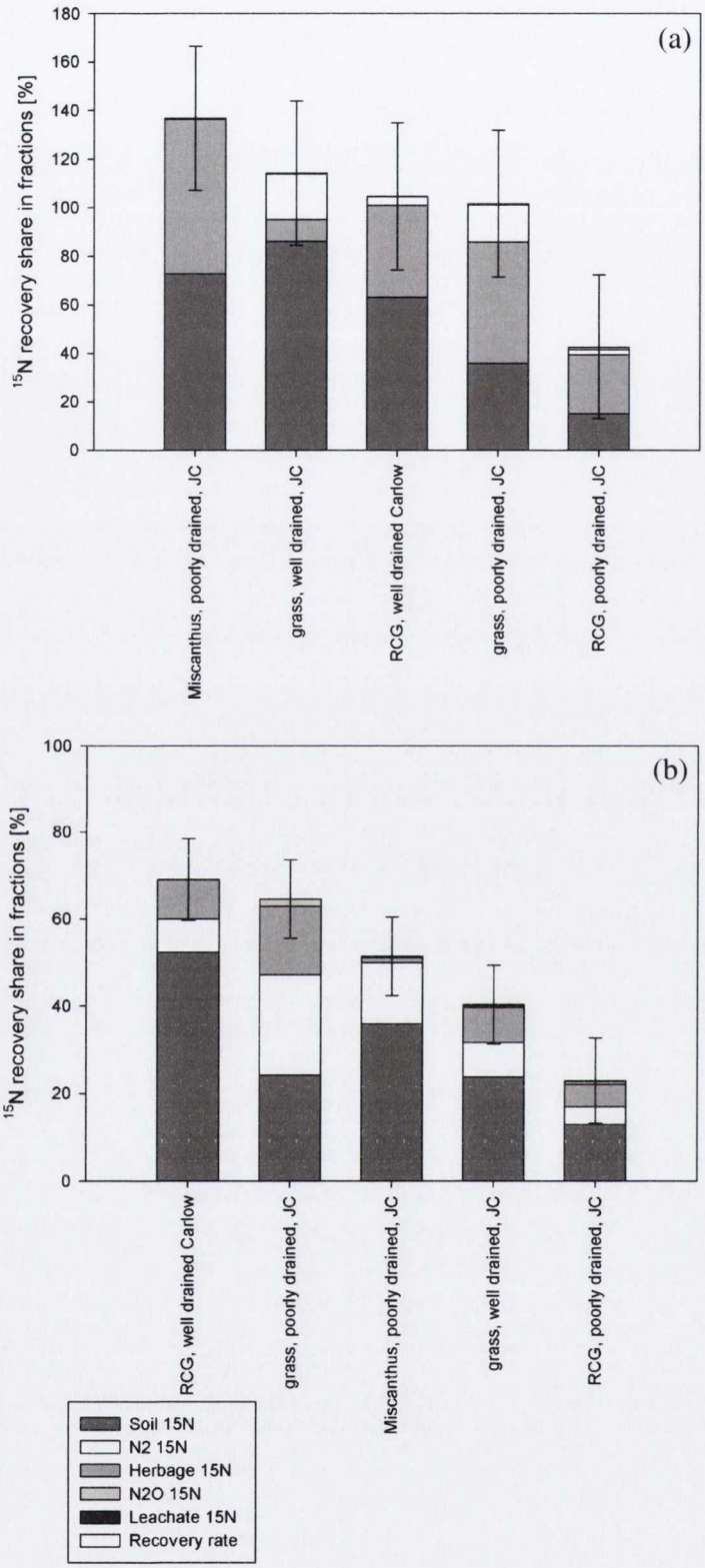


Figure 4.14 Recovery rates of the applied ^{15}N partitioned between soil, biomass, leachate, and gaseous losses following fertilisation with a) $^{15}NH_4NO_3$ and b) $^{15}NH_4{}^{15}NO_3$. Error bars indicate standard error of the mean.

4.4. Discussion

Higher emissions were observed from poorly drained soils and these produced up to 2.1-2.2 kg N_2O-N ha-1 in total over post-treatment period. This is in line with studies by Flechard et al. (2007), Skiba and Ball (2002), and Henault et al (1998) who discovered a large effect of soil type and soil management on N_2O emissions. The study of Flechard *et al.* that monitored N_2O over 3-year period across 10 grassland sites in Europe recorded large differences in N_2O caused by climatic conditions, soil type and soil management (grazing and fertilisation). Intensively managed sites were stronger sources of N_2O than extensive grasslands. Throughout fertilised sites the largest emissions were observed from heavier, poorly drained soils with high clay content. In Skiba and Ball (2002) background emissions varied between 0 and 19 g N_2O-N $ha^{-1}d^{-1}$ from soils ranging from poorly drained silty clay loams to free drained sandy loams, up to between 2 and 123 g N_2O-N $ha^{-1}d^{-1}$ after fertilisation with imperfectly drained clay loam responsible for the largest N_2O losses. Increasing N_2O emissions were a function of bulk density and clay content, usually higher in poorly drained soils. Similarly, Henault *et al* (1998) found much larger N_2O emissions from a gley soil with high clay content than from a freely draining soil.

Emission factors varied substantially between soil-crop systems in this experiment with values observed from below 1% to over 4%. The lowest emission factors of 1% and below were found in *Miscanthus* over poorly drained soil and Reed canarygrass over well drained soil. Poorly drained grassland and Reed canarygrass were characterised by emission factors of approximately 4%. In general, the emission factors were higher than the IPCC (2001) EF of 1% of N applied that is commonly used for GHG inventories. In Ireland, studies of Abdalla *et al.* (2009), Hyde *et al.* (2006), Hsieh *et al.* (2005) and most recently Rafique *et al.* (2011) investigated N_2O emissions following fertiliser application. Abdalla *et al.* (2009) at a research site in Carlow (sandy loam soil), recorded cumulative fluxes of 0.9 ± 0.2 kg and 2.4 ± 0.3 kg N_2O-N ha^{-1} yr^{-1} for the control (no fertilizer) and fertilized (200 kg N ha^{-1} yr^{-1}) grazed grassland sites, respectively. Hyde *et al.*, (2006) measuring N_2O on clay loam soil site in Wexford, recorded between 4.21 (control) and 28.93 kg N ha^{-1} yr^{-1} over two years and several fertilizer rates (0, 225 and 390 kg N ha^{-1} yr^{-1}). Research of Hsieh *et al*

(2005) at a clay loam site in Cork where both organic (130 kg ha^{-1} yr^{-1}) and mineral fertilizer (207 kg N ha^{-1} yr^{-1}) were applied recorded a total loss of 12 kg N_2O-N ha^{-1} yr^{-1}. However while the emission factor found by Abdalla *et al* (2009) was in line with the IPCC default value of 1%, studies of Hyde *et al.*, and Hsieh *et al.*, found the emission factors to be significantly higher. Rafique *et al* (2011) reported an overall emission factor of 1.81% from an eight-site N_2O measurement programme in the south of Ireland over two years. It is concluded in this study that the EF of 1% used in Ireland by the EPA for the national GHG reporting underestimates emissions of N_2O.

Nitrous oxide is a product of two processes: nitrification and denitrification. The proportion of N_2O generated from nitrification and/or nitrifier denitrification increased as soil texture became coarser and soil becoming more freely drained. Between 45% and 85% of N_2O was generated by nitrification in well drained soils over grassland and Reed canarygrass. Drainage properties of the soil affect soil moisture, and consequently oxygen availability of the soil. Therefore, in well drained soils with low% WFPS, nitrification is predominantly responsible for N_2O creation whereas in poorly drained soils it is denitrification (Bateman and Baggs, 2005; Bollman and Conrad, 1998). Weier et al. (1993) as well as Bollman and Conrad (1998) observed more N_2O being produced by denitrification with soil texture becoming finer as these soils become anoxic at lower moisture levels.

Partial denitrification was the main process responsible for N_2O production in poorly drained soils with between 60% and 80% of N_2O being produced through this process. Denitrification derived N_2O is predominantly generated in low oxygen soils. Therefore it is more prevalent in poorly drained soils which maintain higher WFPS (lower oxygen availability) than well drained soils. In unsaturated well-aerated conditions 60% of N_2O can come from nitrification and 40% from denitrification, whereas under saturated conditions this proportion changes to 10-15% from nitrification and 85-90% from denitrification (Mathieu *et al.*, 2006). Denitrification, in contrast to nitrification, can lead to production of N_2 as well as N_2O and therefore is critical in removing reactive N from the environment (Seitzinger *et al.*, 2006). N_2 was the main gaseous N product after N fertilisation with N_2:N_2O ratios high between 10 and 39. Out of all crop-soil systems *Miscanthus* was characterised by the highest denitrification rate of over 80% and at the same time, the lowest N_2O emissions of 0.55 kg N_2O-N ha^{-1}. This

is due to the fact that the majority of gaseous N, between 14 and 17 kg N was lost in the form of N_2. N_2:N_2O ratios found in this experiment were high. However a wide range of values can be found in literature. Stevens and Laughlin (2002) found N_2:N_2O ratios in the fine loamy grassland soil to be 2.2 and 0.5 from control and combined slurry and NO_3 fertiliser, respectively. However Clough *et al.* (1998) recorded ratios varying between ryegrass/white clover pasture soils (silt loam, sandy loam, peat and clay soils) from 6.2 to 33.2 following fertilisation with ^{15}N labelled urine.

Codenitrification is another possible pathway of creating N_2 and N_2O. Codenitrication is a co-metabolic process which uses N compounds such as NH_4^+ or amines differently to denitrification but converts them to the same end products. Gases produced in this process are a hybrid N-N species where one atom of N comes from NO_2^- and the other one from a co-metabolised compound (Spott et al., 2011). According to the ^{15}N enrichment of the gaseous N products codenitrification may be of importance in this study and could explain some of the high N losses following fertilisation.

Many factors can contribute to high N_2 emissions and influence N_2:N_2O ratio. Primarily, N_2O reductase is the enzyme responsible for N_2O reduction to N_2 in soils. Its activity increases with increasing pH values, decreasing nitrate concentrations and decreasing oxygen partial pressure (Chapuis-Lardy *et al.*, 2007, Mørkved *et al.*, 2007; Dannemann *et al.*, 2008). Scheer *et al.* 2009 found that N_2:N_2O ratio varied from 5 at 70% water holding capacity (WHC) to 55 at 130% WHC. When high moisture conditions persist, the balance of gaseous N emissions shifts from N_2O to N_2 affecting N_2:N_2O ratio due to rapid development of nitrate reductase under anoxic conditions but slower development of nitrous oxide reductase responsible for N_2 creation (Letey *et al*, 1981). Higher moisture content and subsequent decrease in soil aeration can be linked to increase in soil bulk density therefore management practices causing compaction will effect in higher N_2O emissions as a result of low oxygen availability (Ruser *et al.*, 2006; Stehfest *et al.*, 2006).

Additionally, soil pH is an important control of the gaseous N emissions (Liu *et al.*, 2010). Cuhel *et al.* (2010) observed large differences in N_2:N_2O ratios between acidic and alkaline grassland environments as N_2 emissions were affected but not N_2O. This caused a shift from 15% to 75% of total N gas produced in the form of N_2. The acidic

soils had high NH_4^+ concentration and low NO_3^- concentrations causing a decrease in nitrifying activity, whereas opposite was found in the alkaline soils. This could cause higher nitrification rate and subsequently stimulate denitrification.

Together with soil NO_3^- and WFPS, readily available carbon (C) is an important factor in controlling denitrification (Saggar *et al.*, 2012) Readily available carbon is required by denitrifiers in order to reduce soil NO_3^- therefore land management practices that increase the amount of readily available carbon stimulate denitrification (Chadwick, 1997). Moreover, increasing C availability increase N_2:N_2O ratio (Smith and Tiedje, 1979). Therefore readily available carbon could have impacted gaseous N emissions and their ratio in this study, especially in the case of *Miscanthus*, due its favourable management for available C accumulation in soil.

Differences between N_2:N_2O ratios may also arise due to community structure and the proportion of fungal to bacterial communities. Biomass of temperate soils can be dominated by fungi capable of nitrification and denitrification (Ruzicka *et al.*, 2000; Stevens and Laughlin, 2002; Laughlin *et al.*, 2008). However as fungi do not possess the NosZ gene, they can only de-nitrify to N_2O therefore affecting the N_2:N_2O ratio of the gaseous N products. Shifts from bacterial to more fungal dominated community structures tend to occur in undisturbed systems, such as grasslands (Frey *et al.*, 1999). Philippot *et al.* (2009) also suggested that the proportion of bacteria able to reduce N_2O could be of importance in determining the nature of the denitrification end products and found a negative correlation between the proportion of denitrifiers possessing the nosZ gene and the N_2:N_2O ratio. Therefore differences in denitrifier community structure are a probable explanation of the differences in N_2:N_2O ratios between grassland and *Miscanthus*, and large N_2O emissions from established grassland contrasting with low emissions from *Miscanthus*.

Oher studies have found a nitrogen-fixing bacterium *Azospirillum doebereinerae sp.* present in *Miscanthus* roots (Eckart *et al.* 2001, Davies *et al.*, 2010). N-fixing bacteria can be a probable explanation of high *Miscanthus* yields together with a lack of response to fertilisation found in literature (Christian *et al.* 2006). This microbial N-fixation in the rhizosphere may be a possible explanation for the low N requirement of *Miscanthus* and the low reactive N loss observed in this experiment.

Leaching is normally the principle N loss pathway following fertilisation (Beaudoin *et al.*, 2005). The magnitude of N losses through leaching depends on soil type, drainage, environmental conditions and agricultural management (Wang and Alva, 1996, Wu *et al.*, 1996, Clough *et al.* 1997). However, no effect of fertilisation was found on temporal ammonium and nitrate patterns in this experiment across soil types. Concentrations of ammonium and nitrate varied over time, though similar changes were observed in both control and fertilised lysimeters. Overall, only between 2.3 and 17.1 g $N\text{-}NH_4^+$ ha^{-1} and between 297 and 1304 g $N\text{-}NO_3^-$ ha^{-1} was lost through leaching. There were no significant differences in N loads in leachates from control and fertilised lysimeters. These losses were very low in comparison to literature values. Bergstrom and Johansson (1991) found losses of between 15.7 and 67.4 kg N $ha^{-1}yr^{-1}$, from spring barley soils of different textures following fertilisation at 100 kg N ha^{-1}. Similarly, Sogbedji *et al.* (2000) recorded between 6.7 and 32.2 kg $N\text{-}NO_3^-$ ha following fertiliser application at 100 kg N ha^{-1} from clay loam and loamy sand maize plots, respectively. Low N loss by leaching is most probably due to low fertilisation rate that reduced the risk of nutrient leaching from the systems. Additionally, high temperature and moisture affected microbial activity shifting the balance of N loss pathways towards gaseous emissions. ^{15}N recovery of the applied fertiliser in this fraction was less than 1%.

The majority of the applied ^{15}N that did not leave the lysimeters through gaseous emissions was recovered in the soil. The presence of ^{15}N in soil approximately five months following fertilisation indicated N retention in soil through immobilisation. During the experiment ^{15}N recovery rate in plant biomass did not exceed 20% with the highest recovery rates were observed in perennial rye grass lysimeters (between 7.9% ± 3.0% and 18.8% ± 4.3%). Recovery rates in biomass of energy crops were low not exceeding 10% (between 0.5 ± 4.3 and 9.0 ± 3.0). In five out of six fertilised energy crop-soil systems no significant increase in biomass ^{15}N was found. These figures are lower than those found by Christian *et al.* (2006). Christian *et al.* study showed that *Miscanthus* fertilised with 60 kg N ha^{-1} utilised between 38% (1yr old plants) and 65% (3yr old plants) of applied N with the remainder either sequestered in soil (between 19% and 26%) or lost via various pathways. Possible explanation of such a large difference between this study and the one of Christian *et al.* is the small planting area

used in the lysimeters, which could cause biomass sampling errors and subsequent underestimation of the ^{15}N recovery in this fraction. However, it is not uncommon for large fractions of the applied N to be immobilised by soil microorganisms. Davies *et al.* (2008) found that 80% of the nitrate-N applied to upland soil was immediately immobilised.

4.5. Conclusions

In this experiment low reactive N losses were found either in gaseous or leached form. Leaching, in particular, did not prove to be an important loss pathway in this experiment, most likely due to unfavourable environmental conditions.

Calculated emission factors were highly variable depending on soil type and / or crop type with very low EF observed in the case of *Miscanthus*. *Miscanthus* is a C4 grass therefore its N use efficiency is higher than the other two grasses used in this experiment. This could be the primary reason for low N losses associated with *Miscanthus* system. Additionally, it is hypothesised that low EF associated with *Miscanthus* could also be due to N fixation by the bacteria found in its roots.

N_2O in this study was produced through both nitrification and denitrification. Denitrification was discovered to be the principle pathway for N_2O generation in heavy soils. High N_2:N_2O ratios found in these soils indicated high levels of total denitrification. Total denitrification is an important pathway of removing reactive nitrogen from the environment through the production of N_2. The high share of N_2 in denitrification end products could have been caused by controls such as temperature, soil moisture content, and pH. Differences between N_2:N_2O ratios between crops could arise from community structure and the proportion of fungal to bacterial communities. As shifts from bacterial to more fungal dominated community structures tend to occur in undisturbed systems such as grasslands, and fungi lack nitrous oxide reductase, more N_2O could be produced by these grass systems.

Most of the applied N was either taken up by plants or immobilised in soil pointing to effective N retention and utilisation in the investigated systems.

Chapter 5: General Discussion

5.1. Overview

N_2O emissions dominate the GHG budget of both tillage and pastoral soils (Rees and Ball, 2010) through organic and mineral N input into these soils (Bouwmann *et al.* 2001; US-EPA 2006; Smith *et al.*, 2007). In OECD countries N fertilisation is responsible for 90% of N_2O emissions from tillage soils (Storey, 1997). Currently approximately 64% of Ireland's land area is dedicated to agriculture, much of it intensely managed grazed grasslands characterised by high fertiliser N inputs and related N losses. N_2O emissions associated with this production system are a growing concern for Irish agriculture, predominantly based on export of ruminant livestock products, which produces 30% of the national GHGs (Breen *et al.*, 2010). The removal of milk quotas in 2015, coupled to national milk production targets is anticipated to lead to an increase in scale and intensity of Irish dairy farming subsequently creating an additional pressure on the environment through gaseous emissions and leaching. In order to counter-balance this rise, non-food crops like willow and *Miscanthus* are expected to make a contribution to emissions reduction within the agricultural sector, while providing diversification in the sector and contributing to EU renewable energy targets. Replacing fossil fuels with bioenergy crops has already expanded recently (Tao and Aden, 2009) however little is known about these crops' effect on the soil N_2O emissions. This study was designed to investigate the effects of the early stages of land use change from grassland into energy crops such as ploughing and fertilisation on N_2O emissions. Additionally differences in fertiliser N loss patterns between established grassland and bioenergy crops were investigated. The key questions raised during this study are:

- Does land use change from grassland into energy crops significantly affect N losses?

- Can these losses be reduced or avoided by changes in tillage management or drainage of soils under change?

- What are soil N_2O emissions from Miscanthus and Reed canarygrass following land use change?

- What is the magnitude of N losses from fertilised mature bioenergy crops in comparison with established grassland?

- What processes govern the fertiliser N losses?

- Can these losses be reduced or avoided by changes in soil drainage?

5.2. Findings of the study

Alternative ploughing techniques and their impact on gaseous and leached N were investigated along a gradient of soil characteristics in Chapter 2 where three different soil types of contrasting drainage were ploughed with either conventional or minimum tillage and fertilised. Results of this chapter found that soil type had a significant effect on the two loss pathways (gaseous and leachate) with the majority of N leaving well draining soils through leaching and poorly draining soils through gaseous emissions. Employing alternative ploughing technique did not reduce the losses. There was no significant decrease between the cumulative total losses (N_2O and leached N) due to the ploughing technique, and the alternative ploughing technique only shifted the balance between the loss pathways. Similarly, Ball *et al.* (1999) observed that both ploughing and fertilisation resulted in large N losses, with the latter proving to be more important in terms of their magnitude. Studies on short-rotation coppice establishment observed an increase in leached N from 1 mg NO_3-N l^{-1} for fallow reference sites to 16.6 NO_3-N l^{-1} and 14.3 NO_3-N l^{-1} for poplar and willow respectively following cultivation and establishment (Schimdt-Walter and Lamersdorf, 2012). Therefore it was concluded that these operations will affect the soil GHG budget and N leaching during land use change.

Clay content of the soil together with tillage significantly affected post-ploughing N_2O emissions. Higher emissions were associated with higher clay content and also with conventional tillage due to degassing process following inversion tillage. No clear correlation was found between N_2O emissions and environmental factors, most

probably due to high variability of the weather data set, however, it could be hypothesised that unusually wet and warm weather over the experimental period did in fact influence the emissions. Summer is an unusual time for ploughing and this timing more than likely caused high N losses as an effect of the environmental conditions. Whilst other studies have also highlighted increased N_2O and leached N emissions following cultivation of grasslands, these emissions are lower than chemical treatment of grass followed by sowing over old grass sward (Dobbie *et al.*, 2003; Vellinga *et al.* 2004, Velthof *et al.* 2010, MacDonald *et al.* 2011). This is most likely due to the fact that chemical amendment and sowing over old grass sward leaves the organic matter near the surface whilst ploughing deposits the organic residues deeper in the soil, where more anaerobic conditions can promote a larger proportion of total denitrification.

Unexpectedly, the lowest cumulative losses of combined N_2O and leached N were observed in the case of the poorly draining gley soil. While the gaseous component (N_2O) was substantial, as also shown in Ball *et al.* (1999), leaching remained a minor loss pathway, therefore leaving the overall losses significantly lower than from other two soils. Minimum tillage, suspected to enhance N_2O emissions (Aulakh *et al.*, 1984), did not have such an effect on the examined soils throughout this study. Minimum tillage through preservation of soil moisture, soil aggregates, and restricting air flow most probably enhanced denitrification causing N_2O reduction to N_2. Therefore, minimum tillage which is believed to reduce the risk of soil organic carbon loss (Paustian *et al.*, 2000) could minimise the negative impact on the environment without adverse effects on N_2O emissions.

To further investigate land use change the second experiment was designed to quantify the N_2O emissions following grassland conversion into energy crops, and to assess the differences between the N_2O emissions of the two energy grasses: *Miscanthus* and Reed canarygrass in field conditions. Most N_2O research is conducted on small plots facilitating replication, and over short time periods, however it is argued that field scale experiments close to real farm situations, despite large spatial and temporal variability provide valuable insight on the N cycling (Cassman *et al.*, 2002, Saggar *et al.*, 2004). Therefore here, a field scale experiment was conducted between April 2009 and April 2011 to allow deployment of real a farm scenario. Experimental plots were prepared

using standard agricultural practice such as herbicide application and conventional inversion tillage.

Results of Chapter 3 found that N_2O emissions from *Miscanthus* sites were not significantly different from the fallow grassland in the first year of the cultivation. The majority of emissions occurred in the summer months and were associated with specific events, similarly to the findings of Jorgensen *et al.* (1997) from a study on *Miscanthus*. It is believed that high summer fluxes were caused by soil disturbance and a subsequent mineralisation of organic nitrogen stored in soil due to the previous land use, combined with favourable weather conditions for enhanced microbial activity. It was difficult to correlate levels of N_2O emissions with soil temperature and moisture. Increasing soil temperature was found to be significantly correlated with increased emission, however due to data variability no clear relationship was found with soil moisture. This could have been due to the fact that the systems examined in this experiment were disturbed with ploughing and change in plant cover. *Miscanthus*' emissions decreased in the second year of cultivation. Fallow grassland plots converted into Reed canarygrass in year two of the experiment produced significantly less N_2O than *Miscanthus*. Economically viable yields were achieved in the third year of *Miscanthus* cultivation, and in the first year of Reed canarygrass cultivation. The amount of N_2O emitted per tonne of yield decreased over time, with both decrease of N_2O emissions and increase of the harvestable above ground biomass.

Fertilisation is the main contributor to N_2O emissions from agricultural fields (Storey, 1999, Mosier and Kroeze, 2000) as well as being a major economic cost for the farmer. A lysimeter study was undertaken to investigate the response of energy crops to nitrogen fertilisation where *Miscanthus*, Reed canarygrass and grassland systems based on a various soil drainage types were fertilised at a low fertilisation rate and the fate of the applied N was followed.

Fertilisation was a major source of N losses from all the soil-crop systems mainly through gaseous N products: N_2 and N_2O emissions. Similar to previous studies by Flechard et al. (2007) on 10 European grasslands, Skiba and Ball (2002) on poorly drained silty clay loams to free drained sandy loams, and Henault et al (1998) on a gley soil with high clay content to a freely draining soil, soil type and soil management had

a large effect of on N_2O emissions. Poorly drained soils were associated with highest N_2O emissions. The exception was an energy crop-*Miscanthus*.

Emission factors found in this study varied widely between the crops and soils (0.3 – 4.4%) and were similar to those reported by Clayton *et al.* (1997), Dobbie *et al.* (1999), Mosier and Kroeze (1999), Crutzen *et al.* (2008), and Skiba and Smith (2000). The lowest emission factor was found in *Miscanthus*. However, in most cases, the emission factors were higher than the IPCC (2001) EF of 1% of N applied that is commonly used for GHG inventories. This is in line with other Irish studies (Abdalla *et al.*, 2009, Hyde *et al.*, 2006, Hsieh *et al.*, 2005, Rafique *et al.*, 2011) reporting that the 1% EF causes underestimation of national GHG emissions.

N_2O was produced through both nitrification and denitrification, with nitrification share highest in well drained soils with coarse texture and denitrification in poorly drained soils. Complete denitrification is an important process leading to N_2O reduction to N_2 and therefore removing reactive nitrogen from the environment (Smil, 1999). Similarly to Clough *et al.* (1998) and Dannenmann *et al* (2008) N_2 was the main gaseous N product after N fertilisation with N_2:N_2O ratios high from all the soils. High share of N_2 in denitrification end products could have been caused by temperature, soil moisture content, and pH. Differences between N_2:N_2O ratios between crops could arise from community structure and the proportion of fungal to bacterial communities. More fungal rather than bacterial dominated communities normally occurring in undisturbed systems such as grasslands, could be responsible for more N_2O release as fungi lack nitrous oxide reductase. Large amounts of N_2 evolution detected in this study on heavier soils correlate well with previous studies demonstrating that a high proportion of total denitrification and/or codenitrification can occur on clay soils and account for 80-90% of gaseous N loss (Clough *et al.* 1998; Stevens and Laughlin 2002; Spott *et al.* 2011).

Miscanthus was characterised by the highest denitrification rate and at the same time, the lowest N_2O emissions. The balance between gaseous N emissions was heavily shifted towards N_2 additionally explaining low emission factor. Miscanthus is an example of a C4 grass which are characterised by high N use efficiency therefore this could be the primary reason of the low N losses from this plant system. Additionally,

the N fixing bacteria found in *Miscanthus*' root system, Azospirillum doebereinerae sp. (Eckert *et al.*, 2001) could also affect its N cycling reducing the overall N losses and fertiliser response from this crop-soil system.

Leaching was not an important N loss pathway from any of the studied crop-soil systems most probably due to environmental conditions favourable to gaseous emissions as a loss pathway rather than leaching. The majority of fertiliser N remaining in the system at the end of the experiment was recovered in soil, most probably in immobilised organic form. No significant effect of applied N was seen in plant biomass suggesting high N efficiency of the energy crops and their low fertiliser requirements. This is comparable with Danalatos *et al.* (2007) where doubling the N-fertilisation rate of *Miscanthus* from 50 kg N ha^{-1} to 100 kg N ha^{-1} had no effect on the yield, and with Himken *et al.* (1997), Boehmel *et al.* (2007), and Maughan *et al.* (2012) where *Miscanthus* was argued not to need fertilisation at all.

5.3 Limitations of the study

Various limitations were encountered during all three experiments included in this thesis. In the first experiment (Chapter 2) it was not possible to include control as all lysimeters had to be ploughed. To overcome this problem N_2O was monitored prior to ploughing and fertilisation and after ensuring that weather conditions were similar pre and post-treatment and therefore would not affect the results, these data were used as an approximation of the background N_2O fluxes. As a result EFs calculated from this study are only a rough approximation however they offer a useful comparison of the treatments.

Due to the fact that leaching is a slower loss pathway than gaseous emissions fractions of leached N resulting from ploughing and fertilisation could not be apportioned. Temporal pattern of leaching exhibited a clear peak following ploughing and fertilisation however leached N from both sources contributed to that peak simultaneously.

Similarly, no control could be included in Chapter 3 due to the scale of the experiment. As CO_2 in the concurrent experiment was measured by eddy covariance technique all of grassland devoted for the study had to undergo land use change to energy crops to ensure areas big enough to use this technique. However as field scale experiments

In Chapter 4 *Miscanthus* based on well drained soil was not included. This was due to the fact that the crop in the collected lysimeters did not survive the experiment preparation phase and this crop-soil system had to be abandoned. Use of lysimeters in this experiment represented tightly managed research situation enabling close monitoring of the N cycling in the crop-soil systems however small areas of the lysimeters made biomass sampling difficult and potentially introduced error when up scaling the results due to small sample size.

Finally, there were some limitations applicable to all chapters. Closed chambers technique commonly used for N_2O measurements dictates the sampling frequencies to be weekly, bi-monthly, or several times a week during periods of expected high fluxes such as post-fertilisation. However, if no fertiliser is added potentially important N_2O peaks might be missed due to sampling frequency (Yao *et al.*, 2009).

5.4 Future work

Despite these limitations this study offers a valuable contribution to the current knowledge of the topic. Concurrent experiments were conducted using the same site in Wexford therefore providing more data for further analyses. The sites used in the above experiments were also used for a carbon cycling study which investigated CO_2 emissions and uptakes (Ní Chonchubhair, unpublished PhD thesis), and a study investigating the effects of future climate changes such as increased temperatures and reduced rainfall on *Miscanthus* and Reed canarygrass yields and associated GHG emissions (Otero *et al.*, 2011). Therefore it is important that these data are used to calculate a complete carbon and nitrogen *Miscanthus* budget for this site and therefore improve our understanding of the effects of such land use change. In a broader context the work described in this thesis presents a valuable opportunity for further research. This work offers an important foundation for calculating GHG balance of the energy

crops in Ireland. Further monitoring of N_2O emissions from mature *Miscanthus* and Reed canarygrass would be beneficial to enable more thorough understanding of the N_2O flux over the crops lifespan. Continuation of this line of research is recommended to gain more understanding on the long term effects of land use change to energy crops on N_2O emissions, especially in various climatic and soil conditions. Together with N_2O, CO_2 and CH_4 should also be investigated in order to achieve a complete GHG budget of energy crops. Ideally, this extended research would contribute to providing detailed best practice guidelines for land use change.

5.5 Implications

In the context of this study the N losses associated with the land use change into energy crops were quantified with ploughing responsible for between 0.4 and 1.0 kg N_2O-N ha^{-1} release of N_2O. Subsequent fertilisation yielded between 1.7 and 8.4 kg N_2O-N ha^{-1}. After including leached losses in the N budget, total losses between ploughing and fertilisation ranged from 6.4 ± 0.7 kg N ha^{-1} and 22.6 ± 2.4 kg N ha^{-1}. Cultivation of perennial, rather than annual energy crops could further reduce the need for tillage and associated losses. Fertilisation being the main source of emissions indicates that the fertiliser requirements and the fertiliser use efficiency play important role in minimising losses and that cultivation of N-efficient energy crops is viable. N_2O emissions from *Miscanthus* plots, however high in the first year after the land use change, were lower than those reported for fertilised Irish grasslands (Hsieh *et al.*, 2005) and decreased further over time, whereas emissions from Reed canarygrass plots significantly lower than from *Miscanthus*. The findings of Roth *et al.*, (2011) on land use change to *Miscanthus* on sandy loam in Ireland show N_2O flux similar to that of adjacent grassland and also point to a frequent N_2O uptake from the energy crop system. Drewer *et al.* (2012) recorded N_2O flux from *Miscanthus* site on fine loam soil in north-east England to be lower than that of annual crops. These two studies are currently the only available datasets of N_2O flux from *Miscanthus* sites in Ireland and the UK and they both suggest no negative effect of land use change to energy crops on N_2O. Energy crops emit between 40% and 99% less N_2O than conventional annual crops (Don *et al.*, 2011) however the figures comparing energy crops with grassland might be substantially different. Carbon balance study on the same experimental site in

Wexford found that both *Miscanthus* and Reed canarygrass were a net GHG sources in the first two and one year of cultivation respectively. However, both crops became strong GHG sinks when mature with *Miscanthus* providing a sink of 14.6 t CO_2 ha^{-1} yr^{-1}, and Reed canarygrass of 12.5 t CO_2 ha^{-1} yr^{-1} in comparison with 2.9 t CO_2 ha^{-1} yr^{-1} sequester by pasture. Therefore land use change to energy crops has a potential for reducing Irish GHG emissions, the extent of these reductions dependent on former and future management practices.

Field nitrous oxide emissions can be defined as follows:

$$N_2O_{total} = (F_{SN}* EF) + (F_{ON}*EF) + (F_{SOM}*EF) + (F_{CR}*EF)$$

Where F_{SN}, F_{ON} F_{SOM} and F_{CR} are the amounts of N inputted from synthetic fertiliser, organic N, soil organic matter mineralisation (on ploughing) and crop residues respectively. EF is the emission factor associated with each of these N sources. Currently there is a single emission factor for all of these N sources (1%, see IPCC 2006). However, the results of this project clearly show that there are variations in both EF_{SOM} and EF_{SN} associated with variations in a) soil type, b) tillage intensity and c) crop type. These results show not only that *Miscanthus'* N use efficiency upon fertilisation was superior to other crops but also highlight the need of further research of emission factors specific for Ireland in order to replace IPCC Tier 1 methodology.

Based on the work presented in this thesis it is believed that biomass crops can, as a result of more widespread cultivation, reduce reactive N emissions in the agricultural sector particularly via enhanced N use and a lower N requirement as well as less intensive management. Especially in terms of EF_{SN}, low fertiliser application rates together with high plant efficiency make energy crops a viable cultivation option. This study demonstrates high N_2O release during the establishment of these crops, but a subsequent reduction in gaseous N losses in a mature crop relative to grass-based systems. High N losses were associated with ploughing, fertiliser application, and the first two years of cultivation of enery crops but they were comparable to or lower than those of Irish grasslands (Hsieh *et al.*, 2005; Hyde *et al.*, 2006; Rafique *et al.*, 2011). In addition, greenhouse gas emissions can be further reduced via enhanced soil C sequestration rates and by the provision of low or carbon neutral indigenous fuels.

5.6. Conclusions

Land use change to energy crops generally led to enhanced N_2O emissions due to soil disturbance and subsequent mineralisation in the first year of cultivation. However, it is unlikely that LUC will have negative impact on medium to long term N_2O emissions. Energy crops, after the intial stage of enhanced emissions, proved to have a GHG budget more favourable to conventionally managed grasslands. Their low N_2O emissions together with low fertilisation requirements and high achievable yields make them a viable option for large scale agricultural deployment.

Previous land use was of great importance as the soil microbial community and nutrient status affected the magnitude of gaseous N losses. Soil type and drainage properties greatly affected the N losses. Accounting for leaching losses in the second experiment provided more detailed N cycling analysis and the magnitude of the leaching component proved to be an important loss pathway for well draining soils that should not be underestimated. On the other hand, poorly draining soils were associated with the lowest overall N losses possibly due to complete denitrification to N_2 and should be considered for land use change into energy crops.

Fluxes following N fertilisation represented the biggest component of the gaseous losses and could be largely avoided with reduced fertilisation which is characteristic for N-efficient energy crops, especially C4 crops such as *Miscanthus*.

Emissions associated with the first year of *Miscanthus* cultivation were comparable to those of managed grassland and did not differ significantly from the experimental set-aside grassland. Approximately 40% reduction in emissions was noted in the second year of cultivation. Newly introduced Reed canarygrass produced even lower N_2O than second year's *Miscanthus*.

N_2O emissions from soils supporting energy crops were driven by environmental factors such as temperature. Moisture has previously been found to affect N_2O fluxes but was not found to be a significant factor in the course of the study.

The majority of N_2O was emitted during summer months. Enhanced fluxes were associated with specific effects of soil disturbance and subsequent mineralisation, weather conditions, or both of these combined.

Biomass yields of commercial value were achieved in the first year of Reed canarygrass, and in the third year of *Miscanthus* cultivation, with yields above 6 and 10 t ha^{-1}, respectively. The environmental cost of the yields in terms of N_2O emitted per yield unit decreased dramatically in the third cultivation season of *Miscanthus* where a vigorous growth of above ground biomass was observed.

Fertilisation of perennial grassland and energy crops led to enhanced N_2O emissions. Lowest emissions and emission factors were found amongst the energy crops. Most of gaseous N emitted from energy crops originated from denitrification and possibly co-denitrification processes and the majority was emitted in the form of inert N_2 with no adverse effects on the environment.

Following fertilisation crop uptake was largely outweighed by soil immobilisation and gaseous losses, however leached losses were negligible. Environmental conditions were believed to favour gaseous emissions compared to leaching.

References:

Abbasi, M.K., Adams, W.A., 2000. Gaseous N emission during simultaneous nitrification-denitrification associated with mineral N fertilization to a grassland soil under field conditions. Soil Biology & Biochemistry 32, 1251-1259.

Abdalla, M., Jones, M., Smith, P., Williams, M., 2009. Nitrous oxide fluxes and denitrification sensitivity to temperature in Irish pasture soils. Soil Use Manage. 25, 376-388.

Aber, J.D., Magill, A., McNulty, S.G., Boone, R.D., Nadelhoffer, K.J., Downs, M., Hallett, R., 1995. Forest biogeochemistry and primary production altered by nitrogen saturation. Water Air Soil Pollut. 85, 1665-1670.

Adler, P.R., Del Grosso, S.J., Parton, W.J., 2007. Life-cycle assessment of net greenhouse-gas flux for bioenergy cropping systems. Ecol. Appl. 17, 675-691.

Agele, S.O., Ewulo, B.S., Oyewusi, I.K., 2005. Effects of some soil management systems on soil physical properties, microbial biomass and nutrient distribution under rainfed maize production in a humid rainforest Alfisol. Nutrient Cycling in Agroecosystems 72, 121-134.

Ammann, A.A., Ruttimann, T.B., Burgi, F., 2000. Simultaneous determination of TOC and TNb in surface and wastewater by optimised high temperature catalytic combustion. Water Res. 34, 3573-3579.

Arah, J.R.M., 1997. Apportioning nitrous oxide fluxes between nitrification and denitrification using gas-phase mass spectrometry. Soil Biology & Biochemistry 29, 1295-1299.

Arah, J.R.M., Smith, K.A., Crichton, I.J., Li, H.S., 1991. Nitrous-oxide production and denitrification in scottish arable soils. Journal of Soil Science 42, 351-367.

Asner, G.P., Elmore, A.J., Olander, L.P., Martin, R.E., Harris, A.T., 2004. Grazing systems, ecosystem responses, and global change. Annu. Rev. Environ. Resour. 29, 261-299.

Aulakh, M.S., Doran, J.W., Walters, D.T., Power, J.F., 1991. Legume residue and soil-water effects on denitrification in soils of different textures. Soil Biology & Biochemistry 23, 1161-1167.

Aulakh, M.S., Rennie, D.A., Paul, E.A., 1984. Gaseous nitrogen losses from soils under zero-till as compared with conventional-till management-systems. Journal of Environmental Quality 13, 130-136.

Badr, O., Probert, S.D., 1993. Environmental impacts of atmospheric nitrous-oxide. Appl. Energy 44, 197-231.

Baggs, E. M. 2008. A review of stable isotope techniques for N_2O source partitioning in soils: recent progress, remaining challenges and future considerations. Rapid Communications in Mass Spectrometry, 22, 1664-1672.

Baggs, E.M., 2011. Soil microbial sources of nitrous oxide: recent advances in knowledge, emerging challenges and future direction. Curr. Opin. Environ. Sustain. 3, 321-327.

Bailey, L.D., Beaucham.Eg, 1973. Effects of temperature on NO^{3-} and $N)^{2-}$ reduction, nitrogenous gas production, and redox potential in a saturated soil. Canadian Journal of Soil Science 53, 213-218.

Ball, B.C., Crichton, I., Horgan, G.W., 2008. Dynamics of upward and downward N_2O and CO_2 fluxes in ploughed or no-tilled soils in relation to water-filled pore space, compaction and crop presence. Soil Tillage Res. 101, 20-30.

Ball, B.C., Scott, A., Parker, J.P., 1999. Field N_2O, CO_2 and CH_4 fluxes in relation to tillage, compaction and soil quality in Scotland. Soil Tillage Res. 53, 29-39.

Barnard, R., Leadley, P.W., Hungate, B.A., 2005. Global change, nitrification, and denitrification: A review. Glob. Biogeochem. Cycle 19.

Bartle, J. R. & Abadi, A. 2009. Toward sustainable production of second generation bioenergy feedstocks. Energy & Fuels, 24, 2-9.

Bateman, E.J., Baggs, E.M., 2005. Contributions of nitrification and denitrification to N2O emissions from soils at different water-filled pore space. Biology and Fertility of Soils 41, 379-388.

Beale, C. V. & Long, S. P. 1997. Seasonal dynamics of nutrient accumulation and partitioning in the perennial C_4 grasses *Miscanthus* x giganteus and Spartina cynosuroides. Biomass and Bioenergy, 12, 419-428.

Beare, M.H., Hendrix, P.F., Coleman, D.C., 1994. Water-stable aggregates and organic-matter fractions in conventional-tillage and no-tillage soils. Soil Science Society of America Journal 58, 777-786.

Beaudoin, N., Saad, J.K., Van Laethem, C., Machet, J.M., Maucorps, J., Mary, B., 2005. Nitrate leaching in intensive agriculture in Northern France: Effect of farming practices, soils and crop rotations. Agriculture Ecosystems & Environment 111, 292-310.

Berglund, O., Berglund, K., 2011. Influence of water table level and soil properties on emissions of greenhouse gases from cultivated peat soil. Soil Biology & Biochemistry 43, 923-931.

Bergstrom, L., Johansson, R., 1991. Leaching of nitrate from monolith lysimeters of different types of agricultural soils. Journal of Environmental Quality 20, 801-807.

Boehmel, C., Lewandowski, I., Claupein, W., 2008. Comparing annual and perennial energy cropping systems with different management intensities. Agric. Syst. 96, 224-236.

Bollmann, A., Conrad, R., 1998. Influence of O_2 availability on NO and N_2O release by nitrification and denitrification in soils. Glob. Change Biol. 4, 387-396.

Bollmann, A., Laanbroek, H.J., 2001. Continuous culture enrichments of ammonia-oxidizing bacteria at low ammonium concentrations. FEMS Microbiol. Ecol. 37, 211-221.

Borjesson, P., 1999. Environmental effects of energy crop cultivation in Sweden - I: Identification and quantification. Biomass Bioenerg. 16, 137-154.

Bouwman, A.F., 1996. Direct emission of nitrous oxide from agricultural soils. Nutrient Cycling in Agroecosystems 46, 53-70.

Bouma, J., G. Varallyay, and N. H. Batjes (1995), Principal land use changes anticipated in Europe, paper presented at Conference on Long-Term Perspectives for Effects of Rural Land Use Changes on Soil Contaminants, Arona, Italy, Jun 08-10.

Bouwman, A.F., Boumans, L.J.M., Batjes, N.H., 2002. Emissions of N_2O and NO from fertilized fields: Summary of available measurement data. Glob. Biogeochem. Cycle 16, 13.

Bransby, D.I., McLaughlin, S.B., Parrish, D.J., 1998. A review of carbon and nitrogen balances in switchgrass grown for energy. Biomass Bioenerg. 14, 379-384.

Breen, J.P., Donnellan, T., Westhoff, P., 2010. Food for Thought: EU Climate Change
Policy Presents New Challenges for Agriculture. EuroChoices 9, 24-29.

Bremner, J.M., 1997. Sources of nitrous oxide in soils. Nutrient Cycling in Agroecosystems 49, 7-16.

Bremner, J.M., Blackmer, A.M., 1979. Effects of acetylene and soil-water content on emission of nitrous-oxide from soils. Nature 280, 380-381.

Brooks, P.D., Stark, J.M., McInteer, B.B., Preston, T., 1989. Diffusion method to prepare soil extracts for automated -N15 analysis. Soil Science Society of America Journal 53, 1707-1711.

Brown, L., Brown, S.A., Jarvis, S.C., Syed, B., Goulding, K.W.T., Phillips, V.R., Sneath, R.W., Pain, B.F., 2001. An inventory of nitrous oxide emissions from agriculture in the UK using the IPCC methodology: emission estimate, uncertainty and sensitivity analysis. Atmos. Environ. 35, 1439-1449.

BSI, 1999. Soil quality - physical methods., Determination of the water retention characteristics - laboratory methods.

BSI, 2006. Soil improvers and growing media. Determination of physical properties —Dry bulk density, air volume, water volume, shrinkage value and total pore space.

Butterbach-Bahl, K., Dannenmann, M., 2011. Denitrification and associated soil N_2O emissions due to agricultural activities in a changing climate. Curr. Opin. Environ. Sustain. 3, 389-395.

Byrne, C.M.P., Hayes, M.H.B., Kumar, R., Novotny, E.H., Lanigan, G., Richards, K.G., Fay, D., Simpson, A.J. 2010. Compositional changes in the hydrophobic acids fraction of drainage water from different land management practices. Water Research, 44, 4379-4390.

Cadoux, S., Riche, A. B., Yates, N. E. & Machet, J.-M. 2012. Nutrient requirements of *Miscanthus* x giganteus: Conclusions from a review of published studies. Biomass and Bioenergy, 38, 14-22.

Cameron, K.C., Smith, N.P., McLay, C.D., Fraser, P.M., McPherson, R.J., Harrison, D.F. and Hardbottle, P. 1992 Lysimeters without edge flow: an improved design and sampling procedure. Soil Science Society of America Journal 56, 1625-1628.

Carpenter, S.R., Caraco, N.F., Correll, D.L., Howarth, R.W., Sharpley, A.N., Smith, V.H., 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecol. Appl. 8, 559-568.

Casler, M.D., Vogel, K.P., Taliaferro, C.M., Ehlke, N.J., Berdahl, J.D., Brummer, E.C., Kallenbach, R.L., West, C.P., Mitchell, R.B., 2007. Latitudinal and longitudinal adaptation of switchgrass populations. Crop Science 47, 2249-2260.

Caslin B., 2008. The Growing Bioenergy Opportunities in Agriculture. Bioenergy 2008, Teagasc Mellows Centre, Athenry, June 19th 2008.

Chadwick D., 1997. Nitrous oxide and ammonia emissions from grassland following applications of slurry: potential abatement practices. Gaseous Nireogen Emissions from Grasslands 257-264. Eds Jarvis S.C. and Pain B.F. Wallingford, UK: CAB International.

Chamberlain, J.F., Miller, S.A., Frederick, J.R., 2011. Using DAYCENT to quantify on-farm GHG emissions and N dynamics of land use conversion to N-

managed switchgrass in the Southern U.S. Agriculture Ecosystems & Environment 141, 332-341.

Chapuis-Lardy, L., Wrage, N., Metay, A., Chotte, J.L., Bernoux, M., 2007. Soils, a sink for N_2O? A review. Glob. Change Biol. 13, 1-17.

Choudhary, M.A., Akramkhanov, A., Saggar, S., 2002. Nitrous oxide emissions from a New Zealand cropped soil: tillage effects, spatial and seasonal variability. Agriculture Ecosystems & Environment 93, 33-43.

Christian, D.G., Poulton, P.R., Riche, A.B., Yates, N.E., 1997. The recovery of N-15-labelled fertilizer applied to *Miscanthus* x Giganteus. Biomass Bioenerg. 12, 21-24.

Christian, D.G., Poulton, P.R., Riche, A.B., Yates, N.E., Todd, A.D., 2006. The recovery over several seasons of N-15-labelled fertilizer applied to *Miscanthus* x giganteus ranging from 1 to 3 years old. Biomass Bioenerg. 30, 125-133.

Christian, D.G., Riche, A.B., 1998. Nitrate leaching losses under Miscanthuus grass planted on a silty clay loam soil. Soil Use Manage. 14, 131-135.

Ciarlo, E., Conti, M., Bartoloni, N., Rubio, G., 2008. Soil N_2O emissions and $N_2O/(N_2O+N_2)$ ratio as affected by different fertilization practices and soil moisture. Biology and Fertility of Soils 44, 991-995.

Clayton, H., McTaggart, I.P., Parker, J., Swan, L., Smith, K.A., 1997. Nitrous oxide emissions from fertilised grassland: A 2-year study of the effects of N fertiliser form and environmental conditions. Biology and Fertility of Soils 25, 252-260.

Clifton-Brown, J. C. & Lewandowski, I. 2000. Overwintering problems of newly established *Miscanthus* plantations can be overcome by identifying genotypes with improved rhizome cold tolerance. New Phytologist, 148, 287-294.

Clifton-Brown, J.C., Neilson, B., Lewandowski, I., Jones, M.B., 2000. The modelled productivity of *Miscanthus* x giganteus (GREEF et DEU) in Ireland. Industrial Crops and Products 12, 97-109.

Clifton-Brown, J.C., Stampfl, P.F., Jones, M.B., 2004. *Miscanthus* biomass production for energy in Europe and its potential contribution to decreasing fossil fuel carbon emissions. Glob. Change Biol. 10, 509-518.

Clough, T.J., Ledgard, S.F., Sprosen, M.S., Kear, M.J., 1998. Fate of N-15 labelled urine on four soil types. Plant and Soil 199, 195-203.

Cole, C.V., Duxbury, J., Freney, J., Heinemeyer, O., Minami, K., Mosier, A., Paustian, K., Rosenberg, N., Sampson, N., Sauerbeck, D., Zhao, Q., 1995.

Global estimates of potential mitigation of greenhouse gas emissions by agriculture.
International Symposium on Soil-Source and Sink of Greenhouse Gases. Kluwer Academic Publ, Nanjing, Peoples R China, pp. 221-228.

Cole, C.V., Duxbury, J., Freney, J., Heinemeyer, O., Minami, K., Mosier, A., Paustian, K., Rosenberg, N., Sampson, N., Sauerbeck, D., Zhao, Q., 1997. Global estimates of potential mitigation of greenhouse gas emissions by agriculture. Nutrient Cycling in Agroecosystems 49, 221-228.

Constantin, J., Mary, B., Laurent, F., Aubrion, G., Fontaine, A., Kerveillant, P., Beaudoin, N., 2010. Effects of catch crops, no till and reduced nitrogen fertilization on nitrogen leaching and balance in three long-term experiments. Agriculture Ecosystems & Environment 135, 268-278.

Corine, 2006. Ireland. Land cover 2006. Overview of land cover & change 2000-2006. In: Agency, E.E. (Ed.).

Coyne, M.S., 2008. Biological Denitrification. In: Schepers J.S., Raun W.R. (Eds.), Nitrogen in Agricultural Systems.

Crutzen, P.J., Mosier, A.R., Smith, K.A., Winiwarter, W., 2008. N_2O release from agro-biofuel production negates global warming reduction by replacing fossil fuels. Atmospheric Chemistry and Physics 8, 389-395.

CSO, 2009. Environmental Indicators Ireland 2012. In: Office, C.S. (Ed.). Stationery Office, Dublin, Ireland, Dublin.

Cuhel, J., Simek, M., Laughlin, R.J., Bru, D., Cheneby, D., Watson, C.J., Philippot, L., 2010. Insights into the Effect of Soil pH on N_2O and N_2 Emissions and Denitrifier Community Size and Activity. Appl. Environ. Microbiol. 76, 1870-1878.

DAFF, 2008. Fact sheet on Irish agriculture. In: Division, E.a.P., Food, D.o.A.a. (Eds.), Dublin.

DAFF, 2010. Food Harvest 2020. In: Division, E.a.P., Food, D.o.A.a. (Eds.), Dublin.

Dalal, R.C., Wang, W.J., Robertson, G.P., Parton, W.J., 2003. Nitrous oxide emission from Australian agricultural lands and mitigation options: a review. Aust. J. Soil Res. 41, 165-195.

Danalatos, N. G., Archontoulis, S. V. & Mitsios, I. 2007. Potential growth and biomass productivity of *Miscanthus* x giganteus as affected by plant density and N-fertilization in central Greece. Biomass and Bioenergy, 31, 145-152.

Dannenmann, M., Butterbach-Bahl, K., Gasche, R., Willibald, G. & Papen, H. 2008. Dinitrogen emissions and the N_2:N_2O emission ratio of a Rendzic

Leptosol as influenced by pH and forest thinning. Soil Biology and Biochemistry, 40, 2317-2323.

Davidson, E.A., Seitzinger, S., 2006. The enigma of progress in denitrification research. Ecol. Appl. 16, 2057-2063.

Davis, S., Parton, W., Dohleman, F., Smith, C., Grosso, S., Kent, A. & Delucia, E. 2010. Comparative biogeochemical cycles of bioenergy crops reveal nitrogen-fixation and low greenhouse gas emissions in a *Miscanthus* × giganteus agro-ecosystem. Ecosystems, 13, 144-156.

Davies, M.G., Smith, K.A., Vinten, A.J.A., 2001. The mineralisation and fate of nitrogen following ploughing of grass and grass-clover swards. Biology and Fertility of Soils 33, 423-434.

de Klein, C.A.M., Eckard, R.J., 2008. Targeted technologies for nitrous oxide abatement from animal agriculture. Australian Journal Of Experimental Agriculture, 48, 14-20.

de Vries, F.T., Hoffland, E., van Eekeren, N., Brussaard, L., Bloem, J., 2006. Fungal/bacterial ratios in grasslands with contrasting nitrogen management. Soil Biology & Biochemistry 38, 2092-2103.

de Vries, W., Kros, J., Reinds, G.J., Butterbach-Bahl, K., 2011. Quantifying impacts of nitrogen use in European agriculture on global warming potential. Curr. Opin. Environ. Sustain. 3, 291-302.

del Prado, A., Merino, P., Estavillo, J.M., Pinto, M., Gonzalez-Murua, C., 2006. N_2O and NO emissions from different N sources and under a range of soil water contents. Nutrient Cycling in Agroecosystems 74, 229-243.

Delgado, J.A., Del Grosso, S.J., Ogle, S.M., 2010. ^{15}N isotopic crop residue cycling studies and modeling suggest that IPCC methodologies to assess residue contributions to N_2O-N emissions should be reevaluated. Nutrient Cycling in Agroecosystems 86, 383-390.

Dennis, S.J., Cameron, K.C., Di, H.J., Moir, J.L., Staples, V., Sills, P., Richards, K.G., 2012. Reducing nitrate losses from simulated grazing on grassland lysimeters in Ireland using a nitrification inhibitor (dicyandiamide). Biology and Environment-Proceedings of the Royal Irish Academy 112B, 79-89.

Department of Environment, H.a.L.G., 2007. Ireland: National Climate Change Strategy 2007-2012. Dublin, Ireland.

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. J. Geophys. Res.-Atmos. 104, 26891-26899.

Dobbie, K.E., Smith, K.A., 2003. Nitrous oxide emission factors for agricultural soils in Great Britain: the impact of soil water-filled pore space and other controlling variables. Glob. Change Biol. 9, 204-218.

Donnelly, A., D. Styles, J. Fitzgerald, and J. Finnan (2011), A proposed framework for determining the environmental impact of replacing agricultural grassland with *Miscanthus* in Ireland GCB Bioenergy, 3(3).

Dowdell, R.J., Colbourn, P., Cannell, R.Q., 1987. A study of mole drainage with simplified cultivation for autumn-sown crops on a clay soil .5. losses of nitrate-n in surface run-off and drain water. Soil Tillage Res. 9, 317-331.

Drewer, J., Finch, J.W., Lloyd, C.R., Baggs, E.M., Skiba, U., 2012. How do soil emissions of N_2O, CH_4 and CO_2 from perennial bioenergy crops differ from arable annual crops? GCB Bioenergy 4, 408-419.

Drury, C.F., Yang, X.M., Reynolds, W.D., Tan, C.S, 2004. Influence of crop rotation and aggregate size on carbon dioxide production and denitrification. Soil & Tillage Research 79, 87-100.

Duffy, P., Hyde, B., Hanley, E., Core, C., O'brien, P., Cotter, E. & Black, K. 2011. Ireland national inventory report 2011, greenhouse gas emissions 1990-2009 reported to the United Nations framework convention on climate change. Wexford: Environmental Protection Agency.

Eck, H.V., Jones, O.R., 1992. Soil-nitrogen status as affected by tillage, crops, and crop sequences. Agron. J. 84, 660-668.

EPA, 2007. Energy White Paper.

EPA, 2009. The EPA & Climate Change
Responsibilities, challenges and opportunities. Environmental Protection Agency, Johnstown Castle estate, Wexford.

EPA, 2011a. Greenhouse Gas Emissions -Distance to Ireland's Kyoto Limit. In: http://www.epa.ie/media/ghgkyoto.pdf (Ed.).

EPA, 2011b. Ireland's Greenhouse Gas Emissions In 2009. In: EPA (Ed.).

EPA, 2011c. Ireland's Greenhouse Gas Emissions Projections 2010-2020. In: EPA (Ed.).

Erickson, H.E., Keller, M., 1997. Tropical land use change and soil emissions of nitrogen oxides. Soil Use Manage. 13, 278-287.

Erisman, J.W., van Grinsven, H., Leip, A., Mosier, A., Bleeker, A., 2010. Nitrogen and biofuels; an overview of the current state of knowledge. Nutrient Cycling in Agroecosystems 86, 211-223.

Estavillo, J.M., Merino, P., Pinto, M., Yamulki, S., Gebauer, G., Sapek, A., Corre, W., 2002. Short term effect of ploughing a permanent pasture on N_2O production from nitrification and denitrification. Plant and Soil 239, 253-265.

Evans, C. D., D. Norris, N. Ostle, H. Grant, E. C. Rowe, C. J. Curtis, and B. Reynolds (2008), Rapid immobilisation and leaching of wet-deposited nitrate in upland organic soils Environ. Pollut., 156(3), 636-643.

Fawcett, R.S., 1995. Agricultural tillage systems: impacts on nutrient and pesticide runoff and leaching., Farming For a Better Environment: A White Paper. Soil and Water Conservation Society, Ankeny, p. 67.

Fenn, M.E., Poth, M.A., Aber, J.D., Baron, J.S., Bormann, B.T., Johnson, D.W., Lemly, A.D., McNulty, S.G., Ryan, D.E., Stottlemyer, R., 1998. Nitrogen excess in North American ecosystems: Predisposing factors, ecosystem responses, and management strategies. Ecol. Appl. 8, 706-733.

Firestone, M.K., 1982. Biological denitrification. Stevenson, F. J., pp. P289-326.

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 Terrestial Ecosystems and the Atmosphere. Wiley, Chichester.

Firestone, M.K., Tiedje, J.M., 1979. Temporal change in nitrous-oxide and dinitrogen from denitrification following onset of anaerobiosis. Appl. Environ. Microbiol. 38, 673-679.

Flechard, C.R., Ambus, P., Skiba, U., Rees, R.M., Hensen, A., van Amstel, A., Pol-van Dasselaar, A.V., Soussana, J.F., Jones, M., Clifton-Brown, J., Raschi, A., Horvath, L., Neftel, A., Jocher, M., Ammann, C., Leifeld, J., Fuhrer, J., Calanca, P., Thalman, E., Pilegaard, K., Di Marco, C., Campbell, C., Nemitz, E., Hargreaves, K.J., Levy, P.E., Ball, B.C., Jones, S.K., van de Bulk, W.C.M., Groot, T., Blom, M., Domingues, R., Kasper, G., Allard, V., Ceschia, E., Cellier, P., Laville, P., Henault, C., Bizouard, F., Abdalla, M., Williams, M., Baronti, S., Berretti, F., Grosz, B., 2007. Effects of climate and management intensity on nitrous oxide emissions in grassland systems across Europe. Agriculture Ecosystems & Environment 121, 135-152.

Flynn, H.C., Smith, J., Smith, K.A., Wright, J., Smith, P., Massheder, J., 2005. Climate- and crop-responsive emission factors significantly alter estimates of current and future nitrous oxide emissions from fertilizer use. Glob. Change Biol. 11, 1522-1536.

Foley, J.A., DeFries, R., Asner, G.P., Barford, C., Bonan, G., Carpenter, S.R., Chapin, F.S., Coe, M.T., Daily, G.C., Gibbs, H.K., Helkowski, J.H., Holloway, T., Howard, E.A., Kucharik, C.J., Monfreda, C., Patz, J.A.,

Prentice, I.C., Ramankutty, N., Snyder, P.K., 2005. Global consequences of land use. Science 309, 570-574.

Fowler, D., Skiba, U., Hargreaves, K.J., 1997. Emissions of Nitrous Oxide from Grasslands. In: S.C., J., B.F., P. (Eds.), Gaseous Nitrogen Emissions from Grasslands. CAB International, Oxon, pp. 147-164.

Freney, J.R., 1997. Emission of nitrous oxide from soils used for agriculture. Nutrient Cycling in Agroecosystems 49, 1-6.

Frey, S.D., Elliott, E.T., Paustian, K., 1999. Bacterial and fungal abundance and biomass in conventional and no-tillage agroecosystems along two climatic gradients. Soil Biology & Biochemistry 31, 573-585.

Galloway, J.N., 1998. The global nitrogen cycle: changes and consequences. Environ. Pollut. 102, 15-24.

Galloway, J.N., Aber, J.D., Erisman, J.W., Seitzinger, S.P., Howarth, R.W., Cowling, E.B., Cosby, B.J., 2003. The nitrogen cascade. Bioscience 53, 341-356.

Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., Karl, D.M., Michaels, A.F., Porter, J.H., Townsend, A.R., Vorosmarty, C.J., 2004. Nitrogen cycles: past, present, and future. Biogeochemistry 70, 153-226.

Galloway, J. N., Townsend, A. R., Erisman, J. W., Bekunda, M., Cai, Z., Freney, J. R., Martinelli, L. A., Seitzinger, S. P. & Sutton, M. A. 2008. Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. Science, 320, 889-892.

Gheysari, M., Mirlatifi, S.M., Homaee, M., Asadi, M.E., Hoogenboom, G., 2009. Nitrate leaching in a silage maize field under different irrigation and nitrogen fertilizer rates. Agric. Water Manage. 96, 946-954.

Goodroad, L.L., Keeney, D.R., 1984. Nitrous-oxide production in aerobic soils under varying ph, temperature and water-content. Soil Biology & Biochemistry 16, 39-43.

Groenigen, J. W., Georgius, P. J., Kessel, C., Hummelink, E. W. J., Velthof, G. L. & Zwart, K. B. 2005. Subsoil ^{15}N-N_2O concentrations in a sandy soil profile after application of ^{15}N-fertilizer. Nutrient Cycling in Agroecosystems, 72, 13-25.

Groffman, P.M., Tiedje, J.M., 1988. Denitrification hysteresis during wetting and drying cycles in soil. Soil Science Society of America Journal 52, 1626-1629.

Guo, L.B., Gifford, R.M., 2002. Soil carbon stocks and land use change: a meta analysis. Glob. Change Biol. 8, 345-360.

Hansen, E.M., Djurhuus, J., 1997. Nitrate leaching as influenced by soil tillage and catch crop. Soil Tillage Res. 41, 203-219.

Hastings, A., Clifton-Brown, J., Wattenbach, M., Mitchell, C. P., Stampfl, P. & Smith, P. 2009. Future energy potential of *Miscanthus* in Europe. GCB Bioenergy, 1, 180-196.

Hayatsu, M., Tago, K., Saito, M., 2008. Various players in the nitrogen cycle: Diversity and functions of the microorganisms involved in nitrification and denitrification. Soil Science and Plant Nutrition 54, 33-45.

Heaton, E., Voigt, T., Long, S.P., 2004a. A quantitative review comparing the yields of two candidate C-4 perennial biomass crops in relation to nitrogen, temperature and water. Biomass Bioenerg. 27, 21-30.

Heaton, E.A., Clifton-Brown, J., Voigt, T.B., Jones, M.B., Long, S.P., 2004b. *Miscanthus* for renewable energy generation: European Union experience and projections for Illinois. Mitigation and Adaptation Strategies for Global Change, 433-451.

Heaton, E.A., Dohleman, F.G., Long, S.P., 2008. Meeting US biofuel goals with less land: the potential of *Miscanthus*. Glob. Change Biol. 14, 2000-2014.

Heincke, M., Kaupenjohann, M., 1999. Effects of soil solution on the dynamics of N_2O emissions: a review. Nutrient Cycling in Agroecosystems 55, 133-157.

Hellebrand, H.J., Kern, J., Scholz, V., 2003. Long-term studies on greenhouse gas fluxes during cultivation of energy crops on sandy soils. Atmos. Environ. 37, 1635-1644.

Hellebrand, H. J., Scholz, V. & Kern, J. R. 2008. Fertiliser induced nitrous oxide emissions during energy crop cultivation on loamy sand soils. Atmospheric Environment, 42, 8403-8411.

Henault, C., Devis, X., Page, S., Justes, E., Reau, R., Germon, J.C. 1998. Nitrous oxide emissions under different soil and land management conditions. Biol Fertil Soils 26, 199-207

Himken, M., Lammel, J., Neukirchen, D., CzypionkaKrause, U., Olfs, H.W., 1997. Cultivation of *Miscanthus* under west European conditions: Seasonal changes in dry matter production, nutrient uptake and remobilization. Plant and Soil 189, 117-126.

Hoekstra, N.J., Lalor, S.T.J., Richards, K.G., O'Hea, N., Lanigan, G.J., Dyckmans, J., Schulte, R.P.O., Schmidt, O., 2010. Slurry (NH_4)-N-^{15}-N recovery in

herbage and soil: effects of application method and timing. Plant and Soil 330, 357-368.

Hooper, A.B., Vannelli, T., Bergmann, D.J., Arciero, D.M., 1997. Enzymology of the oxidation of ammonia to nitrite by bacteria. Antonie Van Leeuwenhoek 71, 59-67.

Horn, R., Domzal, H., Slowinskajurkiewicz, A., Vanouwerkerk, C., 1995. Soil compaction processes and their effects on the structure of arable soils and the environment. Soil Tillage Res. 35, 23-36.

Houghton, R.A., 1994. The worldwide extent of land-use change. Bioscience 44, 305-313.

Houghton, R.A., 2001. Counting terrestrial sources and sinks of carbon. Clim. Change 48, 525-534.

Hsieh, C.I., Leahy, P., Kiely, G., Li, C.S., 2005. The effect of future climate perturbations on N2O emissions from a fertilized humid grassland. Nutrient Cycling in Agroecosystems 73, 15-23.

Humphreys, J., Casey, I.A., Darmody, P., O'Connell, K., Fenton, O., Watson, C.J., 2008. Quantities of mineral N in soil and concentrations of nitrate-N in groundwater in four grassland-based systems of dairy production on a clay-loam soil in a moist temperate climate. Grass Forage Sci. 63, 481-494.

Hutchinson, G.L., Guenzi, W.D., Livingston, G.P., 1993. Soil-water controls on aerobic soil emission of gaseous nitrogen-oxides. Soil Biology & Biochemistry 25, 1-9.

Hutchinson, G.L., Mosier, A.R., 1981. Improved soil cover method for field measurement of nitrous-oxide fluxes. Soil Science Society of America Journal 45, 311-316.

Hyde, B.P., Hawkins, M.J., Fanning, A.F., Noonan, D., Ryan, M., O'Toole, P., Carton, O.T., 2006. Nitrous oxide emissions from a fertilized and grazed grassland in the South East of Ireland. Nutrient Cycling in Agroecosystems 75, 187-200.

IPCC, 2003. Good Practice Guidance for Land Use,
Land-Use Change and Forestry. In: Jim Penman, M.G., Taka Hiraishi, Thelma Krug, Dina Kruger,, Riitta Pipatti, L.B., Kyoko Miwa, Todd Ngara,, Wagner, K.T.a.F. (Eds.). Intergovernmental Panel on Climate Change, Hayama, Kanagawa.

IPCC, 2006. 2006 IPCC Guidelines for National Greenhouse Gas Inventories. In: Eggleston H.S., B.L., Miwa K., Ngara T. and Tanabe K. (Ed.), Prepared by the National Greenhouse Gas Inventories Programme. IGES, Japan, Hayama.

IPCC, 2007. Climate Change 2007: Synthesis Report
An Assessment of the Intergovernmental Panel on Climate Change
Fourth Assessment Report. In: (eds. Bernstein L., B.P., Canziani O., Chen Z., Christ R., Davidson O., Hare W.,, Huq S., K.D., Kattsov V., Kundzewicz Z., Liu J., Lohmann U., Manning M., Matsuno T.,, Menne B., M.B., Mirza M., Nicholls N., Nurse L., Pachauri R., Palutikof J.,, Parry M., Q.D., Ravindranath N., Reisinger A., Ren J., Riahi K., Rosenzweig C.,, Rusticucci M., S.S., Sokona Y., Solomon S., Stott P., Stouffer R., Sugiyama T., Swart R., Tirpak D., Vogel C., Yohe G.) (Eds.). Intergovernmental Panel on Climate Change, Valencia.

Jangid, K., Williams, M.A., Franzluebbers, A.J., Sanderlin, J.S., Reeves, J.H., Jenkins, M.B., Endale, D.M., Coleman, D.C., Whitman, W.B., 2008. Relative impacts of land-use, management intensity and fertilization upon soil microbial community structure in agricultural systems. Soil Biology & Biochemistry 40, 2843-2853.

Jarvis, S.C., Pain, B.F., 1994. Greenhouse-gas emissions from intensive livestock systems - their estimation and technologies for reduction. Clim. Change 27, 27-38.

Johnson, J.M.F., Franzluebbers, A.J., Weyers, S.L., Reicosky, D.C., 2007. Agricultural opportunities to mitigate greenhouse gas emissions. Environ. Pollut. 150, 107-124.

Jones, S. K., Rees, R. M., Skiba, U. M. & Ball, B. C. 2007. Influence of organic and mineral N fertiliser on N_2O fluxes from a temperate grassland. Agriculture, Ecosystems & Environment, 121, 74-83.

Jordan V.W., Leake A.R., Ogilvy S.E., 2000. Agronomic and environmental implications of soil management practices in integrated farming systems. Aspects of Applied Biology 62, 61-66.

Jorgensen, R.N., Jorgensen, B.J., Nielsen, N.E., Maag, M., Lind, A.M., 1997. N_2O emission from energy crop fields of *Miscanthus* "Giganteus" and winter rye. Atmos. Environ. 31, 2899-2904.

Kahle, P., Beuch, S., Boelcke, B., Leinweber, P., Schulten, H.R., 2001. Cropping of *Miscanthus* in Central Europe: biomass production and influence on nutrients and soil organic matter. European Journal of Agronomy 15, 171-184.

Kammann, C., Muller, C., Grunhage, L., Jager, H.J., 2008. Elevated CO_2 stimulates N_2O emissions in permanent grassland. Soil Biology & Biochemistry 40, 2194-2205.

Karp, A., Shield, I., 2008. Bioenergy from plants and the sustainable yield challenge. New Phytol. 179, 15-32.

Kavdir, Y., Hellebrand, H.J., Kern, J., 2008. Seasonal variations of nitrous oxide emission in relation to nitrogen fertilization and energy crop types in sandy soil. Soil Tillage Res. 98, 175-186.

Keeney, D.R., Fillery, I.R., Marx, G.P., 1979. Effect of temperature on the gaseous nitrogen products of denitrification in a silt loam soil. Soil Science Society of America Journal 43, 1124-1128.

Kessavalou, A., Mosier, A.R., Doran, J.W., Drijber, R.A., Lyon, D.J., Heinemeyer, O., 1998. Fluxes of carbon dioxide, nitrous oxide, and methane in grass sod and winter wheat-fallow tillage management. Journal of Environmental Quality 27, 1094-1104.

Killham, K., 1990. Nitrification in coniferous forest soils. Plant and Soil 128, 31-44.

Klemmedtsson, L., Svensson, T., Lindberg, T., Rosswall, T., 1978. The use of acetylene inhibition of nitrous oxide reductase in quantifying denitrification in soils. Swedish Journal of Agricultural Research 7, 179-185.

Koh, L.P., Ghazoul, J., 2008. Biofuels, biodiversity, and people: Understanding the conflicts and finding opportunities. Biological Conservation 141, 2450-2460.

Kong, A.Y.Y., Fonte, S.J., van Kessel, C., Six, J., 2009. Transitioning from standard to minimum tillage: Trade-offs between soil organic matter stabilization, nitrous oxide emissions, and N availability in irrigated cropping systems. Soil Tillage Res. 104, 256-262.

Kramer, A.W., Doane, T.A., Horwath, W.R., van Kessel, C., 2002. Combining fertilizer and organic inputs to synchronize N supply in alternative cropping systems in California. Agriculture Ecosystems & Environment 91, 233-243.

Ladd, J.N., Jackson, R.B., 1982. Biochemistry of ammonification. Stevenson, F. J., pp. P173-228.

Laughlin, R.J., Stevens, R.J., 2002. Evidence for fungal dominance of denitrification and codenitrification in a grassland soil. Soil Science Society of America Journal 66, 1540-1548.

Laughlin, R.J., Stevens, R.J., Muller, C., Watson, C.J., 2008. Evidence that fungi can oxidize NH^{4+} to NO^{3-} in a grassland soil. Eur. J. Soil Sci. 59, 285-291.

Letey, J., Valoras, N., Focht, D.D., Ryden, J.C., 1981. Nitrous-oxide production and reduction during denitrification as affected by redox potential. Soil Science Society of America Journal 45, 727-730.

Letey, J., Valoras, N., Hadas, A., Focht, D.D., 1980. Effect Of Air-Filled Porosity, Nitrate Concentration, And Time On The Ratio Of N_2O-N_2 Evolution During Denitrification. Journal of Environmental Quality 9, 227-231.

Lewandowski, I., Clifton-Brown, J.C., Scurlock, J.M.O., Huisman, W., 2000. *Miscanthus*: European experience with a novel energy crop. Biomass Bioenerg. 19, 209-227.

Lewandowski, I. & Schmidt, U. 2006. Nitrogen, energy and land use efficiencies of *Miscanthus*, Reed canarygrass and triticale as determined by the boundary line approach. Agriculture, Ecosystems & Environment, 112, 335-346.

Lewandowski, I., Scurlock, J.M.O., Lindvall, E., Christou, M., 2003. The development and current status of perennial rhizomatous grasses as energy crops in the US and Europe. Biomass Bioenerg. 25, 335-361.

Li, C.S., Narayanan, V., Harriss, R.C., 1996. Model estimates of nitrous oxide emissions from agricultural lands in the United States. Glob. Biogeochem. Cycle 10, 297-306.

Liu, X.J.J., Mosier, A.R., Halvorson, A.D., Reule, C.A., Zhang, F.S., 2007. Dinitrogen and N_2O emissions in arable soils: Effect of tillage, N source and soil moisture. Soil Biology & Biochemistry 39, 2362-2370.

Liu, B., Mørkved, P. T., Frostegård, Å. & Bakken, L. R. 2010. Denitrification gene pools, transcription and kinetics of NO, N_2O and N_2 production as affected by soil pH. FEMS Microbiology Ecology, 72, 407-417.

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. Appl. Soil Ecol. 4, 5-14.

Macdonald, J. D., Rochette, P., Chantigny, M. H., Angers, D. A., Royer, I. & Gasser, M. O. 2011. Ploughing a poorly drained grassland reduced N_2O emissions compared to chemical fallow. Soil and Tillage Research, 111, 123-132.

Machefert, S.E., Dise, N.B., Goulding, K.W.T., Whitehead, P.G., 2002. Nitrous oxide emission from a range of land uses across Europe. Hydrology and Earth System Sciences 6, 325-337.

Marshall, J.D., Blair, J.M., Peters, D.P.C., Okin, G., Rango, A., Williams, M., 2008. Predicting and understanding ecosystem responses to climate change at continental scales. Front. Ecol. Environ. 6, 273-280.

Mathieu, O., Henault, C., Leveque, J., Baujard, E., Millopux, M.J., Andreux, F. 2006. Quantifying the contribution of nitrification and denitrification to the nitrous oxide flux using N-15 tracers. Environmental Pollution 144, 933-940.

Matson, P.A., Parton, W.J., Power, A.G., Swift, M.J., 1997. Agricultural intensification and ecosystem properties. Science 277, 504-509.

Maughan, M., et al. (2012), *Miscanthus* giganteus productivity: the effects of management in different environments GCB Bioenergy, 4(3), 253-265.

McKendry, P., 2002. Energy production from biomass (part 1): overview of biomass. Bioresource Technology 83, 37-46.

McLaren, R.G., Cameron, K.C., 1996. Soil Science. Sustainable production and environmental protection. Oxford University Press, Melbourne.

McLaughlin, S.B., Walsh, M.E., 1998. Evaluating environmental consequences of producing herbaceous crops for bioenergy. Biomass Bioenerg. 14, 317-324.

McSwiney, C.P., Robertson, G.P., 2005. Nonlinear response of N_2O flux to incremental fertilizer addition in a continuous maize (Zea mays L.) cropping system. Glob. Change Biol. 11, 1712-1719.

Merino, A., Perez-Batallon, P., Macias, F., 2004. Responses of soil organic matter and greenhouse gas fluxes to soil management and land use changes in a humid temperate region of southern Europe. Soil Biology & Biochemistry 36, 917-925.

Miguez, F. E., Villamil, M. B., Long, S. P. & Bollero, G. N. A. 2008. Meta-analysis of the effects of management factors on *Miscanthus* x giganteus growth and biomass production. Agricultural and Forest Meteorology, 148, 1280-1292.

Mishurov, M., Kiely, G., 2010. Nitrous oxide flux dynamics of grassland undergoing afforestation. Agriculture Ecosystems & Environment 139, 59-65.

Mosier, A., Kroeze, C., Nevison, C., Oenema, O., Seitzinger, S., van Cleemput, O., 1998. Closing the global N2O budget: nitrous oxide emissions through the agricultural nitrogen cycle - OECD/IPCC/IEA phase II development of IPCC guidelines for national greenhouse gas inventory methodology. Nutrient Cycling in Agroecosystems 52, 225-248.

Mosier, A., Schimel, D., Valentine, D., Bronson, K., Parton, W., 1991. Methane and nitrous-oxide fluxes in native, fertilized and cultivated grasslands. Nature 350, 330-332.

Mosier, A.R., 1998. Soil processes and global change. Biology and Fertility of Soils 27, 221-229.

Mosier, A.R., Duxbury, J.M., Freney, J.R., Heinemeyer, O., Minami, K., 1998C. Assessing and mitigating N_2O emissions from agricultural soils. Clim. Change 40, 7-38.

Mørkved, P. T., Dörsch, P. & Bakken, L. R. 2007. The N_2O product ratio of nitrification and its dependence on long-term changes in soil pH. Soil Biology and Biochemistry, 39, 2048-2057.

Mulvaney, R.L., Khan, S.A., Mulvaney, C.S., 1997. Nitrogen fertilizers promote denitrification. Biology and Fertility of Soils 24, 211-220.

Murty, D., Kirschbaum, M.U.F., McMurtrie, R.E., McGilvray, A., 2002. Does conversion of forest to agricultural land change soil carbon and nitrogen? a review of the literature. Glob. Change Biol. 8, 105-123.

Myrold, D.D., Bottomley, P.J., 2008. Nitrogen Mineralization and Immobilization. In: J.S., S., W.R., R. (Eds.), Nitrogen in Agricultural Soils.

Neftel, A., Flechard, C., Ammann, C., Conen, F., Emmenegger, L., Zeyer, K., 2006. Experimental assessment of N_2O background fluxes in grassland systems. 1st International Integrated Land Ecosystem Atmosphere Processes Study Science Conference (iLEAPS), Boulder, CO, pp. 470-482.

NZ MoE, 2010. New Zealand's Greenhouse Gas Inventory 1990–2008. Wellington, New Zealand ISSN: 1179-223X Publication number: ME 1009.

Ogle, S.M., Breidt, F.J., Paustian, K., 2005. Agricultural management impacts on soil organic carbon storage under moist and dry climatic conditions of temperate and tropical regions. Biogeochemistry 72, 87-121.

Otero, S., Marsh, D., Lanigan, G.J., Osborne, B.A., 2011. Effects of simulated climate change over energy crops in Ireland. Geophysical Research Abstracts EGU, Vienna 3-8 April 2011

Parkin, T.B., 1987. Soil microsites as a source of denitrification variability. Soil Science Society of America Journal 51, 1194-1199.

Parkin, T.B., Tiedje, J.M., 1984. Application of a soil core method to investigate the effect of oxygen concentration on denitrification. Soil Biology & Biochemistry 16, 331-334.

Partala, A., Mela, T., Esala, M., Ketoja, E., 2001. Plant recovery of N-15-labelled nitrogen applied to Reed canarygrass grown for biomass. Nutrient Cycling in Agroecosystems 61, 273-281.

Parton, W.J., Holland, E.A., Del Grosso, S.J., Hartman, M.D., Martin, R.E., Mosier, A.R., Ojima, D.S., Schimel, D.S., 2001. Generalized model for NO_x and N_2O emissions from soils. J. Geophys. Res.-Atmos. 106, 17403-17419.

Parton, W.J., Mosier, A.R., Ojima, D.S., Valentine, D.W., Schimel, D.S., Weier, K., Kulmala, A.E., 1996. Generalized model for N_2 and N_2O production from nitrification and denitrification. Glob. Biogeochem. Cycle 10, 401-412.

Parton, W.J., Rasmussen, P.E., 1994. Long-term effects of crop management in wheat-fallow .2. century model simulations. Soil Science Society of America Journal 58, 530-536.

Paustian, K., Six, J., Elliott, E.T., Hunt, H.W., 2000. Management options for reducing CO2 emissions from agricultural soils. Biogeochemistry 48, 147-163.

Peake, S., Smith, J., 2003. Climate Change: From Science to Sustainability. The Bath Press, Glasgow.

Peech, M., English, L., 1944. Rapid micro chemical soil tests. Science 57, 167.

Pennock, D.J., T Yates, T., Braidek, J.T., 2006. Towards optimum sampling for regional-scale N_2O emission monitoring in Canada. Canadian Journal of Soil Science 86, 441-450.

Petersen, S.O., Regina, K., Pollinger, A., Rigler, E., Valli, L., Yamulki, S., Esala, M., Fabbri, C., Syvasalo, E., Vinther, F.P., 2006. Nitrous oxide emissions from organic and conventional crop rotations in five European countries. Agriculture Ecosystems & Environment 112, 200-206.

Pielke, R.A., 2005. Land use and climate change. Science 310, 1625-1626.

Powlson, D.S., Riche, A.B., Shield, I., 2005. Biofuels and other approaches for decreasing fossil fuel emissions from agriculture. Annals of Applied Biology 146, 193-201.

Popp, A., Lotze-Campen, H., Leimbach, M., Knopf, B., Beringer, T., Bauer, N. & Bodirsky, B. 2011. On sustainability of bioenergy production: Integrating co-emissions from agricultural intensification. Biomass and Bioenergy.

Prakasam, T.B., Loehr, R.C., 1972. Microbial nitrification and denitrification in concentrated wastes. Water Res. 6, 859-&.

Rabalais, N.N., 2002. Nitrogen in aquatic ecosystems. Ambio 31, 102-112.

Rafique, R., Hennessy, D., Kiely, G., 2011. Nitrous Oxide Emission from Grazed Grassland Under Different Management Systems. Ecosystems 14, 563-582.

Ramankutty, N., Evan, A. T., Monfreda, C. & Foley, J. A. 2008. Farming the planet: 1. Geographic distribution of global agricultural lands in the year 2000. Global Biogeochemical Cycles, 22.

Ramankutty, N., Foley, J.A., 1999. Estimating historical changes in global land cover: Croplands from 1700 to 1992. Glob. Biogeochem. Cycle 13, 997-1027.

Ravishankara, A. R., Daniel, J. S. & Portmann, R. W. 2009. Nitrous Oxide: The dominant ozone-depleting substance emitted in the 21st century. Science, 326, 123-125.

Rees, R.M., Ball, B.C., 2010. Soils and nitrous oxide research. Soil Use Manage. 26, 193-195.

Reicosky, D.C., Kemper, W.D., Langdale, G.W., Douglas, C.L., Rasmussen, P.E., 1995. Soil organic-matter changes resulting from tillage and biomass production. J. Soil Water Conserv. 50, 253-261.

Rochette, P., Angers, D.A., Chantigny, M.H., Bertrand, N., 2008. Nitrous oxide emissions respond differently to no-till in a loam and a heavy clay soil. Soil Science Society of America Journal 72, 1363-1369.

Roelandt, C., van Wesemael, B., Rounsevell, M., 2005. Estimating annual N_2O emissions from agricultural soils in temperate climates. Glob. Change Biol. 11, 1701-1711.

Roth, B., Finnan, J., Jones, M.B., Burke, J.I., Williams, M.W., 2011. The effects of land use change and management on N_2O emissions from *Miscanthus* x giganteus. Nitrogen & Global Change Edinburgh, Scotland.

Rounsevell, M.D.A., Ewert, F., Reginster, I., Leemans, R., Carter, T.R., 2005. Future scenarios of European agricultural land use II. Projecting changes in cropland and grassland. Agriculture Ecosystems & Environment 107, 117-135.

Rounsevell, M. D. A., Reginster, I., Araújo, M. B., Carter, T. R., Dendoncker, N., Ewert, F., House, J. I., Kankaanpää, S., Leemans, R., Metzger, M. J., Schmit, C., Smith, P. & Tuck, G. 2006. A coherent set of future land use change scenarios for Europe. Agriculture, Ecosystems & Environment, 114, 57-68

Rowe, R.L., Street, N.R., Taylor, G., 2009. Identifying potential environmental impacts of large-scale deployment of dedicated bioenergy crops in the UK. Renewable & Sustainable Energy Reviews 13, 260-279.

Rosenkraz, P., Bruggemann, N., Papen, H., Xu, Z., Seufert, G. & Butterbach-Bahl, K. 2005. NO_2, NO and CH_4 exchange, and microbial turnover over a Mediterranean pine forest soil. Biogeosciences Discussions, 2, 673-702.

Ruser, R., Flessa, H., Russow, R., Schmidt, G., Buegger, F., Munch, J.C., 2006. Emission of N_2O, N_2 and CO_2 from soil fertilized with nitrate: Effect of compaction, soil moisture and rewetting. Soil Biology & Biochemistry 38, 263-274.

Ruzicka, S., Edgerton, D., Norman, M., Hill, T., 2000. The utility of ergosterol as a bioindicator of fungi in temperate soils. Soil Biology & Biochemistry 32, 989-1005.

Saggar, S., Jha, N., Deslippe, J., Bolan, N.S., Luo, J., Giltrap, D.L., Kim, D.-G., Zaman, M., Tillman, R.W 2012. Denitrification and N_2O:N_2 production in temperate grasslands: Processes, measurements, modelling and mitigating negative impacts. Science of the Total Environment, article in press.

Sanderson, M.A., Adler, P.R., 2008. Perennial forages as second generation bioenergy crops. International Journal of Molecular Sciences 9, 768-788.

Schimdt-Walter & Lamersdorf (2012), Biomass Production with Willow and Poplar Short Rotation Coppices on Sensitive Areas--the Impact on Nitrate Leaching and Groundwater Recharge in a Drinking Water Catchment near Hanover, Germany BIOENERGY RESEARCH, 5(3).

Schlamadinger, B., Apps, M., Bohlin, F., Gustavsson, L., Jungmeier, G., Marland, G., Pingoud, K., Savolainen, I., 1997. Towards a standard methodology for greenhouse gas balances of bioenergy systems in comparison with fossil energy systems. Biomass Bioenerg. 13, 359-375.

Schmer, M.R., Vogel, K.P., Mitchell, R.B., Moser, L.E., Eskridge, K.M., Perrin, R.K., 2006. Establishment stand thresholds for switchgrass grown as a bioenergy crop. Crop Science 46, 157-161.

Schmidt, J., Seiler, W., Conrad, R., 1988. Emission of nitrous-oxide from temperate forest soils into the atmosphere. J. Atmos. Chem. 6, 95-115.

Seitzinger, S., Harrison, J.A., Bohlke, J.K., Bouwman, A.F., Lowrance, R., Peterson, B., Tobias, C., Van Drecht, G., 2006. Denitrification across landscapes and waterscapes: A synthesis. Ecol. Appl. 16, 2064-2090.

Silver, W.L., Herman, D.J., Firestone, M.K., 2001. Dissimilatory nitrate reduction to ammonium in upland tropical forest soils. Ecology 82, 2410-2416.

Six, J., Elliott, E.T., Paustian, K., Doran, J.W., 1998. Aggregation and soil organic matter accumulation in cultivated and native grassland soils. Soil Science Society of America Journal 62, 1367-1377.

Six, J., Paustian, K., Elliott, E.T., Combrink, C., 2000. Soil structure and organic matter: I. Distribution of aggregate-size classes and aggregate-associated carbon. Soil Science Society of America Journal 64, 681-689.

Skiba, U.,Ball B.C., 2002. The effect of soil texture and soil drainage on emissions of nitric oxide and nitrous oxide Soil Use and Management, 18, 56-60.

Skiba, U., Drewer, J., Tang, Y.S., van Dijk, N., Helfter, C., Nemitz, E., Famulari, D., Cape, J.N., Jones, S.K., Twigg, M., Pihlatie, M., Vesala, T., Larsen, K.S., Carter, M.S., Ambus, P., Ibrom, A., Beier, C., Hensen, A., Frumau, A., Erisman, J.W., Bruggemann, N., Gasche, R., Butterbach-Bahl, K., Neftel, A., Spirig, C., Horvath, L., Freibauer, A., Cellier, P., Laville, P., Loubet, B., Magliulo, E., Bertolini, T., Seufert, G., Andersson, M., Manca, G.,

Laurila, T., Aurela, M., Lohila, A., Zechmeister-Boltenstern, S., Kitzler, B., Schaufler, G., Siemens, J., Kindler, R., Flechard, C., Sutton, M.A., 2009. Biosphere-atmosphere exchange of reactive nitrogen and greenhouse gases at the NitroEurope core flux measurement sites: Measurement strategy and first data sets. Agriculture Ecosystems & Environment 133, 139-149.

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. & Smith, K. A. 2000. The control of nitrous oxide emissions from agricultural and natural soils. Chemosphere - Global Change Science, 2, 379-386.

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 & Biochemistry 25, 1527-1536.

Smil, V., 1999. Nitrogen in crop production: An account of global flows. Glob. Biogeochem. Cycle 13, 647-662.

Smith, K.A., Ball, T., Conen, F., Dobbie, K.E., Massheder, J., Rey, A., 2003. Exchange of greenhouse gases between soil and atmosphere: interactions of soil physical factors and biological processes. Eur. J. Soil Sci. 54, 779-791.

Smith, K.A., Clayton, H., McTaggart, I.P., Thomson, P.E., Arah, J.R.M., Scott, A., 1995. The measurement of nitrous-oxide emissions from soil by using chambers. Philos. Trans. R. Soc. Lond. Ser. A-Math. Phys. Eng. Sci. 351, 327-337.

Smith, K.A., Conen, F., 2004. Impacts of land management on fluxes of trace greenhouse gases. Soil Use Manage. 20, 255-263.

Smith, K.A., Dobbie, K.E., 2001. The impact of sampling frequency and sampling times on chamber-based measurements of N_2O emissions from fertilized soils. Glob. Change Biol. 7, 933-945.

Smith, K.A., Thomson, P.E., Clayton, H., McTaggart, I.P., Conen, F., 1998. Effects of temperature, water content and nitrogen fertilisation on emissions of nitrous oxide by soils. Atmos. Environ. 32, 3301-3309.

Smith M.S., Tiedje J.M., 1979. Phases of denitrification following oxygen depletion in soil. Soil Biol Biochem 11, 261-267.

Smith, P., Martino, D., Cai, Z., Gwary, D., Janzen, H., Kumar, P., McCarl, B., Ogle, S., O'Mara, F., Rice, C., Scholes, B., Sirotenko, O., Howden, M., McAllister, T., Pan, G., Romanenkov, V., Schneider, U., Towprayoon, S.,

Wattenbach, M., Smith, J., 2008. Greenhouse gas mitigation in agriculture. Philos. Trans. R. Soc. B-Biol. Sci. 363, 789-813.

Smith, P., Martino, D., Cai, Z.C., Gwary, D., Janzen, H., Kumar, P., McCarl, B., Ogle, S., O'Mara, F., Rice, C., Scholes, B., Sirotenko, O., Howden, M., McAllister, T., Pan, G.X., Romanenkov, V., Schneider, U., Towprayoon, S., 2007. Policy and technological constraints to implementation of greenhouse gas mitigation options in agriculture. Agriculture Ecosystems & Environment 118, 6-28.

Sogbedji, J.M., van Es, H.M., Yang, C.L., Geohring, L.D., Magdoff, F.R., 2000. Nitrate leaching and nitrogen budget as affected by maize nitrogen rate and soil type. Journal of Environmental Quality 29, 1813-1820.

Soussana, J.F., Allard, V., Pilegaard, K., Ambus, P., Amman, C., Campbell, C., Ceschia, E., Clifton-Brown, J., Czobel, S., Domingues, R., Flechard, C., Fuhrer, J., Hensen, A., Horvath, L., Jones, M., Kasper, G., Martin, C., Nagy, Z., Neftel, A., Raschi, A., Baronti, S., Rees, R.M., Skiba, U., Stefani, P., Manca, G., Sutton, M., Tubaf, Z., Valentini, R., 2007. Full accounting of the greenhouse gas (CO_2, N_2O, CH_4) budget of nine European grassland sites. Agriculture Ecosystems & Environment 121, 121-134.

Spott O., Russow, R., Stange, C.F., 2011. Formation of hybrid N_2O and hybrid N_2 due to codenitrification: First review of a barely considered process of microbially mediated N-nitrosation. Soil Biology and Biochemistry 43, 1995-2011.

Stark, C.H. and Richards, K.G. 2008 The continuing challenge of nitrogen loss to the environment: Environmental consequences and mitigation strategies. Dynamic Soil, Dynamic Plant 2, 41-55.

St Clair, S., Hillier, J., Smith, P., 2008. Estimating the pre-harvest greenhouse gas costs of energy crop production. Biomass Bioenerg. 32, 442-452.

Stark, J.M., Firestone, M.K., 1995. Mechanisms for soil-moisture effects on activity of nitrifying bacteria. Appl. Environ. Microbiol. 61, 218-221.

Stark, J.M., Hart, S.C., 1996. Diffusion technique for preparing salt solutions, Kjeldahl digests, and persulfate digests for nitrogen-15 analysis. Soil Science Society of America Journal 60, 1846-1855.

Stehfest, E., Bouwman, L. 2006. N_2O and NO emission from agricultural fields and soils under natural vegetation: summarizing available measurement data and modeling of global annual emissions. Nutrient Cycling in Agroecosystems 74, 207-228.

Stevens, R. J., Laughlin, R. J. 2002. Cattle slurry applied before fertilizer nitrate lowers nitrous oxide and dinitrogen emissions. Soil Sci. Soc. Am. J. 66, 647-652

Stevens, R. J., Laughlin, R. J. & Malone, J. P. 1998. Soil pH affects the processes reducing nitrate to nitrous oxide and di-nitrogen. Soil Biology and Biochemistry, 30, 1119-1126.

Stevens, R.J., Laughlin, R.J., Burns, L.C., Arah, J.R.M., Hood, R.C., 1997. Measuring the contributions of nitrification and denitrification to the flux of nitrous oxide from soil. Soil Biology and Biochemistry 29, 139-151.

Storey, M., 1997. The climate implications of agricultural policy reform. 'Policies and and measures for common action' Working Paper 16. Annex I. Experts Group on the FCCC, OECD/IEA.

Struwe, S., Kjoller, A., 1994. Potential for n2o production from beech (fagus-silvaticus) forest soils with varying pH. Soil Biology & Biochemistry 26, 1003-1009.

Styles, D., Jones, M.B., 2007a. Current and future financial competitiveness of electricity and heat from energy crops: A case study from Ireland. Energy Policy 35, 4355-4367.

Styles, D., Jones, M.B., 2007b. Energy crops in Ireland: Quantifying the potential life-cycle greenhouse gas reductions of energy-crop electricity. Biomass Bioenerg. 31, 759-772.

Styles, D., Jones, M.B., 2008a. Life-cycle environmental and economic impacts of energy-crop fuel-chains: an integrated assessment of potential GHG avoidance in Ireland. Environ. Sci. Policy 11, 294-306.

Styles, D., Jones, M.B., 2008b. *Miscanthus* and willow heat production - An effective land-use strategy for greenhouse gas emission avoidance in Ireland? Energy Policy 36, 97-107.

Styles, D., Thorne, F., Jones, M.B., 2008. Energy crops in Ireland: An economic comparison of willow and *Miscanthus* production with conventional farming systems. Biomass Bioenerg. 32, 407-421.

Su, F., Takaya, N. & Shoun, H. 2004. Nitrous oxide-forming codenitrification catalyzed by cytochrome P450nor. Bioscience, Biotechnology, and Biochemistry, 68, 473-475.

Šimek, M., Jisova, L. & Hopkins, D. W. 2002. What is the so-called optimum pH for denitrification in soil? Soil Biology and Biochemistry, 34, 1227-1234.

t Mannetje, L., 2007. Climate change and grasslands through the ages: an overview. Grass Forage Sci. 62, 113-117.

Tampfl, P.F.S., Clifton-Brownw, J.C., Jones, M.B., 2007. European-wide GIS-based modelling system for quantifying the feedstock from *Miscanthus* and the

potential contribution to renewable energy targets. Glob. Change Biol. 13, 2283-2295.

Tao, L. & Aden, A. 2009. The economics of current and future biofuels. In Vitro Cellular & Developmental Biology - Plant, 45, 199-217.

Teagasc, 2008. Nutrient Guidance for Energy Crops. In: Teagasc (Ed.).

Thomson, A.J., Giannopoulos, G., Pretty, J., Baggs, E.M., Richardson, D.J., 2012. Biological sources and sinks of nitrous oxide and strategies to mitigate emissions. Philosophical Transactions of the Royal Society B-Biological Sciences 367. 1157-1168

Tortoso, A.C., Hutchinson, G.L., 1990. Contributions of autotrophic and heterotrophic nitrifiers to soil no and N_2O emissions. Appl. Environ. Microbiol. 56, 1799-1805.

Townsend, A.R., Howarth, R.W., Bazzaz, F.A., Booth, M.S., Cleveland, C.C., Collinge, S.K., Dobson, A.P., Epstein, P.R., Keeney, D.R., Mallin, M.A., Rogers, C.A., Wayne, P., Wolfe, A.H., 2003. Human health effects of a changing global nitrogen cycle. Front. Ecol. Environ. 1, 240-246.

Tuck, G., Glendining, M.J., Smith, P., House, J.I., Wattenbach, M., 2006. The potential distribution of bioenergy crops in Europe under present and future climate. Biomass Bioenerg. 30, 183-197.

UN, 1997. Kyoto Protocol to the United Nations Framework Convention on Climate Change.

US-EPA, 2006. Global Anthropogenic Non-CO_2 Greenhouse Gas Emissions: 1990-2020. United States Environmental Protection Agency, EPA 430-R-06-003, June 2006. Washington, DC, USA. www.epa.gov/nonco2/econ-inv/dow.

Vellinga, T.V., van den Pol-van Dasselaar, A., Kuikman, P.J., 2004. The impact of grassland ploughing on CO_2 and N_2O emissions in the Netherlands. Nutrient Cycling in Agroecosystems 70, 33-45.

Velthof, G., Hoving, I., Dolfing, J., Smit, A., Kuikman, P. & Oenema, O. 2010. Method and timing of grassland renovation affects herbage yield, nitrate leaching, and nitrous oxide emission in intensively managed grasslands. Nutrient Cycling in Agroecosystems, 86, 401-412.

Velthof, G.L., Oenema, O., 1995. Nitrous oxide fluxes from grassland in the Netherlands .1. Statistical analysis of flux-chamber measurements. Eur. J. Soil Sci. 46, 533-540.

Verburg, P. H., Rounsevell, M. D. A. & Veldkamp, A. 2006. Scenario-based studies of future land use in Europe. Agriculture, Ecosystems & Environment, 114, 1-6.

Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H., Tilman, G.D., 1997. Human alteration of the global nitrogen cycle: Sources and consequences. Ecol. Appl. 7, 737-750.

Wang, F.L., Alva, A.K., 1996. Leaching of nitrogen from slow-release urea sources in sandy soils. Soil Science Society of America Journal 60, 1454-1458.

Weier, K.L., Doran, J.W., Power, J.F., Walters, D.T., 1993. Denitrification and the dinitrogen nitrous-oxide ratio as affected by soil-water, available carbon, and nitrate. Soil Science Society of America Journal 57, 66-72.

Whitehead, D.C., 1995. Grassland Nitrogen. CAB International, Wallingford, Oxon, UK.

(WMO), W.M.O., 2006. Annual Greenhouse Gas Bulletin. WMO-GAW, Geneva, Switzerland.

Wolfe, A.H., Patz, J.A., 2002. Reactive nitrogen and human health: Acute and long-term implications. Ambio 31, 120-125.

Wrage, N., Lauf, J., del Prado, A., Pinto, M., Pietrzak, S., Yamulki, S., Oenema, O., Gebauer, G., 2004a. Distinguishing sources of N_2O in European grasslands by stable isotope analysis. Rapid Communications in Mass Spectrometry 18, 1201-1207.

Wrage, N., Velthof, G.L., Laanbroek, H.J., Oenema, O., 2004b. Nitrous oxide production in grassland soils: assessing the contribution of nitrifier denitrification. Soil Biology & Biochemistry 36, 229-236.

Wrage, N., Velthof, G.L., van Beusichem, M.L., Oenema, O., 2001. Role of nitrifier denitrification in the production of nitrous oxide. Soil Biology & Biochemistry 33, 1723-1732.

Wu, Q.J., Ward, A.D., Workman, S.R., 1996. Using GIS in simulation of nitrate leaching from heterogeneous unsaturated soils. Journal of Environmental Quality 25, 526-534.

Xu, X., Liu, W., Zhang, C., Kiely, G., 2011. Estimation of soil organic carbon stock and its spatial distribution in the Republic of Ireland. Soil Use Manage. 27, 156-162.

Yao, Z., Zheng, X., Xie, B., Liu, C., Mei, B., Dong, H., Butterbach-Bahl, K. & Zhu, J. 2009. Comparison of manual and automated chambers for field measurements of N_2O, CH_4, CO_2 fluxes from cultivated land. Atmospheric Environment, 43, 1888-1896.