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The Effects of Land-use Change and Management on Soil Nitrous Oxide Emissions from *Miscanthus x giganteus*

PhD Thesis

2011

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Abstract

The use of bioenergy crops as fossil fuel replacements has expanded in the recent past and is likely to continue to grow in the near future as countries attempt to meet greenhouse gas reduction targets outlined in various policies. *Miscanthus x giganteus* is a tropical C_4 grass which is being increasingly exploited as an energy crop due to its substantial yields in temperate climates and low nitrogen input requirements. While *Miscanthus* has been subject to much research little is known about its relationship with soil N_2O emissions. Here the effects of land-use change from grassland to *Miscanthus,* as well as nitrogen fertiliser management, on soil N_2O emissions have been investigated. Additionally the suitability of the process based model DNDC for simulating N_2O emissions from *Miscanthus* was assessed.

Two separate twelve-month field trials were carried out at the Teagasc Oak Park Research Centre, Co. Carlow. The effects of land-use change were examined by measuring N_2O emissions, soil NO_3^- and NH_4^+ concentrations, and soil temperature and water filled pore space from a grassland, newly planted *Miscanthus* crop, and long established *Miscanthus* crop. Similarly the effects of fertiliser addition were examined by measuring the same parameters from a long established *Miscanthus* crop subject to nitrogen fertiliser application rates of 0, 63, and 125 kg-N ha^{-1}. Data collected during these trials were used to parameterise, run, and validate the process-based model DNDC.

N_2O fluxes from the land-use change trial ranged from 0.22 kg-N ha^{-1} yr^{-1} in the grassland to 0.63 kg-N ha^{-1} yr^{-1} in the newly planted *Miscanthus*. However no significant difference was found between the plots when analysed by a repeated measures two-way ANOVA. This was due to large amounts of variation observed throughout the year as evidenced by frequent N_2O uptake. N_2O uptake was seen in over 39% of all daily N_2O fluxes measured.

Nitrogen fertiliser application was found to have a significant effect on N_2O flux, but increasing the rate of fertiliser from 63 to 125 kg-N ha^{-1} did not. N_2O fluxes measured 0.46, 0.86 and 1.05 kg-N ha^{-1} yr^{-1} in the 0, 63 and 125 kg-N ha^{-1} fertiliser treatments respectively. Additionally nitrogen fertiliser application was not found to significantly affect above ground biomass. An economic cost analysis revealed fertiliser application led to a loss in the region of €15 ha^{-1}. This cost was not significantly impacted when the cost of N_2O as CO_2 equivalents was included.

DNDC was parameterised for use with *Miscanthus* and validated using data collected during the previous fertiliser trial. Model performance was initially poor. The model was then calibrated by adjusting pH. This calibration procedure significantly enhanced model performance with overall relative deviation improving from 106.6% to -1.3%, a significant improvement in RMSE was also seen. This indicates that DNDC can be a useful tool for predicting soil N_2O emissions from *Miscanthus.*

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Chapter 1

General Introduction

1.1 Introduction

Globally the contribution of greenhouse gas emissions to a number of environmental issues has been broadly accepted. Evidence highlighting the relationship between anthropogenic greenhouse gas emissions and the earth's changing climate patterns is wide-spread (IPCC, 2007b). The need to reduce greenhouse gas emissions has been recognised, as has the essential role of policy in this issue. This is highlighted by a number of international, European and national policies. The United Nations Framework Convention on Climate Change Kyoto Protocol of 1997 has called for annex 1 countries (40 industrialised nations, including all members of the OECD) to collectively reduce their green house gas emissions by 5.2% from 1990 baseline levels, for the reporting period 2008-2012, while the EU has committed to a reduction of 8%. Despite this Ireland has been allowed an increase of 13% due to projected growth at the time. With the end of this agreement approaching progress has been positive, although reductions in emissions have been strongly affected by the global economic downturn as well as policy effects. The EU is currently on track to meet their Kyoto agreement targets, and may actually overachieve by as much as 5%. Ireland too would seem to seem to be on target. Average emissions from 2008-2010 were 14.4% above 1990 levels, however with predicted further decreases due to a struggling economy and decreasing livestock herds, the use of Land-Use, Land-Use Change and Forestry (LULUCF) activities and the EU Emissions Trading Scheme (ETS) it is expected that the target of a 13% increase will be achieved (European Energy Agency, 2011). Post-Kyoto legally binding future targets have yet to be agreed but many individual targets for the year 2020 have been published. The EU have announced a 'Climate and Energy Package' through which they intend to reduce emissions by 20% compared 1990 levels, and have further pledged to increase that target to 30% if all other countries take part (Jaekyu, 2011). Based on these objectives Ireland has committed to a reduction in emissions of 20% below a 2005 baseline (Government of Ireland, 2011). Furthermore the government's energy white paper calls for 33% of energy produced to come from renewable sources by 2020 (Government of Ireland, 2007).

The agriculture and energy supply sectors are major contributors to greenhouse gas emissions. Globally accounting for 13.5% and 25.9% of all anthropogenic emissions respectively (IPCC, 2007b). Figure 1.1 illustrates contribution of different sectors to Irish greenhouse gas emissions in 2009. Here agriculture was responsible for 28% of all national anthropogenic greenhouse gas emissions, the highest proportion of any sector. This was followed by energy use at 28% and energy supply at 21% (Duffy *et al.*, 2011). A potential mechanism to reduce greenhouse gas emissions from both these sectors is to use existing agricultural land for bio-energy crop production. The Kyoto Protocol encourages the sequestration of atmospheric CO_2 through changes in land use and management as a way to offset CO_2 emissions (Kyoto Protocol Articles 3.3 & 3.4), while bio-energy crops have been identified to show the greatest potential for carbon mitigation of

all the available agricultural options (Smith *et al.*, 2000). As crops grow they fix CO_2 from the atmosphere, this CO_2 is then released when the crops undergo combustion meaning that the net atmospheric CO_2 levels remain unchanged. This has led to the use of the term 'Carbon neutral' in reference to energy produced from biomass (Price *et al.*, 2004). Yet in reality this can be a misrepresentation. There can be a net CO_2 output associated with energy crops due to the external energy costs associated with the planting, fertilisation, harvesting, and processing of the crops as well as consequent direct soil emissions (Lewandowski *et al.*, 1995). This term also excludes other important greenhouse gases, N_2O and CH_4 - although they are often implied and measured as CO_2 equivalents.

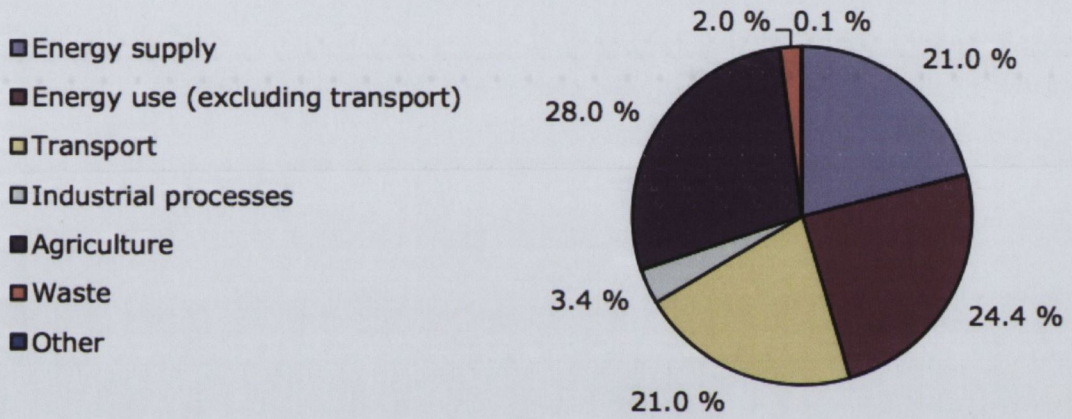


Figure 1.1 Share of greenhouse gas emissions in Ireland by sector (Duffy *et al.*, 2011).

Recent work has indicated that for many common first generation bioenergy crops such as rapeseed and maize the negative impacts of N_2O emissions on radiative forcing outweigh the benefits achieved by the subsequent fossil fuel savings, although the same study has suggested that these results will be more favourable for grasses and woody coppices (Crutzen *et al.*, 2008). As such it is clearly important that in order to understand the total impact of the use of such crops on total greenhouse budgets further research is carried out to investigate the impacts of their establishment and production on N_2O emissions. This thesis aims to address this by examining deviations in soil N_2O emissions resulting from land-use change from grassland to a second-generation bioenergy crop *Miscanthus x giganteus*, as well as subsequent nitrogen fertiliser management. Furthermore an approach to modelling N_2O emissions from *Miscanthus x giganteus* is examined. This chapter outlines important information from the current literature relative to the work found later in this thesis.

1.2 Land-use change

The human species has impacted global land-use throughout history in the extreme. Between one-third and one-half of the earth surfaces are thought to have changed directly as a consequence of the expansion of human development and resource requirements (Avissar & Werth, 2005). Since the industrial revolution (1850 – 2000) up to 572 Pg CO_2 have been emitted as the result of anthropogenically driven land-use change (Houghton, 2003). These emissions are caused by changes in biogeochemical processes, such as altering the rates of nutrient cycles (Feddema *et al.*, 2005), and the release of stable soil carbon stores through deforestation and the conversion of grasslands to cultivated land (IPCC, 2007a). In addition to releasing large amounts of CO_2, the conversion of previously stable ecosystems such as forests or grasslands to cultivated agricultural land can also result in large increases in N_2O emissions. This is largely due to increased substrate availability caused by the incorporation of above ground biomass into the soil and the disturbance of below ground organic matter, and the consequent adjustment of rates of nutrient cycling (Velthof *et al.*, 2010, MacDonald *et al.*, 2011).

Globally around 35% of ice-free land is used for agriculture (Ramankutty *et al.*, 2008), however this figure is ever changing. The period between the 1960's and 1990's saw a 12% increase in agricultural lands around the world (Mann, 1999). According to Houghton (1999) 68% of all land-use change associated greenhouse gas emissions are caused by the establishment and abandonment of agricultural land. The expansion of agricultural land has been largely driven by the need to produce food for an expanding global population and changes in dietary habits (Tilman *et al.*, 2001). However these factors are less influential in western countries and as such agricultural land use in Europe has in fact declined by approximately 13% in the past four decades (Rounsevell *et al.*, 2003). In Europe 34% of all land is used for arable crop production, while 14% is dedicated to grassland (Verburg *et al.*, 2006). In a European context it has been predicted that there will be a large reduction in future agricultural land cover due to changes in food demand and developments in crop production, though it is also noted that this may be offset by an increase in bioenergy crop production (Rounsevell *et al.*, 2006). An availability of possible future surplus agricultural land is likely to drive land-use changes, of which afforestation and agricultural land-use change to bioenergy crop production offer some of the most environmentally sound options (Houghton, 1999, Smith *et al.*, 2000). Furthermore the EU has committed to a target of 20% of all energy to be supplied by renewable means by 2020. Bioenergy crops currently account for over 60% of the total renewable energy in Europe and this trend is likely to continue, with a 10% share of EU transport fuels due to be provided by biofuels by 2020 (Don *et al.*, 2011). In order to reach these targets it is estimated that 17-21 million hectares of additional land will need to be converted to bioenergy crop production (Hastings *et al.*, 2009, Özdemir *et al.*, 2009).

This trend may be reflected in Ireland where there is an above average proportion of agricultural land, 61%. The majority of this is dedicated to livestock supporting pasture, 90%, while 10% is used for arable crop production (Agriculture, 2009). A decoupling of EU direct payments from agricultural production, to land-area-based payments has led to a trend for decreasing livestock numbers throughout the country (Styles & Jones, 2008). This has highlighted the need for alternative farming practices. Agricultural land-use change to bioenergy crops is one such option that is likely to be popular due in part to a government subsidy scheme announced in 2008.

1.3 Bio-energy crops

Bioenergy crops can be defined as any plant material used to produce bioenergy (Lemus & Lal, 2005). The utilisation of bioenergy crops has spread rapidly due to policies promoting the use of biofuels and commercial opportunities created by increasing petroleum prices (Hettinga *et al.*, 2009, van den Wall Bake *et al.*, 2009). Between 2003 and 2008 the production of bioethanol increased from 10 to 34 billion litres in the USA alone, while Government policy mandates biofuel production of 100 billion litres by 2022 (Tao & Aden, 2009). Meanwhile the EU have set targets for 10% of all vehicle fuel to be provided by biofuels by 2020 (Antizar-Ladislao & Turrion-Gomez, 2008). Most of this increase has involved the use of first generation bioenergy crops, utilised due to familiar agronomy and readily available technology. First generation biofuels refer to conventional food crops that can be processed to provide energy. Crops commonly used for this purpose such as maize, wheat, soybean, sugar cane, rapeseed and oil palm are generally converted to bioethanol by fermentation or biodiesel by transesterification (Smeets *et al.*, 2009). However many first generation biofuels are sugar and starch rich crops subject to input intensive agricultural processes (Jakob *et al.*, 2009), often leading to an overall energy production process with relatively low outputs compared to inputs (Farrell *et al.*, 2006, Wu *et al.*, 2007).

Second generation biofuels have the potential to provide a more efficient alternative. Second generation biofuels largely refer to lignocellulosic feedstocks (Naik *et al.*, 2010). This can refer to primary sources – purpose grown bioenergy crops, secondary – residues from agriculture and forestry, or tertiary – urban/industrial wastes and by-products. Work has also been carried out investigating the utilisation microalgae as a source in biodiesel production (Schenk *et al.*, 2008). Recently large amounts of investment have been applied to developing second generation conversion technologies and biomass sources (Bartle & Abadi, 2009). Crops chosen for use as primary sources tend to be robust perennial herbaceous and woody species, selected due to low input requirements during growth, good yields, and high energy outputs. One such crop subject to large amounts of research in Europe, and more recently the USA, is *Miscanthus x giganteus*.

1.3.1 Miscanthus

The genus Miscanthus is naturally distributed in East Asia. They are rhizomatous perennial grasses using a C_4 photosynthetic pathway, and are found growing optimally in tropical and subtropical regions (Clifton-Brown *et al.*, 2000). There are many Miscanthus genotypes, however of particular interest here is a naturally occurring hybrid – *Miscanthus x giganteus* (Greef & Deuter ex Hodkinson & Renvoize). *M. x giganteus* has been subject to considerable amounts of research in Europe due to it's ability to produce relatively large yields in temperate climates, while utilising a C_4 photosynthetic pathway. This particular hybrid was first cultivated in Denmark in 1935 after being introduced from Japan by Aksel Olsen (Linde-Laursen, 1993). Its parent species have been found to be *Miscanthus sacchiriflorus* – a diploid genotype – and *Miscanthus sinensis* – a tetraploid – meaning that *Miscanthus x giganteus* (from here on in simply referred to as *Miscanthus*) is a sterile triploid genotype (Hodkinson *et al.*, 2002). *Miscanthus* is of interest as a bioenergy crop due to a number of physiological advantages.

Crops using C_4 photosynthetic pathways have been shown to have greater radiation use efficiency than C_3 plants. This is particularly true in tropical climates, but several species – importantly *Miscanthus* – have been shown to respond well in temperate climates. Due to an improved mechanism for trapping CO_2, C_4 plants are on average 40% more efficient at converting intercepted radiation into dry matter than C_3 plants (Monteith, 1978). In the UK, *Miscanthus* has been shown to be up to 37% more efficient in terms of light use than native C_3 plants (Beale & Long, 1995). Water use efficiency has also been found to be greater in many C_4 plants than C_3 plants, this is due a lower stomatal conductance per molecule of fixed CO_2 in C_4 plants (Downes, 1969). Beale *et al.* (1999) determined water use efficiency in *Miscanthus* to be in the range of typical C_4 plants and exceed that found in C_3 species.

Significantly *Miscanthus* is a particularly efficient crop in terms of nutrient use. C_4 plants frequently exhibit better nutrient use efficiencies than C_3 plants (Öztürk *et al.*, 1981), this is particularly true in relation to nitrogen and phosphorus. Greater nitrogen use efficiency (NUE) in C_4 crops can be attributed to higher CO_2 concentrations at the site of Rubisco in the photosynthetic pathway; it has been shown that C_4 species contain 10-100 times more CO_2 at the site of Rubisco (Furbank & Hatch, 1987). Hence less Rubisco is required and the crops nitrogen use efficiency is improved (Long, 1999). When compared to other C_4 crops *Miscanthus* has also shown favourable nutrient use efficiencies. Compared with another common C_4 crop, maize, it has been shown to be up to 70 g g^{-1} more efficient in terms of NUE (Beale & Long, 1997). While some C_4 crops have been found to compare unfavourably with some C_3 crops at low nitrogen levels (Sage & Pearcy, 1987, Hocking & Meyer, 1991), this is unlikely to be the case with *Miscanthus* due to the plants rhizomatous nature. As well as relocating substantial amounts of nutrients to the rhizomes at the

end of the growing season, the rhizomes provide an extensive network below ground, which can intercept nutrients and thus reduce leaching. Beale and Long (1997) found nitrate loss through leaching in *Miscanthus* to be negligible, largely because of its below ground network, but also as the crops nitrogen demand is greatest at the beginning of the growing season not long after fertiliser application generally occurs. *Miscanthus*' nutrient use efficiency may also be improved by the presence of symbiotic relationships with nitrogen fixing bacteria (Eckert *et al.*, 2001, Boddey *et al.*, 2003). These relationships have been proposed to play an important role in *Miscanthus* nutrient supply. Davis *et al.* (2010) predicted up to 250 kg-N ha^{-1} could be derived from nitrogen fixing bacteria using the model DAY-CENT, although the extent of these relationships is likely to vary from site to site.

As previously mentioned *Miscanthus* is a rhizomatous crop. At the end of the growing season as the crop senesces much of the above ground biomass nutrient content relocates to the rhizomes, where they are stored over the winter. During the following growing season these nutrients are recycled, meaning that fertiliser requirements are greatly reduced (Beale & Long, 1997). Himken *et al.* (1997) calculated that between 20-46% of the nitrogen utilised by *Miscanthus* was remobilized from the rhizomes. This is an important factor when considering biomass as a fuel source due to the large energy demand required for fertiliser manufacture (Lewandowski & Schmidt, 2006). This also accounts for the relatively low concentration of contaminants released during combustion. As nutrients relocate to the rhizomes before harvesting, little are left in the above ground organs that are harvested and eventually undergo combustion. It has been shown that *Miscanthus* biomass has a suitable chemical composition for combustion. It has a lower mineral content than straw, and it's reactivity and ignition stability are favourable when compared with coal (Lewandowski *et al.*, 2000).

As a perennial rhizomatous grass *Miscanthus* can be harvested each year but only requires planting approximately every 15 years. This reduces external energy costs – and therefore CO_2 output – resulting from the use of machinery for annual planting, as well as eradicating the need for tilling, a large source of greenhouse gas emissions. It has also been stated that *Miscanthus* has good pest and disease resistance, this further reduces external energy costs by eliminating the need for pesticide application and also increases potential yield (Price *et al.*, 2004). Additionally as a triploid genotype it is sterile (Hodkinson *et al.*, 2002), and the inability to produce seeds greatly reduces the risk of becoming an invasive species.

All of the above factors indicate *Miscanthus*' suitability as an energy crop but importantly it also has been shown to produce substantial yields in European temperate climates. *Miscanthus* is suitable for delayed harvesting, that is harvesting the crop in spring (February to April). *Miscanthus* can be harvested once the crop has senesced, usually late autumn to early winter. Yield at this time

is referred to as peak yield, but a delayed harvest is often desirable as it results in lower crop moisture and mineral contents, which is useful for combustion, as well as reducing energy costs associated with drying. However a delayed harvest can also lead to significant yield reductions; losses of up to 25% have been reported for crops harvested in March (Lewandowski *et al.*, 2000). Field trials across Europe have produced yields of 10-30 t DM ha^{-1} yr^{-1} at delayed harvest, with variability largely caused by climatic variation (Clifton-Brown *et al.*, 2004b), while in the USA peak yields of up to 61 t DM ha^{-1} yr^{-1} have been recorded (Heaton *et al.*, 2008). In Ireland Clifton-Brown *et al.* (2000) have predicted peak yields of 26 t DM ha^{-1} yr^{-1} using the productivity model MISCANMOD. Such high yields rarely occur before the third growing season when the stand has reached maturity and fully established. Despite having a superior cold tolerance to many C_4 species (Farage *et al.*, 2006) perhaps the main obstacle to cultivating *Miscanthus* in temperate climates is its vulnerability to frost during the first growing season. This may also account for much of the observed variability in yield. Experiments have shown that winter soil temperatures of -3.4°C will kill rhizomes during the first winter (Clifton-Brown & Lewandowski, 2000). It has also been noted that a long winter frost in the first year may kill the young sprouts leading to the failure of the rhizomes to re-sprout (Christian & Haase, 2001).

Due to *Miscanthus*' high nutrient use efficiency high yields can be achieved with relatively low rates of nitrogen fertiliser application. Beale & Long (1997) propose that a nitrogen fertiliser application rate of 92 kg-N ha^{-1} is suitable to produce a yield of 15 t DM ha^{-1} while maintaining good soil quality, also taking into account that mineralisation and atmospheric deposition will also fulfil some of the crops nitrogen requirements. More recently Cadoux *et al.* (2011) have recommended a nitrogen application rate of 70 kg-N ha^{-1} using the same criteria, while Holland *et al.* (2005) state that average European nitrogen deposition rates of 9-12 kg-N ha^{-1} yr^{-1} may be sufficient to replace nitrogen lost through harvesting. It is broadly acknowledged that fertilisation is unnecessary during the first two years after planting, due to a minimal effect on yield and a high risk of nitrate leaching during this period (Miguez *et al.*, 2008, Cadoux *et al.*, 2011).

Nonetheless the relationship between external nitrogen inputs and *Miscanthus* yields is still subject to differing opinions. Due to its high nitrogen use efficiency and rhizomatous nature fertiliser application may not be necessary at all in soils with high rates of organic nitrogen mineralisation (Lewandowski *et al.*, 2000). Whilst field trials carried out in the UK found only 38% of ^{15}N applied as fertiliser at a rate of 60 kg-N ha^{-1} was taken up by the crop (Christian *et al.*, 1997). Danalatos *et al.* (2007) have found biomass production to show no significant response to nitrogen application rates of 50 and 100 kg-N ha^{-1} during field trials in Volos, Greece. While Ercoli *et al.* (1999) and Lewandowski & Schmidt (2006) have discussed the positive impacts of nitrogen on biomass production. Lewandowski & Schmidt (2006) have shown a positive correlation between nitrogen supply and yield, up to a peak level of 114 kg N ha^{-1}. Beyond this peak they suggest that increasing

nitrogen levels have a decreasing effect on yield due to toxicity. Ercoli *et al.* (1999) have also identified a positive correlation, where biomass production was still increasing significantly at nitrogen application rates of 200 kg N ha^{-1}. Although they imply an important relationship between soil moisture content, nitrogen level and yield. It was found that irrigation showed no positive impact on yield in the absence nitrogen fertilisation. This indicates that nitrogen level is the limiting factor in this situation, but also that the positive peak seen in the relationship between nitrogen level and yield is proportional to soil moisture content. A possible relationship between soil moisture content and nitrogen uptake has also been proposed by a number of members of the European Miscanthus Network (Clifton-Brown *et al.*, 2001). Cadoux et al. (2011) have carried out a review of available studies on *Miscanthus* yield. Of the eleven studies investigated five showed no yield response to nitrogen fertiliser, whilst six found positive responses, highlighting the uncertainties in the present knowledge of the relationship between nitrogen fertiliser application rate and *Miscanthus* yield.

Miscanthus has much potential as a bioenergy crop and can be processed to generate, power, heat, fuel, and reduce greenhouse gas emissions (Heaton *et al.*, 2004). Life cycle analyses of *Miscanthus* have shown the crop to have positive environmental impact when used as a fossil fuel substitute. This is evidenced by a reported ratio of energy outputs to inputs of 14.5-19.7, compared to a ratio of 8.5 for conventional grain crops (Lewandowski & Kicherer, 1997). Lewandowski *et al.* (1995) have calculated that replacing coal with *Miscanthus* during energy production could lead to a reduction in related greenhouse gas emissions of 90%. While for Ireland Styles & Jones (2008) calculated that growing *Miscanthus* for use as heat production fuel on 2.1% of available agricultural land would lead to a reduction of 2.5% in the national greenhouse gas inventory based on 2004 levels. It has also been shown that *Miscanthus* has the potential to produce up to 260% more ethanol per hectare than commonly used corn, and that by utilising the land currently used to grow corn for ethanol production with *Miscanthus* the USA could currently meet its 2016 renewable fuel use targets (Heaton *et al.*, 2008). However it should be noted that the greenhouse gas balances described here are highly dependant on nitrogen fertiliser application rates. In the previously mentioned study by Lewandowski *et al.* (1995) nitrogen fertiliser was found to contribute to 43% of total greenhouse gas emissions. This is caused by emissions resulting from the manufacture and spreading of fertiliser, and direct soil N_2O emissions.

1.4 Nitrous Oxide

Atmospheric nitrous oxide represents a considerable environmental threat due to its effects on stratospheric ozone and impacts as a greenhouse gas. N_2O has long been known to be damaging to the ozone layer (Crutzen, 1976, Williams *et al.*, 1992), with up to 13 Tg N_2O reacting in the stratosphere forming harmful oxygen free radicals each year (Butenhoff & Khalil, 2007). It is currently recognised as the most important ozone depleting compound and is likely to remain so for the remainder of this century (Ravishankara *et al.*, 2009). N_2O represents around 8% of all anthropogenic greenhouse gases and is third behind only CO_2 and CH_4 in terms of its damaging impact. Although atmospheric concentrations are relatively low its effect is significant due to the fact that it has a global warming potential 298 times greater than that of CO_2 over a 100 year period, and an atmospheric lifespan of 114 years. This has been compounded by a steady increase in atmospheric concentration of 0.26% yr^{-1} observed over the past few decades. The period between 1998 and 2005 alone saw an increase of 5 ppb, bringing global atmospheric concentrations to 319 ppb, representing an 11% increase in its contribution to global radiative forcing. Whilst the period between 1960 and 1999 saw concentrations grow at a rate over two times faster than any seen in the two millennia before 1800 (IPCC, 2007b). These changes have been strongly influenced by changes to food production systems over the past 150 years, in particular a rapid increase in the use of nitrogen fertiliser (Kroeze, 1999), as well as – albeit to a lesser extent – increased levels of NO_x deposition due to the combustion of fossil fuels (Butterbach-Bahl *et al.*, 1997).

Approximately 44% of global N_2O production is anthropogenic (Mosier *et al.*, 1998), arising from sources such as agriculture, fossil fuel combustion and industrial processes, aircraft, biomass and biofuel burning, human excreta, rivers, estuaries and coastal zones, and atmospheric deposition. The majority of nitrous oxide is produced in soil by microorganisms involved in nutrient cycling. Aerobic autotrophic nitrification and anaerobic heterotrophic denitrification are broadly recognised as the main soil N_2O production processes (Flechard *et al.*, 2007), however a number of other processes such as heterotrophic nitrification (Odu & Adeoye, 1970), aerobic denitrification (Robertson & Kuenen, 1990), fungal denitrification (Laughlin & Stevens, 2002), nitrifier denitrification (Wrage *et al.*, 2001), co-denitrification (Su *et al.*, 2004), nitrate ammonification (Silver *et al.*, 2001), and chemodenitrification (Christianson & Cho, 1983), are also known to substantially contribute. In total soil based processes are responsible for approximately 69% of all anthropogenic N_2O released into the atmosphere (IPCC, 2007b).

1.4.1 Agricultural N_2O Emissions

Agriculture has an important impact on global N_2O emissions, accounting for 10-12% of total greenhouse gas emissions. In terms of N_2O agriculture is single largest source of anthropogenic emissions accounting for 60% of total global emissions in 2005. It is responsible for 65% of total N_2O emissions within the EU15 (European Energy Agency, 2001), and an above average 79% in Ireland (Hyde *et al.*, 2006). Of this soil emissions make up the largest source (IPCC, 2007a). It has been estimated that agriculture has been responsible for over 70% of the increase in global atmospheric N_2O concentrations since 1850 (Kroeze, 1999). This is expected to continue, with agricultural N_2O emissions predicted to rise by 35-60% by 2030 (Bruinsma, 2003). This has been driven by a steadily increasing population and associated stresses on food demand (Smith, 1997), which lead to increasing nitrogen input into agricultural systems by the use of synthetic fertilisers, manure, human waste, and N_2 fixing crops. These large inputs increase rates of nitrogen cycling and consequent N_2O emissions from soil microbial processes (Groenigen *et al.*, 2005). 90% of all agricultural N_2O emissions from within the EU15 are reported to result from external nitrogen inputs (European Energy Agency, 2001). Fertiliser induced soil N_2O emissions have risen drastically in the recent past, with an 800% growth in nitrogen fertiliser use seen between the years 1960 and 2000, while synthetic fertilisers account for around half of all nitrogen reaching croplands globally today (Fixen & West, 2002).

Expanding agricultural land requirements have led to the conversion of enormous areas of temperate and tropical forest to pasture and cropland (Ramankutty & Foley, 1999). During the clearing process the combustion and biological decomposition of accumulated biomass results in an increase in available mineral N and hence can lead to increased N_2O emissions lasting for several years (Keller & Reiners, 1994). Agricultural lands used for livestock grazing also provide a significant source of N_2O. Urine and cattle droppings can provide a large input of nitrogen and readily available carbon substrates leading to increased N_2O emissions. It has been estimated that this accounts for approximately 16% of total global N_2O emissions (Flessa *et al.*, 1996), while in some countries with intensive livestock farming this figure can be much higher. For grazed pasture in New Zealand this accounts for up to 52% of anthropogenic N_2O emissions (de Klein *et al.*, 2001). A similar scenario is likely to exist in Ireland where 90% of all agricultural land supports livestock (Agriculture, 2009). However as previously mentioned recent changes in EU subsidy payments is likely to encourage alternative agricultural land uses, such as bioenergy crop production (Styles & Jones, 2008).

Hellebrand *et al.* (2008) measured soil N_2O emissions from a range of bioenergy crops over a period of nine years, finding mean N_2O emissions to be 0.8 ± 0.2% of N fertiliser applied, well within range of the IPCC default emission factor of 1 ± 0.3-3% (IPCC, 2007a). However the same

study did find emissions from perennial grasses and woody coppices to be consistently lower than those from annual crops. Similarly a review carried out by Don *et al.* (2011) suggested that second generation bioenergy crops emit 40% to >99% less N_2O than conventional annual crops. Crutzen *et al.* (2008) re-examined the accepted IPCC emission factor using a 'top down' approach resulting in an emission factor of 3-5%. This was then applied to two common first generation bioenergy crops, maize and rapeseed, to assess the impact of soil N_2O emissions on their greenhouse gas lifecycle budget. It was found that potential soil N_2O emissions could outweigh the greenhouse gas emissions saved through fossil fuel saving, but again it was accepted that a more positive situation may exist for woody coppices and grasses, such as *Miscanthus*. Current research discussing N_2O emissions from *Miscanthus* is rare, one of the few currently published studies of this sort was carried out by Jorgensen *et al.* (1997). Here N_2O was measured directly from fertilised and unfertilised *Miscanthus* for 207 days, with 1.5% of fertiliser N applied being lost as N_2O, similar to the rates observed by Hellebrand *et al.* (2008) across a range of other bioenergy crops. More recently Drewer *et al.* (2011) have found N_2O emissions from *Miscanthus* to be significantly lower than those from wheat – oilseed rape crop rotations. The previously mentioned review by Don *et al.* (2011) includes unpublished data on N_2O emissions from *Miscanthus*, which were found to be low when compared with wheat and maize.

1.4.2 Soil N_2O Production

N_2O is produced in the soil as a product of a number of microbial processes involved in ecosystem N cycling. Naturally nitrogen is available to ecosystems through two pathways; atmospheric deposition and nitrogen fixation. It is estimated that in 1995 100 Tg-N yr^{-1} of reactive nitrogen was deposited from the atmosphere, while by 2050 this figure could reach 200 Tg-N yr^{-1} (Galloway *et al.*, 2004). Biological N fixation by cultivated crops also represents a significant input into global nitrogen cycles, with an input of 40 Tg-N yr^{-1} calculated for 2005. Nitrogen fixation in soils occurs through non-symbiotic free-living bacteria and symbiotic bacteria in the root nodules of legumes and a number of other plants. Nonetheless anthropogenic nitrogen fertiliser additions still represent the largest input of nitrogen globally. In 2005 synthetic fertiliser production by the Haber-Bosch process reached 121 Tg-N yr^{-1} (Galloway *et al.*, 2008). While these nitrogen additions are intended for crop uptake, often only around 50% is consumed through crop growth (Mosier *et al.*, 1996). The balance remains in the soils and is either lost through leaching or utilised in microbial processes, often resulting in N_2O emissions.

The most commonly used conceptual model of soil N_2O emissions is the "hole in the pipe" model suggested by Firestone & Davidson (1989) (Figure 1.2). This model proposes that N_2O production in soil is controlled at three levels (Stark *et al.*, 2002). The first level is the gross rate of nitrification

and denitrification combined. The second level is the "size of the holes in the pipe", which represents the factors that control partitioning of N among the end products like inorganic nitrogen availability, soil temperature and pH. The third level represents transport processes, in which water filled pore space plays a major role.

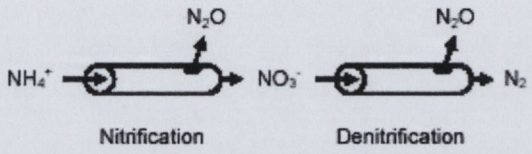


Figure 1.2 The 'hole in the pipe' conceptual model of N_2O production (Firestone & Davidson, 1989).

As previously mentioned N_2O is produced via a number of microbial processes. However the two broadly recognised as most important are aerobic autotrophic nitrification and anaerobic heterotrophic denitrification (Flechard *et al.*, 2007). Nitrification refers to the stepwise oxidation of ammonia to nitrite, and nitrite to nitrate, producing N_2O as a by-product (Troeh & Thompson, 2005). Of particular interest is the first step of this process, ammonia oxidation (Baggs, 2011), however the literature commonly simply refers to N_2O production via this pathway as nitrification. Nitrification is commonly carried out by autotrophic bacterial species such as *Nitrosomonas* and *Nitrobacter* which grow using the energy generated through the nitrification process (Robertson & Kuenen, 1991). It is also possible for nitrification to be carried out by a number of heterotrophic organisms, which must use an external source of organic carbon as an energy source. This process is more commonly found in fungi than bacteria (Odu & Adeoye, 1970). Whilst autotrophic nitrification is considered to be the dominant form of nitrification in arable soils (Robertson & Groffman, 2007), heterotrophic nitrification is thought to be dominant in acid soils, and under suitable conditions will produce more N_2O per cell than autotrophic nitrification (Anderson *et al.*, 1993, Bremner, 1997). Additionally there is also evidence to suggest that the microorganism group archaea may be involved in N_2O production by nitrification (Schleper, 2010). Nitrification is an aerobic process, as the oxidation steps require oxygen as the terminal electron acceptor, and is likely to occur when NH_4^+ is readily available (Robertson & Vitousek, 1981). If oxygen is in anyway limited, NH_4^+ may not undergo full oxidation to NO_3^- and can produce large amounts of N_2O (Poth & Focht, 1985). It has also been shown that under further short-term oxygen limiting conditions these autotrophic bacteria may produce N_2O through nitrifier denitrification (Wrage *et al.*, 2001). Rates of nitrification are affected by a number of environmental factors and tend to be lower at low pH, low O_2 availability, high C/N ratios, and high soil moisture contents (Robertson, 1982, Bramley & White, 1990, Bremner, 1997). Nitrification has been found to be a significant

source of N_2O at WFPS of 35-65% (Linn & Doran, 1984, Abbasi & Adams, 2000). At WFPS below 40% N_2O production is often restricted as NO is favoured as a by-product of the nitrification process (Davidson & Verchot, 2000).

Denitrification refers to the stepwise reduction of nitrate to nitrite and then to NO, N_2O, and N_2. Although N_2 is the ultimate end product of the process, it often does not reach completion leading to considerable emissions of N_2O (Tiedje, 1988). The process is carried out by a number of heterotrophic microorganisms requiring organic compounds as an energy source. These tend to be facultative bacteria. *Pseudomonas* species are the most commonly occurring denitrifiers, but other species such as *Alcaligenes* are known to make substantial contributions (Robertson & Groffman, 2007). Fungi have also been found to have an appreciable influence in N_2O production by denitrification. Laughlin & Stevens (2002) discovered fungi to be the dominant driver of N_2O production in a grassland soil, and also concluded that fungal denitrification could be of ecological significance due to the fact that N_2O – as opposed to N_2 – is the dominant end product of the process. N_2O production by denitrification is predominantly an anaerobic process. Denitrifiers are capable of using NO_3^- as an electron acceptor when O_2 is limited, allow them to cope with anaerobic conditions (Šimek *et al.*, 2002). Davidson *et al.* (2000) report that above 60% WFPS N_2O production is favoured by denitrification, while Bateman & Baggs (2005) found all soil N_2O production at 70% WFPS resulted from denitrification. However aerobic denitrification has been found to occur in situations where O_2 is not limiting (Patureau *et al.*, 2000). Heterotrophic bacterial species such as *Pseudomonas*, *Aeromas* and *Moraxella* have been shown to be able to use NO_3^- for respiration in the presence of O_2 (Carter *et al.*, 1995, Patureau *et al.*, 2000). Co-denitrification has also been found to play a role in soil N_2O production. This refers to the process whereby one nitrogen atom from either NO or N_2O produced during denitrification combines with another from a parallel process resulting in a hybrid product (Su *et al.*, 2004). Rates of N_2O production by denitrification are dependant on a number of factors. High levels of N_2O emissions resulting from denitrification are promoted by high soil temperature and moisture, large concentrations of NO_3^- and available carbon substrate, and low soil pH (Tiedje, 1988, Stevens *et al.*, 1998, Šimek & Cooper, 2002).

Nitrate ammonification is also now known to have some role in soil N_2O production in a range of ecosystems (Baggs, 2011). This process describes the dissimilatory reduction of nitrate to ammonium and is often referred to as DNRA. Nitrate ammonification is thought to occur at high pH, C/N ratio, bulk density, and soil nitrate concentrations (Schmidt *et al.*, 2011). It is clear from the number of processes presented here that soil N_2O production is a complex subject, it has been discussed that the existence of undiscovered N_2O production processes is also possible if not likely (Baggs, 2011). Figure 1.3 demonstrates the key pathways and reactions involved in the processes considered above.

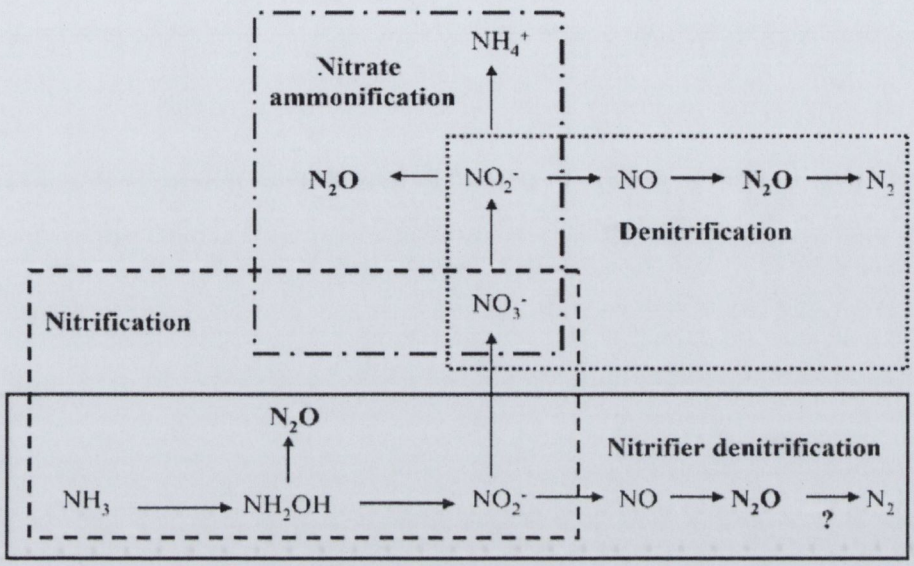


Figure 1.3 Key reactions and pathways of microbial N_2O production in soil (Baggs, 2008).

In general denitrification is believed to be responsible for higher rates of N_2O production than nitrification (Williams *et al.*, 1992). Their relative contributions are controlled by a range of factors including climate, ecosystem type, soil characteristics, patterns of organic carbon production and decomposition, as well as agricultural soil management practices (Khalil *et al.*, 2002). WFPS has often been cited as the most important controlling variable as it is directly linked with aeration and oxygen availability in the soil (Davidson, 1991, Davidson *et al.*, 2000, Smith *et al.*, 2003). High WFPS reduces the fraction of oxygenated pores in the soil, while also restricting the diffusion of plant root respired O_2 through the soil (Smith *et al.*, 2003). Denitrification is also favoured by high WFPS as it increases the solubility of organic carbon and nitrate (Bowden & Bormann, 1986). Well *et al.* (2006) found that nitrification was the dominant source of N_2O production at 55% WFPS, while above 75% WFPS denitrification was dominant. However a maximum WFPS of 80-85% for peak N_2O production by denitrification has also been suggested, as beyond this NO_3^- production by nitrification is seriously impeded (Veldkamp *et al.*, 1998, Dobbie & Smith, 2003a). As with nitrification the production of NO is favoured at lower WFPS. At WFPS between 50-60% NO production has been found to be in the region of 3-5 times greater than N_2O. However this is rarely observed in the field as NO has a greater tendency to be further reduced to N_2 by denitrifying microorganisms (Firestone & Davidson, 1989). Overall rates of N_2O production are thought to be greatest at intermediate WFPS in the range of 50-80% (Davidson, 1991, Dobbie & Smith, 2003a), but within this range N_2O emissions have been found to increase exponentially (Keller & Reiners, 1994, Dobbie & Smith, 2001).

As WFPS is derived directly from soil moisture and soil porosity, soil structure is also an important factor in determining rates of nitrification, denitrification, and overall N_2O production. A large soil porosity and high number of macro-pores is likely to lead to large N_2O emissions. As well as affecting important drainage properties (Choudhary *et al.*, 2002) soil structure heavily influences rates of N_2O production by denitrification by providing mixtures of anaerobic sites were denitrification can occur and aerobic pores through which the N_2O produced can escape to the atmosphere (Smith *et al.*, 2003). This mixture of aerobic and anaerobic sites, and the general heterogeneous nature of soil also means that soil N_2O production is not restricted to any one process at a given time. It has been noted that different processes may produce N_2O simultaneously in different microsites in the same soil (Robertson & Tiedje, 1987).

Soil N_2O emissions are strongly affected by soil temperature. Rates of both nitrification and denitrification increase with temperature as microbial activity is heavily temperature dependant (Addiscott, 1983). Strong evidence of this relationship can be seen when monitoring diurnal N_2O fluxes. Flessa *et al.* (2002) found 89% of diurnal variation in N_2O emissions correlated with temperature, while similar results have also been seen by Skiba *et al.* (1996) and Christensen (1983). Similar to WFPS N_2O emissions have been found to increase exponentially with soil temperature. This can be seen when looking at Q_{10} values – a measure of the rate of change when adjusting temperature by 10 °C – for soil N_2O emissions. Q_{10} for biochemical processes tend to be in the range of 2-3, however for soil N_2O emissions they frequently measure up to 10 or above (Smith *et al.*, 2003). Dobbie & Smith (2001) calculated Q_{10} from an arable field ranging from 9-50, while Brumme (1995) found a Q_{10} of around 14 for a forest soil. A frequent explanation for this large response is given by the theory of anaerobic zone development in soils, whereby anaerobic sites increase with temperature due to increased microbial activity, as consequently larger O_2 gradients leave sizeable areas of soil O_2 deficient (Leffelaar, 1986, Li *et al.*, 2000). While high temperatures are known to increase N_2O emissions, positive changes at very low temperatures are known to cause significant emissions too. In some field studies winter fluxes have been found to account for up to 70% of the total annual N_2O budget (Wagner-Riddle *et al.*, 1997, Lemke *et al.*, 1998). A large proportion of these emissions are caused by freeze-thaw cycles. Due to the physical disruption of soil organic matter and release of readily degradable carbon compounds from dead soil microbes (Christensen & Tiedje, 1990).

Soil pH affects soil N_2O production both directly and indirectly. Soil pH has been referred to as the 'master variable' of soil as it influences many physical, chemical, and biological properties and processes in the soil (Brady & Weil, 1999), a number of which are involved in N_2O production. Vitally it is an important controller of the size, diversity, and activity of soil bacterial communities (Fierer & Jackson, 2006). Šimek & Cooper (2002) concluded that the size of the active denitrifying community is directly related to pH. It is well established that pH has an important impact on the

N_2O product ratios of both nitrification ($N_2O/NO_2^- + NO_3^-$ and denitrification ($N_2O/N_2 + N_2O$) (Mørkved *et al.*, 2007). Lower pH values have been found to result in greater N_2O emissions from denitrification and a greater N_2O product ratio, while above pH 7 denitrification is more likely reach completion resulting in the production of more N_2 than N_2O (Šimek *et al.*, 2002). This is thought to be caused by the inhibition of the enzyme N_2O reductase which is responsible for reducing N_2O to N_2 during denitrification (Thomsen *et al.*, 1994). However it has also been pointed out that due to the ability of denitrifying bacteria to adapt to changes in soil pH and the influence of other factors it is difficult to define an optimum pH for N_2O production by denitrification (Šimek *et al.*, 2002). As denitrification often results in larger emissions of N_2O than nitrification it is has been suggested that liming slightly acidic agricultural soils may reduce overall N_2O emissions (Liu *et al.*, 2010). Conversely low soil pH is known to reduce rates of nitrification (Bremner, 1997). This is related to the inability of autotrophic ammonia-oxidizing bacteria to grow below pH 5-5.5 (Prosser, 1990, De Boer & Kowalchuk, 2001). It has been suggested that this is caused by NO_2^- toxicity (Stevens *et al.*, 1998).

Overall soil N_2O production is driven by a range of climatic factors, soil properties, and agricultural management practices. In any given situation different variables may operate as the dominant controlling influence, frequently even within micro-sites in the same soil (Robertson & Tiedje, 1987). In general soil N_2O production is controlled by a range of interactions between these factors, and due to the complex nature of these interactions it can often be difficult to define a correlation between these factors and N_2O emissions.

1.4.3 Soil N_2O Consumption

Stratospheric ozone destruction represents the largest global sink of N_2O (Butenhoff & Khalil, 2007), however soils also offer a potentially significant sink. Blackmer & Bremner (1976) demonstrated in a laboratory experiment that some soils consume more N_2O than they produce, resulting in a net uptake. This has since been found in numerous field studies in a wide range of ecosystems including forests (Dong *et al.*, 1998, Butterbach-Bahl *et al.*, 2002), grasslands (Ryden, 1983, Yamulki *et al.*, 1997, Yates *et al.*, 2006), and arable systems (Yamulki *et al.*, 1995, Goossens *et al.*, 2001). Anaerobic heterotrophic denitrification is considered the most important soil N_2O consumption pathway, as atmospheric N_2O may be reduced to N_2 by the enzyme N_2O reductase during this process (Bremner, 1997, Yu *et al.*, 2000). As such conditions that favour denitrification with a low N_2O product ratio encourage N_2O uptake, such as warm, wet soils with high organic matter contents. High soil moisture also aids N_2O consumption by increasing concentrations of dissolved N_2O, hence lengthening its residence time in the soil and increasing the likelihood of reduction to N_2 (Chapuis-Lardy *et al.*, 2007). Low soil concentrations of inorganic nitrogen have

been strongly linked with N_2O consumption (Ryden, 1983, Khalil *et al.*, 2002). Low NO_3^- availability is likely to increase the use of N_2O as an electron acceptor during denitrification (Butterbach-Bahl *et al.*, 2002). Despite these observations N_2O uptake has been found under a large variety of conditions, making it difficult to define an ideal set of parameters (Chapuis-Lardy *et al.*, 2007). This variability in conditions may be explained by the involvement of alternative N_2O consumption pathways. Rosenkranz *et al.* (2005) proposed that aerobic denitrification may have been the main consumption process in a well aerated sandy soil, while the involvement of nitrifying bacteria through nitrifier denitrification has also been suggested (Chapuis-Lardy *et al.*, 2007).

1.5 Modelling N_2O Emissions

The importance of models for simulating soil N_2O emissions has been highlighted by recent work calling into question the validity of applying the IPCC default emission factor for N_2O. Using a top down approach Crutzen *et al.* (2008) have shown that the IPCC default emission factor may underestimate global soil N_2O emission by up to a factor of five. Models can offer a valuable tool to predict N_2O emissions under a large range of scenarios, and often offer much more accurate and site-specific predictions than simply applying default IPCC emission factors. Additionally they offer an opportunity to predict future emissions as dictated by potential climate change scenarios.

In general models used to simulate N_2O emissions are process based, built on a series components linking physical, chemical, and biological processes. A number of models have been successfully used to predict agricultural soil N_2O emissions; these include DAYCENT, SOILN, ANIMO, DAISY, CENTURY, ECOSSE, and DNDC. DNDC is a popular model used to simulate a number of crop and soil dynamics. Its use it widespread with its website listing over 70 publications involving the model (University of New Hampshire, 2011).

1.5.1 DNDC

The DNDC (DeNitrification-DeComposition) model is a process-based model that can be used to predict greenhouse gas emissions, crop growth and soil carbon and nitrogen dynamics (Li *et al.*, 2001). DNDC was first designed for use with agriculture soils in the USA (Li *et al.*, 1992a), but variations have been used with considerable success for a number of land-uses and climates, ranging from tropical rainforests (Kiese *et al.*, 2005), to temperate grasslands (Brown *et al.*, 2002). DNDC has been successfully used to simulate N_2O emissions from Irish agricultural soils on a number of occasions (Hsieh *et al.*, 2005, Abdalla *et al.*, 2009a, Rafique *et al.*, 2011).

DNDC is a process-based model capable of predicting N_2O emissions as well as CO_2, CH_4, Crop growth, and soil C and N dynamics. DNDC carries out these calculations based on the interactions of five sub-models. These are, thermal-hydraulic, aerobic decomposition, denitrification, fermentation, and plant growth (Giltrap *et al.*, 2010). The thermal hydraulic sub-model calculates soil climatic conditions such as WFPS and temperature. The aerobic decomposition sub-model predicts rates of decomposition, CO_2 production, nitrification and ammonia volatilization. The denitrification sub-model simulates the various stages of the denitrification process, reducing NO_3^- to N_2. The fermentation sub-model simulates fermentation processes based on soil redox potential, whilst the crop growth sub-model predicts crop growth, partitioning and nutrient accumulation. Further description of the model is available in Li *et al.* (2000) and Li, C. (2007).

1.6 Aims

This thesis consists of three primary aims; firstly to investigate the effects of land-use change from grassland to *Miscanthus* on soil N_2O emissions, secondly to investigate the effects of varying rates of nitrogen fertiliser application on *Miscanthus*, and finally to assess the suitability of DNDC as a tool to predict soil N_2O emissions from *Miscanthus.* In order to do this two separate twelve-month field trials were carried out at the Teagasc Oak Park Research Centre, Co. Carlow, Ireland. In Chapter 2 the effects of land-use change from grassland to *Miscanthus* are examined. N_2O emissions as well as soil NO_3^- and NH_4^+ concentrations, and soil temperature and WFPS from a grassland, newly planted *Miscanthus* crop, and long established *Miscanthus* crop where measured at least bi-monthly, and the results discussed relative to statistical analyses by repeated measures two-way ANOVA and best fit multiple regression. Meanwhile Chapter 3 examines the effects of fertiliser addition by measuring the same parameters from a long established *Miscanthus* crop subject to three rates of nitrogen fertiliser application. The results are discussed relative to statistical analyses similar to those in Chapter 2. Furthermore Chapter 3 investigates the relationship between nitrogen fertiliser application rate, above ground biomass yield, and soil N_2O emissions. This relationship is further assessed by integrating the market prices of these elements into an economic analysis, deriving the farm-gate cost of nitrogen fertiliser application to *Miscanthus* during this experiment. In Chapter 4 data collected during the above trials are used to parameterise, run, and validate the process-based model DNDC for use with *Miscanthus*. Further calibration of the model is performed using a procedure presented in Chapter 4. Finally Chapter 5 discusses the results of the previous chapters relative to each other. A further economic analysis is also carried out demonstrating how farm-gate cost of nitrogen fertiliser application to *Miscanthus* may change under future climate change scenarios, combining work from Chapters 3 & 4.

Chapter 2

The effects of land-use change from Lolium perenne *to* Miscanthus x giganteus *on soil nitrous oxide flux*

2.1 Introduction

The agriculture and energy sectors are major contributors to greenhouse gas emissions, globally accounting for 13.5% and 25.9% of all anthropogenic emissions respectively (Denman *et al.*, 2007). In 2007 Irish agriculture was responsible for 26.8% of all national anthropogenic greenhouse gas emissions, the highest proportion of any sector, followed by energy at 21.5% (EPA, 2009). In Western Europe soil N_2O emissions are the dominant source of agricultural greenhouse gas emissions (IPCC, 2007a). In Ireland N_2O accounts for 38% of all agricultural GHG emissions, the vast majority of which come from agricultural soils (Duffy *et al.*, 2011).

A potential mechanism to reduce greenhouse gas emissions from both these sectors is to use existing agricultural land for bio-energy crop production. Worldwide 34% of all ice-free land is used for agriculture (Ramankutty *et al.*, 2008). In Ireland this figure is above average at 61%, of this 79% is dedicated to pasture, hay and grass silage, 11% to grazing and 10% to crop production (Government of Ireland, 2009). The Kyoto Protocol encourages the sequestration of atmospheric CO_2 through changes in land use and management as a way to offset CO_2 emissions (Kyoto Protocol Articles 3.3 & 3.4), and bio-energy crops have been identified to show the greatest potential for carbon mitigation of all the available agricultural options (Smith *et al.*, 2000). Ireland has a significant potential for land-use change to bio-energy crops due to the large availability of agricultural land. The need for alternate farming practices has also been emphasised by the decoupling of EU direct payments from agricultural production, to land-area-based payments, hastening a trend for decreasing livestock numbers in Ireland (Styles & Jones, 2008).

As crops grow they fix CO_2 from the atmosphere, this CO_2 being released when the crops undergo combustion implying that the net atmospheric CO_2 levels remain unchanged. This has led to the use of the term 'Carbon neutral' in reference to energy produced from biomass (Price *et al.*, 2004). Yet in reality this is a misrepresentation. There is a net CO_2 output associated with energy crops due to the external energy costs associated with the planting, fertilisation, harvesting, and processing of the crops (Lewandowski *et al.*, 1995). Other greenhouse gas costs – importantly N_2O emissions – are often overlooked or crudely calculated when analysing the environmental impacts of bio-energy crops. Recent work has indicated that for many common bio-energy crops such as rapeseed and maize the negative impacts of N_2O emissions on radiative forcing outweigh the benefits achieved by the subsequent fossil fuel savings, but the same study did suggest that these results may be more favourable for grasses and woody coppices (Crutzen *et al.*, 2008). One such grass gaining popularity as an energy crop is *Miscanthus x. giganteus*.

Miscanthus x giganteus is a C_4 grass originating from Asia, which has shown positive results when grown as a bio-energy crop in temperate climates. Field trials have produced yields of 10-30 t DM ha^{-1} yr^{-1} across Europe and 14 t DM ha^{-1} yr^{-1} in Ireland at delayed harvest (Clifton-Brown *et al.*,

2004b). As *Miscanthus* is a perennial rhizomatous crop with limited fertiliser requirements (Beale & Long, 1997), and planting required roughly only every 15 years (Price *et al.*, 2004). These attributes are favourable for bio-energy crops as they significantly reduce the external energy costs associated with their life cycle. Studies have shown *Miscanthus* to exhibit superior radiation use efficiency (Beale & Long, 1995) and water use efficiency (Lewandowski *et al.*, 2000) than many C_3 plants in temperate climates. Importantly its nutrient use has also been found to be more efficient than many C_3 species (Beale & Long, 1997). This is likely to be aided by the presence of nitrogen fixing bacteria in soils associated with *Miscanthus* (Eckert *et al.*, 2001, Boddey *et al.*, 2003) which are proposed to be an important factor in *Miscanthus* N-cycling (Davis *et al.*, 2010). A land use change from conventional agricultural crops to *Miscanthus* may also to lead to an increase in the quantity and quality of plant litter. Plant litter can account for up to almost 20% of total *Miscanthus* above ground biomass (Christian *et al.*, 1997), when combined with large yields there is potential for a large quantity of litter to become incorporated into the soil. The quality of this litter is likely to be affected by the fact that C_4 crops tend to contain larger proportions of recalcitrant tissues than C_3 species (Craine *et al.*, 2003, Camill *et al.*, 2004). These changes affect below ground substrate availability and consequent microbial community dynamics (Carney & Matson, 2005, Zavaleta & Hulvey, 2007), in turn influencing the soil nitrogen balance impacting processes such as nitrification, denitrification, and therefore N_2O emissions.

A better understanding of the environmental impacts of land-use change to bio-energy crops is clearly required. This experiment has focused on the effects of land-use change from an unfertilised, un-grazed grassland (*Lolium perenne*) to *Miscanthus x giganteus,* on soil N_2O fluxes. Here the hypothesis is proposed that "land-use change from *Lolium perenne* to *Miscanthus x giganteus* will not lead to a significant increase in soil N_2O emissions".

2.2 Materials and Methods

2.2.1 Site Description

This experiment was carried out at Teagasc, Crop Research Centre, Oak Park, Co. Carlow, Ireland (52°51' N 6°54' W, 50 m a.s.l.). The soil is classified as sandy loam with a mean C/N ratio of 10.7 (Dondini *et al.*, 2009).

The site has a mean annual precipitation of 830mm and temperature of 9.3°C for the period 1982-2002. During the study period there was an annual rainfall of 946 mm and mean annual temperature of 9.4°C, with minimum and maximum temperatures of -7°C to 26.8°C. February was the driest month receiving only 22.8 mm of rainfall, while July was the wettest with 159.3 mm (Climate records supplied by Met Eireann, Irish Meteorological Service).

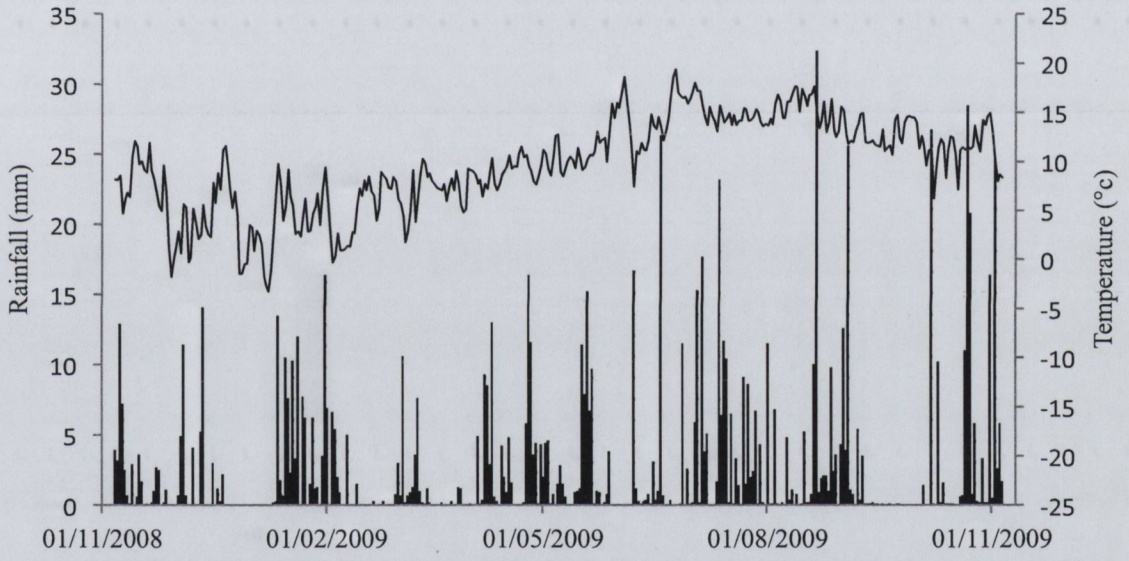


Figure 2.1 Temperature (line) and rainfall (bars) from November 2008 to November 2009 as recorded by Met Eireann at Oak Park, Co. Carlow, Ireland.

This experiment was carried out over a one-year period from November 2008 to November 2009, in three adjacent plots; an eighteen year old grassland (*Lolium perenne*), a fourteen year old established *Miscanthus* crop, and a seven month old newly planted *Miscanthus* crop. All three plots receive minimum management, where no fertiliser has been applied since 2002. The grassland is cut bi-annually in May and September, while the *Miscanthus* is harvested annually in April. The grassland and established *Miscanthus* plot were converted from a former arable site in 1990 and 1994 respectively, while the newly planted *Miscanthus* plot was converted from the grassland in 2008. Here one hectare of grassland was conventionally tilled and planted with *Miscanthus* at a density of two rhizomes per m^2. Herbicide was applied to this plot in June 2008 (AllySX and Compirox) and August 2008 (MCPA). All three plots have a pH of 6.8 (Dondini *et al.*, 2009).

2.2.2 Experimental Design

This experiment utilises a split-plot design with land-use as the main treatment. The three plots are located in close proximity to each other (Figure 2.2). They are referred to as grassland (G), newly planted *Miscanthus* (NM) and established *Miscanthus* (EM). Each plot consists of four subplots. The positioning of the subplots was decided during a previous, related experiment. Two samples were taken from opposing corners of each subplot resulting in a zigzag sampling pattern.

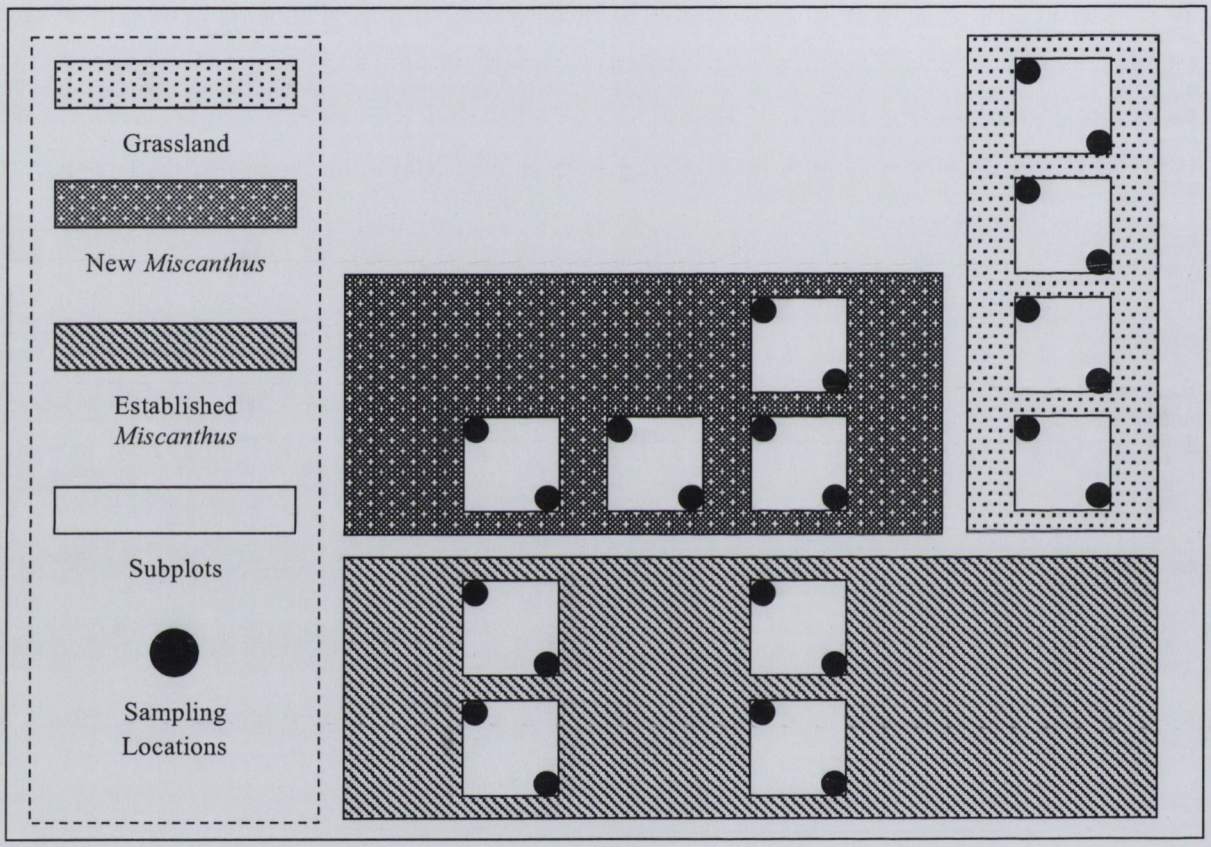


Figure 2.2 Diagram of experimental layout, (not to scale).

2.2.3 Chamber Design

Non-Flow-Through, Non-Steady-State (NFT-NSS) chambers were used to measure soil N_2O emissions (Figure 2.3). The chambers have been vented in order to minimize negative feedbacks caused by accumulating gas concentrations in the chamber (Hutchinson & Livingston, 2001, Davidson & Savage, 2002). The chambers have been designed to fit amongst the dense *Miscanthus* crop, with a surface area of 0.0314 m^2, a height of 0.194 m and a volume of 5 l. The vents attached to each chamber measure 0.2 m in length and have an internal volume of 25 ml. These measurements were chosen in order to minimize diffusive losses and to allow air to move in

response to pressure changes inside the chamber (Hutchinson & Mosier, 1980), and also to have an internal volume greater than the volume of each sample removed from the chamber. Each chamber attaches firmly to a collar planted permanently in the soil. The collars measure 0.12 m in height, 0.1 m of which is buried below the soil surface, and have an internal surface area of 0.0314 m^2. These have been tested using the criteria set out by Rochette and Eriksen-Hamel (2008) scoring in the 'very good' category for chamber design.



Figure 2.3 Non-Flow-Through, Non-Steady-State chambers used in this experiment.

2.2.4 N_2O Flux

N_2O emissions were usually measured weekly using the chambers described above. On occasion measurements were only taken fortnightly. These exceptions occurred between 21/11/08 and 04/12/08, 16/12/08 and 07/01/09, 13/01/09 and 04/02/09, 24/06/09 and 09/07/09, 14/08/09 and 25/08/09, and 22/09/09 and 08/10/09. Eight chambers per treatment were attached to collars positioned permanently in the soil. 22 ml gas samples were taken using a 60 ml syringe and stored in an evacuated 22 ml glass vial with a butyl rubber/PTFE septum. Samples were taken at 0.5 minutes after chamber deployment to allow for equilibration inside the chamber, and again at 40.5 minutes. Measurements were always taken between 10.30 and 13.30, in order to achieve the best

approximation of daily average flux. The chambers were tested for linearity over five points at the start of the experiment, and then again over three points seasonally.

Samples were analysed for N_2O within one month of collection using a gas chromatograph fitted with an electron capture device (Perkin-Elmer Clarus 500 GC). The GC was calibrated every three months using standards concentrations of 0, 2, and 10 ppm N_2O. These standards were stored in evacuated 22 ml glass vials with butyl rubber/PTFE septa, filled using 60 ml syringes.

N_2O fluxes are calculated using the following formula derived from Hutchinson and Livingston (1993):

$$F = \left(\frac{\partial C}{\partial t}\right)\left(\frac{M_m}{V_m}\right)\left(\frac{V}{A}\right)$$

Where C is the N_2O concentration in the chamber (ppm), t is chamber deployment time, M_m is the molar mass of N_2O (g), V_m is the molar volume of N_2O (m^3), t is time (hours), V is the chamber volume (m^3) and A is the surface area covered by the chamber (m^2). Cumulative fluxes were calculated by plotting N_2O flux against time, interpolating linearly between the points and then integrating beneath the line (Dobbie & Smith, 2003b).

2.2.5 Soil Temperature, Moisture, Bulk Density and WFPS

Soil temperature and volumetric water content (θ) were measured weekly (n=8), coinciding with N_2O measurements, using a WET sensor (Delta-T). Measurements were taken from a depth of 0-10 cm in a 0.25 m^2 area surrounding each soil collar. Mean bulk density (ρ) for each treatment (0-10 cm) was measured once in March 2009. Eight 172 cm^3 soil cores were collected from each treatment. Bulk density was calculated as the dry weight of soil (g) contained in a soil core of a known volume (cm^3). Soil porosity (f) was determined assuming a particle density of 2.65 as:

$$f = \left(1 - \frac{\rho}{2.65}\right)(100)$$

Water filled pore space (*WFPS*) was then calculated using the following equation:

$$WFPS = \frac{(\theta)(100)}{f}$$

2.2.6 Soil Nitrate and Ammonium Content

Soil samples were collected monthly from a depth of 0-10 cm for analysis of nitrate and ammonium concentrations (n=8). Samples were collected from a 1 m^2 area surrounding each soil collar. Nitrate and ammonium were extracted from the soil samples based on the techniques described in Compton and Boone (2000). Samples were homogenized and manually sieved through a 2 mm mesh. A 10 g subsample was taken from each sample. This was then added to 100 ml of 2 M KCl and shaken for one hour a 135 rpm. The resulting extract was filtered through filter paper (Whatman no. 42), and the filtrate frozen until ready for analysis using a colorimeter (Lachat QuickChem QC8500 Automated Ion Analyzer).

2.2.7 Statistical Analysis

Data was analysed using Prism 5 (Graph Pad) and Data Desk 6.2 (Data Description Inc.). The experiment was analysed as a split-plot design with land use as the main plot treatment. All data were tested for normal Gaussian distribution and transformed as appropriate. A repeated measures two-way ANOVA followed by a Bonferroni post-test was used in order to test the effects of plot and time on N_2O flux, WFPS, soil temperature, soil nitrate concentration and soil ammonium concentration. A best fit multiple regression analysis was carried out for N_2O flux versus soil temperature, soil moisture, WFPS, soil nitrate and ammonium. A one-way ANOVA followed by a Tukey's multiple comparison post-test was performed on bulk density data, and yearly cumulative N_2O emissions. All data were expressed as mean ± standard error values.

2.3 Results

2.3.1 Bulk Density

Mean bulk density varied between the plots from 0.76 g cm^{-3} in G to 0.87 g cm^{-3} in EM and 0.94 g cm^{-3} in NM (Table 2.1). 'Plot' was shown to have a significant effect on bulk density by a one-way ANOVA (Table 2.2). Specifically a significant difference was seen between plots G and NM, but not between G and EM, or NM and EM (Table 2.3).

Table 2.1 Mean bulk density (g cm^{-3}) and standard error (n=8) for all plots.

Plot	G	NM	EM
Bulk Density	0.76 ± 0.02	0.94 ± 0.04	0.87 ± 0.05

Table 2.2 Summary of one-way ANOVA on bulk density data.

P	**P Summary**	**F**	**R^2**	**Groups**
0.0086	**	6.013	0.3641	3

Table 2.3 Summary of Tukey's Multiple Comparison Test after one-way ANOVA on bulk density data.

Plots	**Mean Diff.**	**q**	**P < 0.05**	**Summary**
G v NM	-0.1862	4.866	Yes	**
G v EM	-0.1134	2.963	No	ns
NM v EM	0.0728	1.903	No	ns

2.3.2 Water Filled Pore Space

Soil moisture (0-10 cm) was measured on each sampling date. WFPS was calculated using these measurements and bulk density. Figure 2.3 shows mean WFPS and standard error (n=8) in each plot measured from November 2008 to November 2009. The highest mean WFPS (63.38%) occurred on 04/02/09 in plot EM, while the lowest (14.94%) was seen in 24/06/09 in G.

Analysis by repeated measures two-way ANOVA showed 'plot', 'time' and 'interaction' to all have a significant effect on WFPS (Table 2.4). Further analysis found 'plot' to lead to a significant difference in WFPS between G and EM (19 dates), and NM and EM (22 dates) on a much greater number of occasions than between G and NM (3 dates) (Annex 2.1). In order to further examine these differences they have been plotted against time. In Figures 2.5, 2.6 & 2.7 it can be seen that the greatest differences in WFPS are seen between plots G and EM, and NM and EM, occurring most frequently between the dates 22/04/09 and 22/07/09.

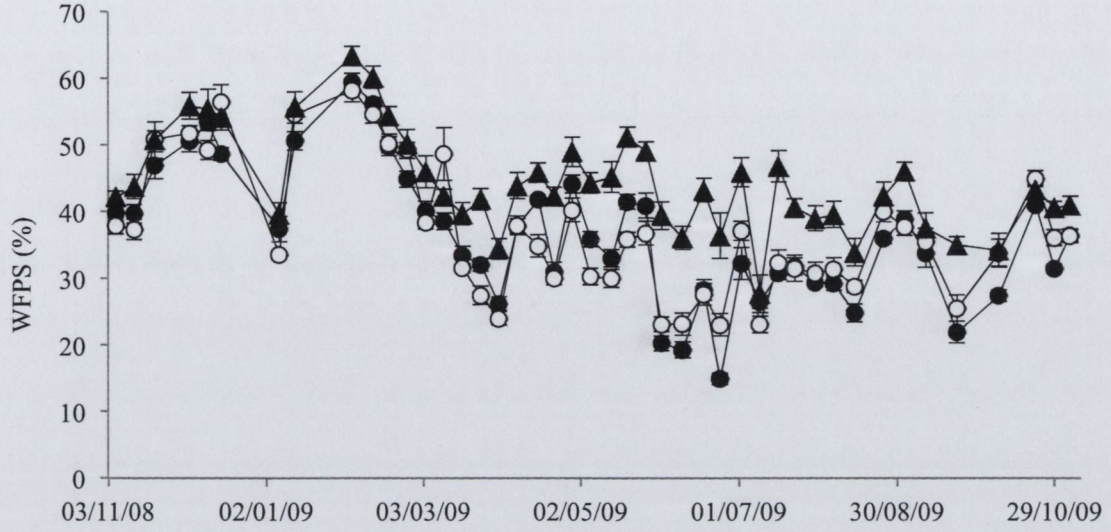


Figure 2.4 Mean WFPS (%) ± SE (n=8) from November 2008 to November 2009 from three plots; G (●), NM (○) and EM (▲).

Table 2.4 Summary of Repeated Measures Two-way ANOVA on WFPS data.

Source of Variation	Df	F	P	P Summary
Interaction	86	4.747	< 0.0001	***
Time	42	97.49	< 0.0001	***
Plot	2	59.68	< 0.0001	***

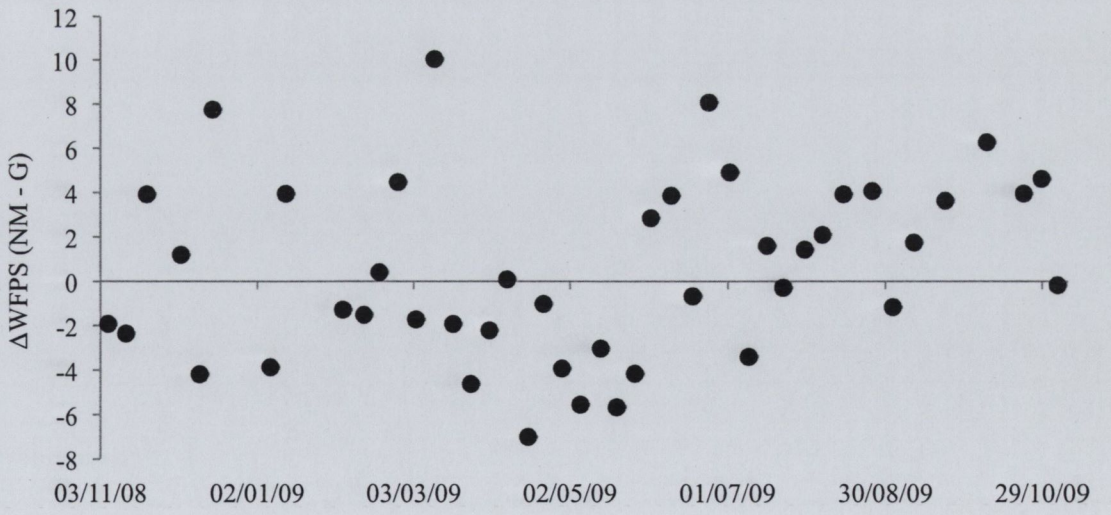


Figure 2.5 Difference in mean WFPS between NM and G from November 2008 to November 2009.

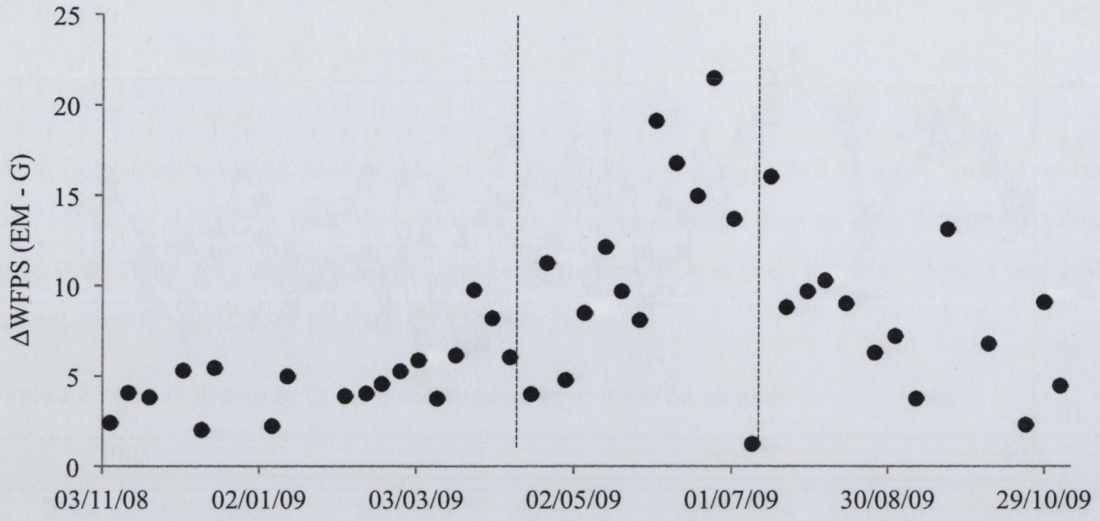


Figure 2.6 Difference in mean WFPS between EM and G from November 2008 to November 2009. Dashed lines indicate the dates 22/04/09 and 22/07/09.

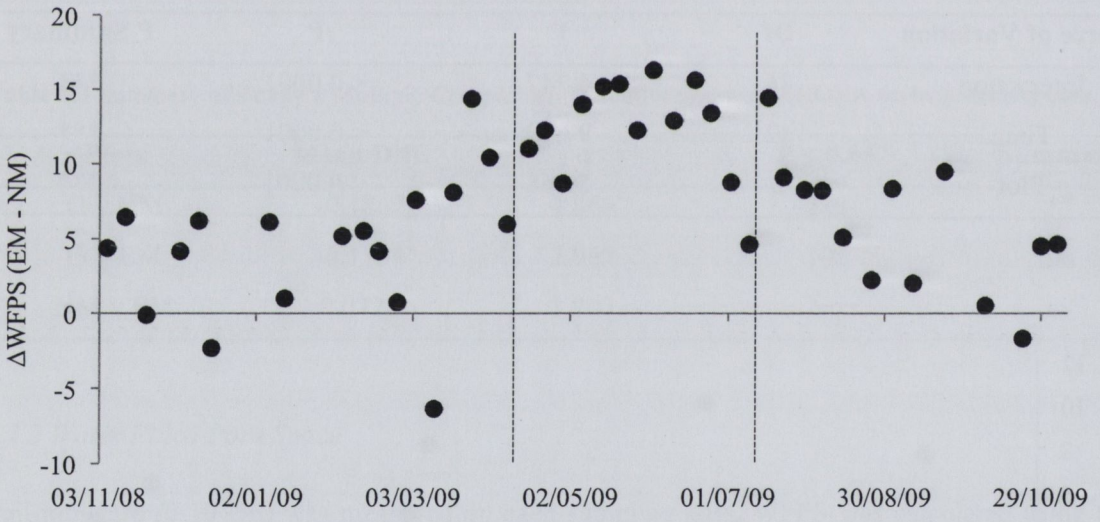


Figure 2.7 Difference in mean WFPS between EM and NM from November 2008 to November 2009. Dashed lines indicate the dates 22/04/09 and 22/07/09.

Table 2.5 Summary of Repeated Measures Two-way ANOVA on soil temperature data.

Source of Variation	Df	F	P	P Summary
Interaction	86	31.29	< 0.0001	***
Time	43	1751	< 0.0001	***
Plot	2	86.60	< 0.0001	***

2.3.3 Soil Temperature

Soil temperature (0-10 cm) was measured on each sampling date. Figure 2.8 shows mean soil temperature and standard error (n=8) in each plot measured from November 2008 to November 2009. The highest mean soil temperature (26.5°C) occurred on 24/06/09 in plot NM, while the lowest (-4.7°C) occurred on 04/02/09 in G. This low was an exception with mean soil temperatures consistently found to be lower in plot EM.

Analysis by repeated measures two-way ANOVA showed 'plot', 'time' and 'interaction' to all have a significant effect on soil temperature (Table 2.5). Further analysis by a Bonferroni post-test has shown 'plot' to lead to a significant difference in soil temperature between all plots, with significant differences found on a large number of occasions (Annex 2.2).

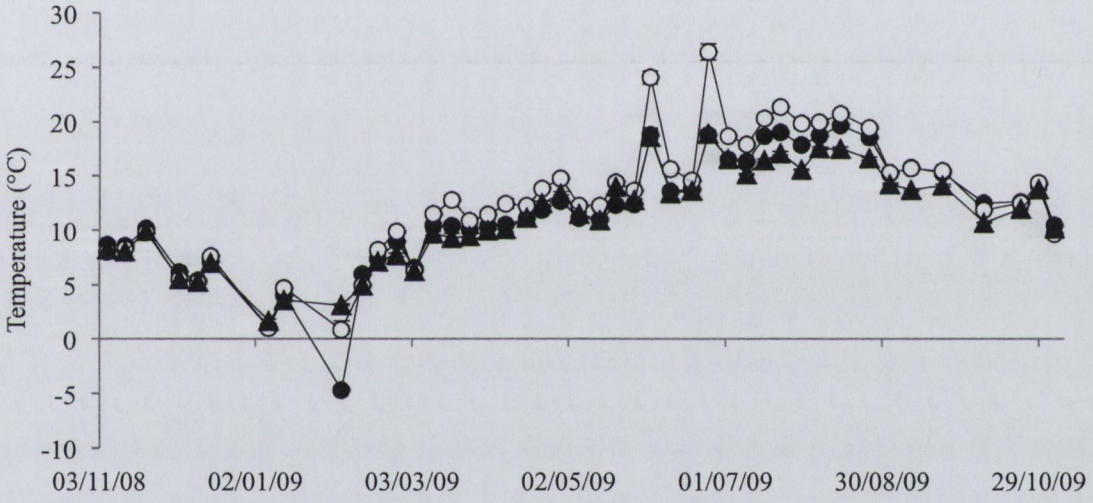


Figure 2.8 Soil temperature (°C) ± SE (n=8) from November 2008 to November 2009 for three plots; G (●), NM (○) and EM (▲).

2.3.3 Soil Nitrate & Ammonium

Soil NO_3^- concentration (0-10 cm) was measured monthly (n=8) from all three plots (Figure 2.9). Plot G frequently contained the lowest mean concentrations. The least being 1.49 mg kg^{-1} measured on 21/11/08. Both plots NM and EM tended to contain larger concentrations, with the highest (12.22 mg kg^{-1}) being measured in NM on 24/06/09. Analysis by repeated measure two-way ANOVA showed 'plot', 'time' and 'interaction' to all have a significant effect on soil NO_3^- concentration (Table 2.6). Comparing each individually by a Bonferroni post-test (Table 2.7) found a greater number of dates with significantly different soil NO_3^- concentrations between plots

G and NM (5), and G and EM (4) than NM and EM (1), with the majority of differences occurring between March and July.

As with NO_3^-, Soil NH_4^+ concentration (0-10 cm) was measured monthly (n=8) from all three plots (Figure 2.10). NH_4^+ showed more variation between plots than NO_3^- but had similar results when statistically analysed. The lowest mean soil NH_4^+ concentration (1.19 mg kg^{-1}) was measured in plot NM on 22/04/09, while the highest (9.13 mg kg^{-1}) was also measured in NM on 16/12/08. Analysis by repeated measure two-way ANOVA showed 'plot', 'time' and 'interaction' to all have a significant effect on soil NH_4^+ concentration (Table 2.8). Again comparing each plot individually by a Bonferroni post-test (Table 2.9) found a greater number of dates with significantly different soil NH_4^+ concentrations between plots G and NM (7), and G and EM (5) than NM and EM (2), although differences did occur over a longer time period from December to September.

To illustrate further the effect of 'plot' and 'time' on inorganic soil nitrogen the difference in mean soil nitrate and ammonium concentrations between plots EM and G (Figure 2.11), and NM and G (Figure 2.12), were plotted against time. Interestingly variation in both compounds exhibited opposing patterns throughout the year. While the difference in NO_3^- increased from spring through summer and then began to decrease, the opposite was seen with the difference in NH_4^+, which decreased from spring through summer and then began to increase. The difference between plots NM and G show similar patterns. Again the difference in NO_3^- increased from spring through summer and then began to decrease, while the opposite was seen with the difference in NH_4^+, which decreased from spring through summer and then began to increase.

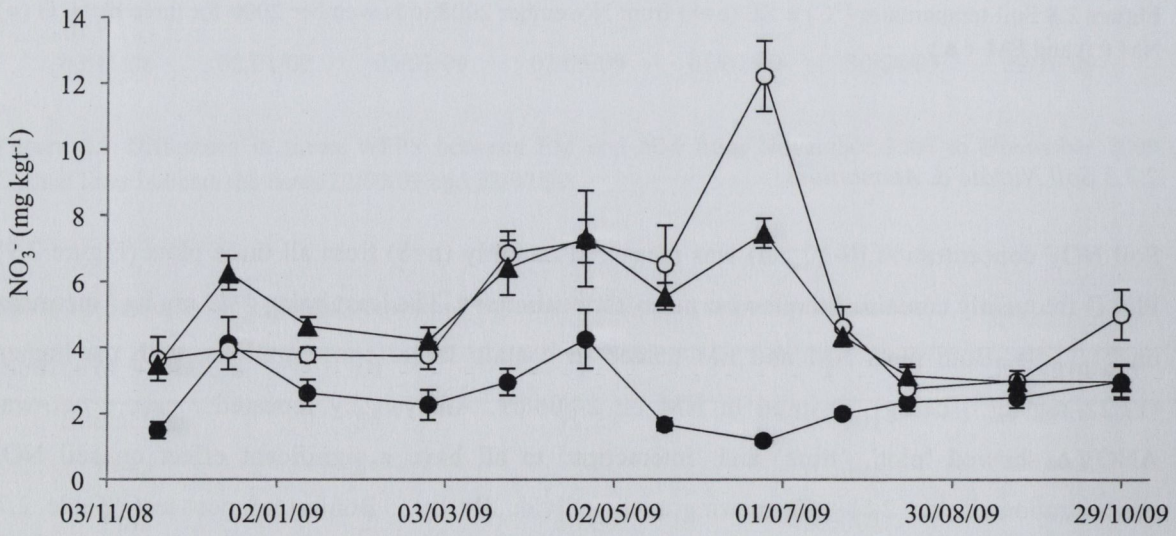


Figure 2.9 Soil NO_3^- (mg kg^{-1}) ± SE (n=8) for three plots; G (●), NM (○) and EM (▲).

Table 2.6 Summary of Repeated Measures Two-way ANOVA on Soil NO_3^- data.

Source of Variation	Df	F	P	P Summary
Interaction	22	8.736	< 0.0001	***
Time	11	20.27	< 0.0001	***
Plot	2	28.03	< 0.0001	***

Table 2.7 Summary of significant dates from a Bonferroni post-test on soil NO_3^- data.

Plot	Date	Difference	t	P	P Summary
G v NM	25/03/09	3.902	4.750	< 0.001	***
G v NM	22/04/09	2.964	3.607	< 0.01	**
G v NM	20/05/09	4.841	5.892	< 0.001	***
G v NM	24/06/09	11.03	13.43	< 0.001	***
G v NM	22/07/09	2.626	3.196	< 0.05	*
G v EM	25/03/09	3.458	4.209	< 0.001	***
G v EM	22/04/09	3.074	3.709	< 0.01	**
G v EM	20/05/09	3.868	4.709	< 0.001	***
G v EM	24/06/09	6.272	7.635	< 0.001	***
NM v EM	24/06/09	-4.763	5.798	< 0.001	***

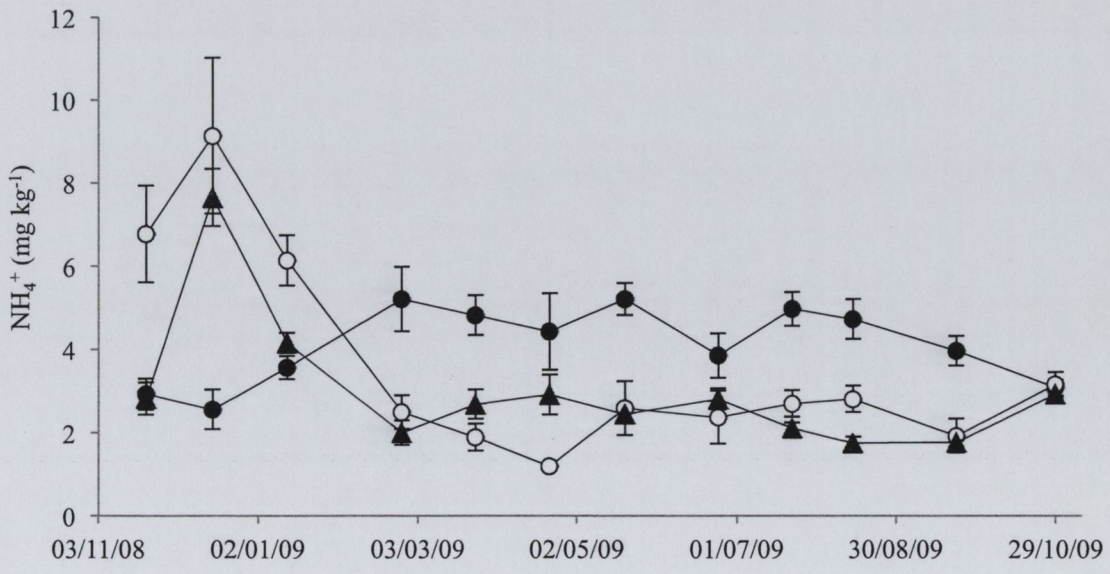


Figure 2.10 Soil NH_4^+ (mg kg^{-1}) ± SE (n=8) for three plots; G (●), NM (○) and EM (▲).

Table 2.8 Summary of Repeated Measures Two-way ANOVA on soil NH_4^+ data.

Source of Variation	Df	F	P	P Summary
Interaction	22	8.354	< 0.0001	***
Time	11	5.325	< 0.0001	***
Plot	2	20.77	< 0.0001	***

Table 2.9 Summary of significant dates from a Bonferroni post-test on soil NH_4^+ ($1/NH_4^+$) data.

Plot	Date	Difference	t	P	P Summary
G v NM	16/12/09	-0.2792	3.504	< 0.01	**
G v NM	25/02/09	0.2318	2.910	< 0.05	*
G v NM	25/03/09	0.4088	5.131	< 0.001	***
G v NM	22/04/09	0.5806	7.288	< 0.001	***
G v NM	20/05/09	0.3556	4.463	< 0.001	***
G v NM	24/06/09	0.2667	3.347	< 0.05	*
G v NM	22/09/09	0.3438	4.303	< 0.001	***
G v EM	16/12/09	-0.4264	5.352	< 0.001	***
G v EM	25/02/09	0.3423	4.296	< 0.001	***
G v EM	22/07/09	0.2813	3.531	< 0.01	**
G v EM	14/08/09	0.3905	4.902	< 0.001	***
G v EM	22/09/09	0.3107	3.900	< 0.01	**
NM v EM	22/04/09	-0.4393	5.514	< 0.001	***
NM v EM	14/08/09	0.2312	2.902	< 0.05	*

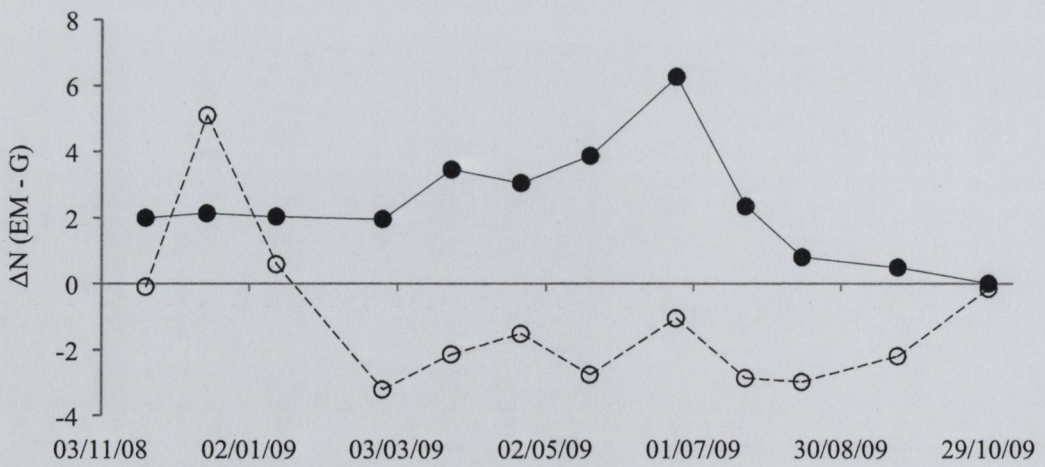


Figure 2.11 Difference in mean soil nitrate (•) and soil ammonium (○) concentrations between EM and G, from November 2008 to November 2009.

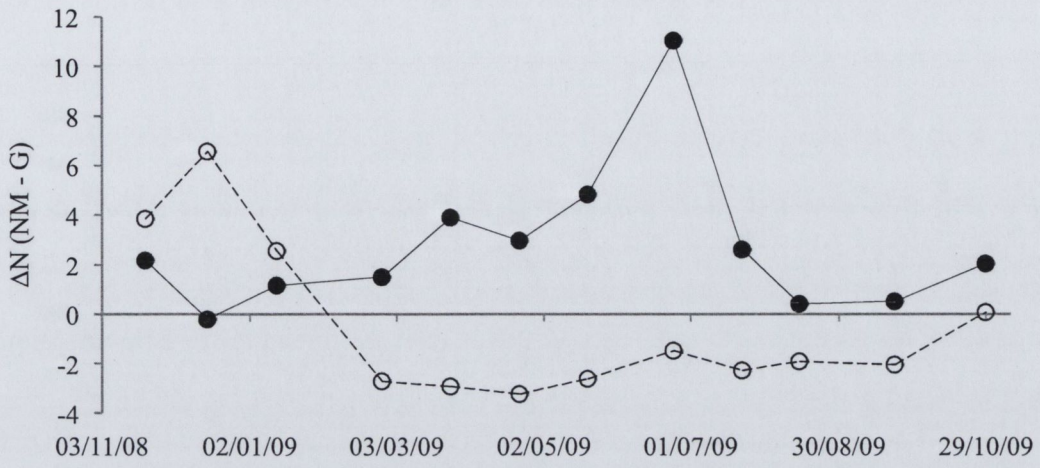


Figure 2.12 Difference in mean soil nitrate (•) and soil ammonium (○) concentrations between EM and NM, from November 2008 to November 2009.

2.3.4 Nitrous Oxide Flux

Figure 2.13 shows the mean N_2O flux and standard error (n=8) from plots G, NM and EM from November 2008 to November 2009. Mean fluxes showed large variation within treatment, with plot G ranging from -75.31 to 36.72 μg-N m^{-2} hr^{-1}, NM ranging from -59.35 to 70.40 μg-N m^{-2} hr^{-1} and EM ranging from -42.97 to 76.29 μg-N m^{-2} hr^{-1}. The majority of the positive and negative peaks occurred between the dates 22/04/09 and 22/07/09. Analysing these data by repeated measures two-way ANOVA (Table 2.10) showed that 'time' and 'interaction' have a significant effect on N_2O flux, while 'plot' has no significant effect. Unsurprisingly a Bonferroni post-test found significant differences only once between each of the plots, all occurring between April and June (Table 2.11). It should also be noted that N_2O consumption was frequent, with uptake observed in 36, 40 and 42 % of all measurements in plots NM, EM and G respectively.

Table 2.12 shows mean yearly cumulative N_2O flux and standard error (n=8). Fluxes were highest from plot NM (613.6 ± 294.1 g-N ha^{-1} yr^{-1}), this was the case for the majority of the year as can be seen in Figure 2.14. Cumulative fluxes from plot EM were 377.8 ± 132.7 g-N ha^{-1} yr^{-1}, Figure 2.14 shows that this was influenced by a period of consistent uptake between December 2008 and April 2009, and then a period of relatively high emissions between April and August 2009. The lowest mean yearly cumulative flux was seen in plot G (216.5 ± 163.4 g-N ha^{-1} yr^{-1}). A one-way ANOVA of these data (Table 2.13) showed that 'plot' had no significant effect. A subsequent Tukey's multiple comparison post-test (Table 2.14) also showed no significant difference in yearly cumulative N_2O flux between any of the plots. Best fit multiple regression analyses were carried out to test which factors control N_2O flux in each of the plots. WFPS, soil temperature, soil nitrate

and ammonium concentrations, and their interactions were examined. Results were inconsistent, with N_2O flux poorly explained across the board.

25% of N_2O fluxes from plot NM were found to be controlled by a large array of factors and complex interactions. Of these the interaction between soil NO_3^- concentration, WFPS, and soil temperature ($p \leq 0.0001$), and the interaction between soil NO_3^- concentration, soil NH_4^+ concentration, WFPS, and soil temperature ($p \leq 0.0001$), were found to be highly significant (Table 2.15). Meanwhile plots G and EM responded very poorly with only 2% and 6% of N_2O fluxes accounted for by the regression analyses respectively. No factor was found to significantly control N_2O fluxes from plot G (Table 2.16), while soil NO_3^- concentration ($p = 0.0049$), soil NH_4^+ concentration ($p = 0.0467$), the interaction between soil NO_3^- concentration and soil temperature ($p = 0.0119$), and the interaction soil NO_3^- concentration and WFPS ($p = 0.0094$) were all found to be significant factors in plot EM (Table 2.17).

Table 2.10 Summary of Repeated Measures Two-way ANOVA on N_2O flux data.

Source of Variation	Df	F	P	P Summary
Interaction	86	1.396	0.0129	*
Time	43	1.582	0.0109	*
Plot	2	0.6559	0.5293	ns

Table 2.11 Summary of significant dates from a Bonferroni post-test on N_2O flux ($Log(N_2O + 250)$) data.

Plot	Date	Difference	t	P	P Summary
G v NM	18/06/09	-0.2289	4.663	< 0.001	***
G v EM	29/04/09	0.1833	3.733	< 0.01	**
NM v EM	18/06/09	0.2022	4.118	< 0.01	**

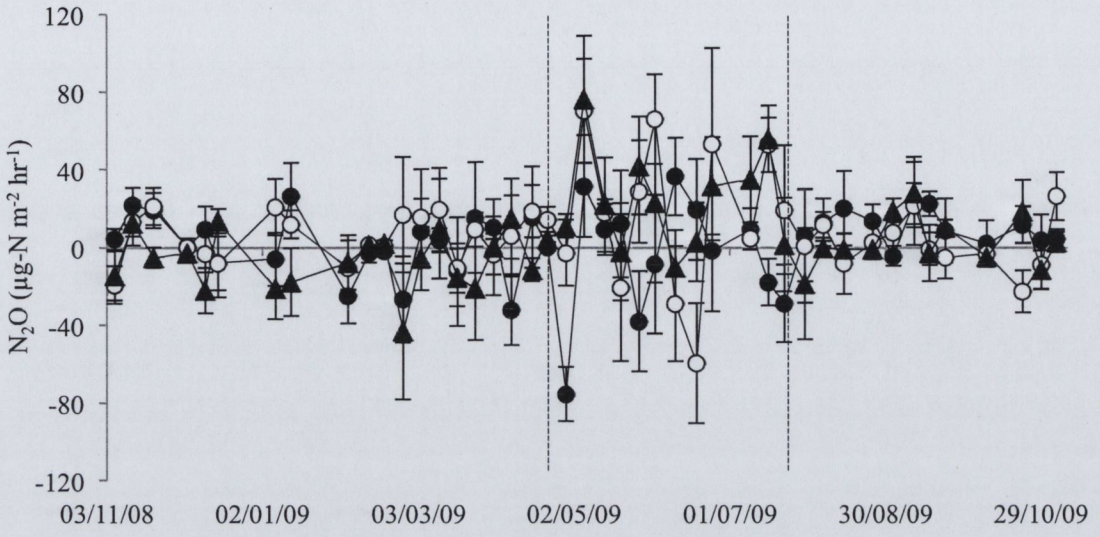


Figure 2.13 Mean N_2O Flux (µg-N m^{-2} hr^{-1}) ± SE (n=8) from three plots; G (●), NM (○) and EM (▲). Dashed lines indicate the dates 22/04/09 and 22/07/09.

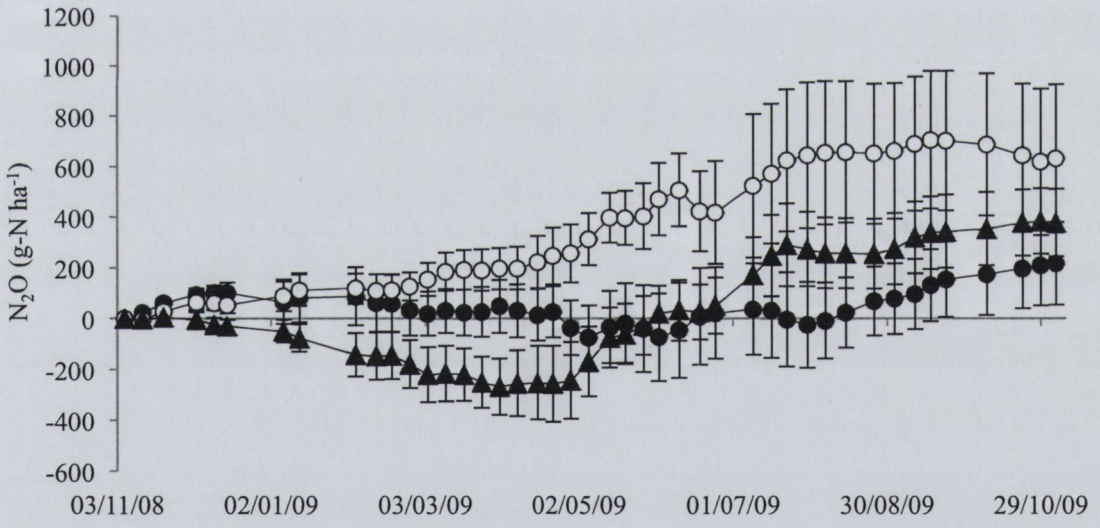


Figure 2.14 Cumulative N_2O Flux (g-N ha^{-1}) ± SE (n=8) over one year from three plots; G (●), NM (○) and EM (▲).

Table 2.12 Mean cumulative N_2O flux (g-N ha^{-1} yr^{-1}) and standard error (n=8) for all plots.

Plot	G	NM	EM
N_2O	216.49 ± 163.43	613.59 ± 294.13	377.83 ± 132.71

Table 2.13 Summary of one-way ANOVA of plot on cumulative yearly N_2O data set.

P	P Summary	F	R^2	Groups
0.3833	ns	1.004	0.0873	3

Table 2.14 Summary of Tukey's Multiple Comparison Test after one-way ANOVA on cumulative yearly N_2O data.

Plots	Mean Diff.	q	P < 0.05	Summary
G v NM	-415.1	1.988	No	ns
G v EM	-161.3	0.7726	No	ns
NM v EM	253.8	1.215	No	ns

Table 2.15 Summary of best fit multiple regression analysis of N_2O ($\sqrt{(N_2O+165)}$) flux data, with soil temperature, WFPS, soil NO_3^- ($\log(NO_3^-)$) and soil NH_4^+ ($\log(NH_4^+)$) from plot NM, (Adjusted $R^2 = 0.25$).

Source	Sum of Square	df	Mean Square	F-ratio
Regression	162.322	12	13.5269	3.53
Residual	310.367	81	3.83169	
Variable	**Coefficient**	**Coefficient S.E.**	**t-ratio**	**Probability**
Constant	39.5317	7.384	5.35	≤ 0.0001 (***)
Temp	-0.395418	0.3345	-1.18	0.2406
WFPS	-0.240363	0.0974	-2.47	0.0157 (*)
NO_3	-29.0904	7.880	-3.69	0.0004 (**)
NH_4	-53.1587	13.94	-3.81	0.0003 (**)
Temp*WFPS	-0.030441	0.0144	-2.12	0.0370 (*)
NO_3*Temp	-0.769748	0.3246	-2.37	0.0201 (*)
NH_4*WFPS	0.304351	0.1113	2.73	0.0077 (**)
NO_3*NH_4	69.6034	19.28	3.61	0.0005 (**)
NO_3*WFPS*Temp	0.093523	0.0193	4.86	≤ 0.0001 (***)
NH_4*WFPS*Temp	0.092166	0.0275	3.35	0.0012 (**)
NO_3*NH_4*Temp	1.52667	0.4248	3.59	0.0006 (**)
NO_3*NH_4*WFPS*Temp	-0.202331	0.0430	-4.71	≤ 0.0001 (***)

Table 2.16 Summary of best fit multiple regression analysis of N_2O flux data, with soil temperature, WFPS, soil NO_3^- ($\log(NO_3^-)$) and soil NH_4^+ from plot G, (Adjusted $R^2 = 0.02$).

Source	Sum of Square	df	Mean Square	F-ratio
Regression	7873.42	1	7873.42	2.50
Residual	296253	94	3151.63	
Variable	**Coefficient**	**Coefficient S.E.**	**t-ratio**	**Probability**
Constant	149.767	11.73	12.8	≤ 0.0001 (***)
NH_4*Temp	-0.306071	0.1936	-1.58	0.1173

Table 2.17 Summary of best fit multiple regression analysis of N_2O ($\sqrt{N_2O}$) flux data, with soil temperature, WFPS, soil NO_3^- ($\log(NO_3^-)$) and soil NH_4^+ ($\log(NH_4^+)$) from plot EM, (Adjusted $R^2 = 0.06$).

Source	Sum of Square	df	Mean Square	F- ratio
Regression	51.6375	7	7.37679	1.52
Residual	237.475	49	4.84643	
Variable	**Coefficient**	**Coefficient S.E.**	**t-ratio**	**Probability**
Constant	9.22322	3.314	2.78	0.076
NO_3	-46.9953	15.93	-2.95	0.0049 (**)
NH_4	43.0300	21.08	2.04	0.0467 (*)
NO_3*Temp	1.38166	0.5292	2.61	0.0119 (*)
NO_3*WFPS	0.667520	0.2469	2.70	0.0094 (**)
NH_4*Temp	-0.977431	0.6562	-1.49	0.1428
NH_4*WFPS	-0.680202	0.3500	-1.94	0.0577
WFPS*Temp	-0.010701	0.0059	-1.81	0.0764

2.4 Discussion

In this experiment the difference in N_2O fluxes, and common factors that are known to affect them, were examined in three adjacent plots containing grassland (G), a newly planted *Miscanthus* crop (NM) and a long established *Miscanthus* crop (EM) (Figure 2.15). These plots were chosen in order to study the short and long-term impacts of land use change from grassland to *Miscanthus*. A randomized block experimental layout was not possible due to the need to use a well-established *Miscanthus* plot. Hence a split-plot design with land-use as the main plot treatment was used. All three plots share a very close geographical location, soil type, and land-use history. Recently a comparable study has been carried out using these three plots (Dondini *et al.*, 2009).

An important point to make relative to this discussion relates to crop growth observed in the plots. The winter of 2008 was particularly cold, with an average temperature of 4.1°C, and a minimum temperature of -5°C in December, and average and minimum temperatures of 4°C and -7°C respectively in January (Met Eireann). This will have damaged the rhizomes of the newly planted *Miscanthus*, which are very susceptible to cold damage in the first winter after planting (Greef *et al.*, 1997). Clifton-Brown et al. (2000) found 50% of *Miscanthus* rhizomes were killed at a temperature of -3.4°C. This damage was evident, as much of the crop failed to establish in 2009 resulting in very low crop density and poor growth among those that did emerge. This was also exacerbated by competition from other plants as can be seen in Figure 2.15b. No effect of the harsh winter on growth was seen in the long established *Miscanthus* (Figure 2.15c).

As well as N_2O fluxes, soil bulk density, WFPS, temperature, nitrate and ammonium concentrations were measured. Bulk density was found to be lowest in plot G and highest in NM (Table 2.1). This is due to the fact that plot NM was found to contain a high density of stones beneath the soil surface. These are likely to have been moved to the surface when the plot was conventionally tilled 11 months prior to these measurements. Bulk density was found to differ significantly between G and NM (Table 2.3). This is unsurprising as plot G is long established and with no significant disturbance in its recent history, while plot NM was subject to considerable disturbance when tilled. Bulk density did not differ significantly between the other plots. Bulk density has been used to calculate WFPS, as such 'plot' was found to have a significant effect on WFPS (Table 2.4). WFPS was found to be significantly different between plots G and EM, and NM and EM, on a substantially greater number of dates than was seen between G and NM (Annex 2.1). Canopy cover is likely to have had an impact on these differences. A dense canopy was present at peak growth in plot EM compared to NM and G (Figure 2.15). A large leaf area will have affected soil water by increasing leaf evapotranspiration (Denmead & Shaw, 1962), as well as increasing the interception of radiant energy hence decreasing soil moisture losses by evaporation in plot EM (Cooper *et al.*, 1983). Figure 2.5 shows these differences plotted against time.



Figure 2.15 Photographs displaying all three plots; G (a), NM (b) & EM (c).

It can be seen in both instances that the largest differences between the plots occur between the sampling dates 22/04/09 and 22/07/09, with WFPS being consistently higher in EM than G and NM. This is due to the emergence of crop cover as discussed above, but could also be affected by water use efficiency during this period of intense growth. C_4 plants are known to be capable of better water use efficiency than C_3 plants (Long, 1983). 'Plot' was also found to affect soil temperature (Table 2.5), with significant differences seen between all plots (Figure 2.8). This will have been affected by crop cover as discussed previously, but could also be linked with the differing bulk densities observed among the plots (Table 2.1), as bulk density can directly affect soil temperature (Letey, 1985).

As none of the plots were subject to additional nitrogen treatments soil nitrate and ammonium concentrations were found to be relatively low (1.49 – 12.22 mg-NO_3^- kg^{-1} & 1.19 – 9.13 mg-NH_4^+ kg^{-1}) when compared those found in fertilised *Miscanthus* plots discussed in the next chapter. Both soil NO_3^- and NH_4^+ concentrations were found to be significantly affected by 'plot' (Tables 2.7 & 2.9). Significant differences occurred more frequently between plots G and NM, and G and EM, than NM and EM for both soil nitrate and ammonium concentrations (Tables 2.7 & 2.9). Looking at these differences graphically (Figures 2.11 & 2.12) it is apparent that temporal differences between NO_3^- and NH_4^+ occur. NO_3^- increased in the *Miscanthus* plots compared to the grassland throughout the growing season before decreasing again, whilst NH_4^+ increased in the grassland compared to the *Miscanthus* plots. These trends suggest differences in soil nitrogen cycling pathways between the *Miscanthus* and grassland plots. Foereid *et al.* (2004) found a higher rate of soil N mineralisation in C_3 grass (*Lolium* spp) plots than *Miscanthus* plots during soil incubation experiments, while field studies have shown higher rates of soil N mineralisation in C_3 communities than C_4 during the growing season (Epstein *et al.*, 1998, Mahaney *et al.*, 2008). Slower rates of nitrogen mineralisation beneath C_4 species has been linked with an increase in the quantity and quality of plant litter, this in turn can lead to an increase in rates of microbial nitrogen immobilisation (Evans *et al.*, 2001). This could explain the large positive difference in soil NH_4^+ between the Grass and *Miscanthus* plots during the growing season. Differing rates of mineralisation have also been linked with changes in soil pH (Curtin *et al.*, 1998, Högberg *et al.*, 2007), however as there is no difference in pH between the plots this is not a viable explanation here. The difference in NO_3^- observed between the Grass and *Miscanthus* plots during the growing season may indicate *Miscanthus* has a preference for root uptake of NH_4^+. Suspension cultures of a similar species *Miscanthus* × *ogiformis* Honda 'Giganteus' have been found to show a strong preference for ammonium as a source of nitrogen for growth (Holme, 1998).

N_2O flux displayed a lot of variation in all three plots, with few noteworthy peaks measured throughout the year (Figure 2.13). Overall cumulative yearly fluxes were relatively low (Table

2.12), and fell within the range commonly observed for unfertilised grasslands dominated by Italian ryegrass (*Lolium perenne*). In a nearby unfertilized grazed pasture yearly fluxes of 1000 g-N ha^{-1} have been recorded (Abdalla *et al.*, 2010), while yearly N_2O fluxes of 219 – 511 g-N ha^{-1} have been observed in an unfertilised grassland in Scotland (Jones *et al.*, 2007). A recent study monitoring N_2O emissions from a 3-4 year old unfertilised *Miscanthus* crop over two years found mean annual emissions of 324 g-N ha^{-1} yr^{-1}, again very similar to those found here (Drewer *et al.*, 2011). Likewise the yearly cumulative N_2O emissions measured during this experiment compare favourably with those from other bio-energy crops. Hellebrand *et al.* (2003) monitored N_2O emissions from a range of bio-energy crops. Yearly emissions from unfertilised plots in 2000 were; willow (540 g-N ha^{-1}), poplar (530 g-N ha^{-1}), hemp (940 g-N ha^{-1}), and rape (1110 g-N ha^{-1}). Elsewhere, Jin *et al.* (2010) have found yearly N_2O emissions ranging from 600-700 g-N ha^{-1} from reed canary grass. While recognising that factors other than crop type play an important role in soil N_2O production, it is clear that soil N_2O emissions from *Miscanthus* occupy a range commonly seen for bio-energy crops.

N_2O consumption was common, occurring in 36%, 40% and 42% of all measurements in plots NM, EM and G respectively. The frequently cited link between N_2O consumption and low concentrations of soil inorganic N (Ryden, 1983, Clayton *et al.*, 1997, Glatzel & Stahr, 2001) is likely to have been an important factor in this case. N_2O consumption is thought to generally occur when N_2O is reduced to N_2 by denitrifiers during denitrification (Hutchinson & Davidson, 1992, Bremner, 1997), although nitrifiers are also known to contribute to N_2O consumption via the nitrifier denitrification pathway (Beaumont *et al.*, 2002, Schmidt *et al.*, 2004, Chapuis-Lardy *et al.*, 2007). It is unclear as to what was the main N_2O consumption pathway here. Most of the peaks observed in N_2O emission and uptake occurred between 22/04/09 and 22/07/09. This period coincides with peak crop growth at the site, similar results were found by Du, R. et al (2006) who found an increase in N_2O flux variation during the growing season in grassland soils. The difference in WFPS between the sites was also found to increase during this time and may be linked. Dobbie and Smith (2003b), have reported WFPS to be the main driving factor behind N_2O flux during the growing season. Analyses by best fit multiple regressions were inconsistent amongst the plots, with little more than a vague relationship observed. Adjusted R^2 squared values were poor ranging from 0.25 in plot NM (Table 2.15) to 0.02 and 0.06 in G (Table 2.16) and EM (Table 2.17) respectively. Of the 25% of N_2O fluxes accounted for from plot NM the interaction between soil NO_3^- concentration, WFPS, and soil temperature, and the interaction between soil NO_3^- concentration, soil NH_4^+ concentration, WFPS, and soil temperature, were found to be highly significant (Table 2.15). These analyses indicate that a large number of complex factors and interactions as opposed to any single variable are likely to influence the rate of background N_2O flux throughout the year.

While 'plot' was not found to significantly affect N_2O flux (Table 2.10), a trend towards higher fluxes from plot NM can be seen when looking at cumulative yearly fluxes (Figure 2.14). This difference may be due to soil disturbance and substrate incorporation caused by conventional tilling before *Miscanthus* was planted. Studies have found N_2O emissions to increase significantly following the disturbance of previously undisturbed soils. MacDonald *et al.* (2011) found a significant increase in N_2O emissions in the year following the ploughing of a managed grassland, while Velthof *et al.* (2010) have found that the renovation of a grassland increased N_2O emissions by a factor of 1.8-3.0.

2.5 Conclusions

Based on the results of this experiment, land-use change from grassland to *Miscanthus* will not have a negative impact on medium to long-term N_2O emissions. No significant difference was found in N_2O flux between the grassland and *Miscanthus* plots. Furthermore once established, *Miscanthus* can be a particularly low source of N_2O, with yearly N_2O fluxes found in this experiment similar to those from unfertilised un-grazed grassland. The short-term effects of land-use change of this type on soil N_2O emissions are a little more ambiguous. Although yearly N_2O fluxes were not found to differ significantly between plots, they were found to be considerably higher in the new *Miscanthus* plot than the grassland, increasing by a factor of almost 3. This is unsurprising as increases in N_2O emissions are expected after the ploughing of previously undisturbed grassland. Many have also reported the majority of this effect occurs in a short period following ploughing. Davies *et al.* (2001) measured N_2O emissions of 1.5-3.7 kg N ha^{-1} in the seven weeks following ploughing. MacDonald *et al.* (2011) have linked this with rapid rate of N mineralisation following soil disturbance. This experiment has not looked at these effects. Work measuring these fluxes following land use change to *Miscanthus* is currently being carried out in Ireland, and should be taken into account when considering the full N loss implications of land-use change to *Miscanthus*. Another important consideration is the use of nitrogen fertiliser. While this study examines a scenario involving no nitrogen addition, fertilisation will be a key factor in N_2O emissions from many land use change scenarios. *Miscanthus*' low fertiliser requirements (Danalatos *et al.*, 2007, Cadoux *et al.*, 2011) could potentially lead to reduced N_2O emissions in other land-use change scenarios.

Chapter 3

The effects and costs of inorganic nitrogen fertiliser application on soil nitrous oxide emissions from Miscanthus x giganteus

3.1 Introduction

The previous chapter examined soil N_2O flux from a grassland and newly planted and established *Miscanthus x giganteus* stands receiving no nitrogen fertiliser. As a continuation this chapter investigates N_2O flux from an established *Miscanthus* stand receiving variable rates of nitrogen fertiliser. It has been reported that 42% of all N_2O emissions from cultivated land globally result directly from synthetic nitrogen fertiliser application (Smith, 1997), but our understanding of *Miscanthus* is poor in this regard. This chapter aims to address this by examining the effects and costs of synthetic nitrogen fertiliser application on soil N_2O fluxes and above ground biomass yield in *Miscanthus*.

Soil nitrous oxide emissions are of the upmost importance when considering the environmental costs of growing *Miscanthus x giganteus* as a bio-energy crop. As discussed in the previous chapter N_2O flux in unfertilised *Miscanthus* crops is unlikely to differ significantly from similarly managed grasslands. As with most agricultural crops a vast amount of soil N_2O emissions are likely to be caused by fertiliser addition (Bouwman *et al.*, 2002). The nutrient requirements of *Miscanthus* are known to be low compared to other conventional crops, due to it's high nutrient use efficiency, very efficient nutrient recycling to the rhizomes, extensive rooting (Beale & Long, 1997, Cadoux *et al.*, 2011), and symbiotic relationships with N-fixing bacteria (Davis *et al.*, 2010). Optimum rates of fertilisation are unclear. *Miscanthus'* response to N fertiliser is subject to a number of controlling variables in particular soil type (Miguez *et al.*, 2008) and soil water content (Ercoli *et al.*, 1999). Cadoux et al. (2011) have carried out a review of available studies on *Miscanthus* yield. Of the eleven studies investigated five showed no yield response to nitrogen fertiliser, whilst six found positive responses. Of these, high yield responses were only seen in two studies where irrigation was present. It has been acknowledged that no fertilisation is required in the first two years after planting *Miscanthus* due to minimal effect on yield and a high risk of leaching of applied fertiliser during this period (Miguez *et al.*, 2008, Cadoux *et al.*, 2011). Beyond this Cadoux et al. (2011) conclude in this review that for a yield of 15 t ha^{-1} a fertiliser application at a rate of 70 kg-N ha^{-1} is sufficient to replace nitrogen lost through harvesting and maintain good soil quality, while Beale and Long (1997) suggest a rate of 92 kg-N ha^{-1}. Excess fertilisation is commonplace worldwide, nutrient management alone is estimated to have the potential to reduce N_2O emissions from croplands by up to 0.68 kg-N ha^{-1} yr^{-1} (Smith *et al.*, 2008a). Nutrient management is also important to ensure that the level of nitrogen fertiliser application is economically viable. Maximum Economic Yield values, often used to recommend fertiliser application rate (Neeteson & Wadman, 1987), are unavailable for *Miscanthus,* and its uncertain and often poor yield response to nitrogen fertiliser could lead to financial losses at the farm gate.

An understanding of how such additions of nitrogen fertiliser will effect N_2O emissions from *Miscanthus* is vital. Currently very little information is available in this regard, with most studies simply adopting the IPCC default value of 1 ± 0.3-3 % of fertiliser N applied (IPCC, 2007a). One of the few currently published work of this sort was carried out by Jorgensen *et al.* (1997). Here N_2O was measured directly from fertilised and unfertilised *Miscanthus* for 207 days, with 1.5% of fertiliser N applied being lost as N_2O. More work is clearly required on this topic in order to fully understand the contribution of N_2O to the environmental costs of producing *Miscanthus* as a bio-energy crop. The experiment discussed in this chapter was designed to investigate the effect of varying rates of nitrogen fertiliser application on soil N_2O production and above ground biomass yield in *Miscanthus* grown in coarse textured soil. Here it is proposed that as with most agricultural crops, increasing the rate of nitrogen fertiliser application will lead to a direct increase in soil N_2O emissions resulting in the following hypothesis:

> *H1: Increasing the rate of nitrogen fertiliser application to* Miscanthus x giganteus *will significantly increase N_2O emissions.*

Additionally it is proposed that due to *Miscanthus*' high N use efficiency and limited nitrogen fertiliser requirements, much of the applied N will not be utilised by the crop and as such will lead to N_2O emissions disproportionate to any observed increases in yield.

> *H2: The observed increase in N_2O emissions will not be reflected by a proportional increase in above ground biomass yield.*

Finally, it is proposed that due to a low yield response, adding nitrogen fertiliser to *Miscanthus* will not be commercially profitable to an operational farm.

> *H3: Nitrogen fertiliser application to* Miscanthus *will not be economically profitable.*

3.2 Materials and Methods

3.2.1 Site Description

This experiment was carried out at Teagasc, Crop Research Centre, Oak Park, Co. Carlow, Ireland (52°51' N 6°54' W, 50 m a.s.l.). The soil is classified as loamy sand with a pH of 6.8 and a C/N ratio of 12.9 (Dondini *et al.*, 2009).

The site has a mean annual precipitation of 830mm and mean temperature of 9.3°C for the period 1982-2002. Meanwhile during the study period there was an annual rainfall of 718 mm and mean annual temperature of 8.8°C, with minimum and maximum temperatures of -12.9°C to 33.3°C. August was the driest month receiving only 25.5 mm of rainfall, while September was the wettest with 108.7 mm (Climate records supplied by Met Eireann, Irish Meteorological Service).

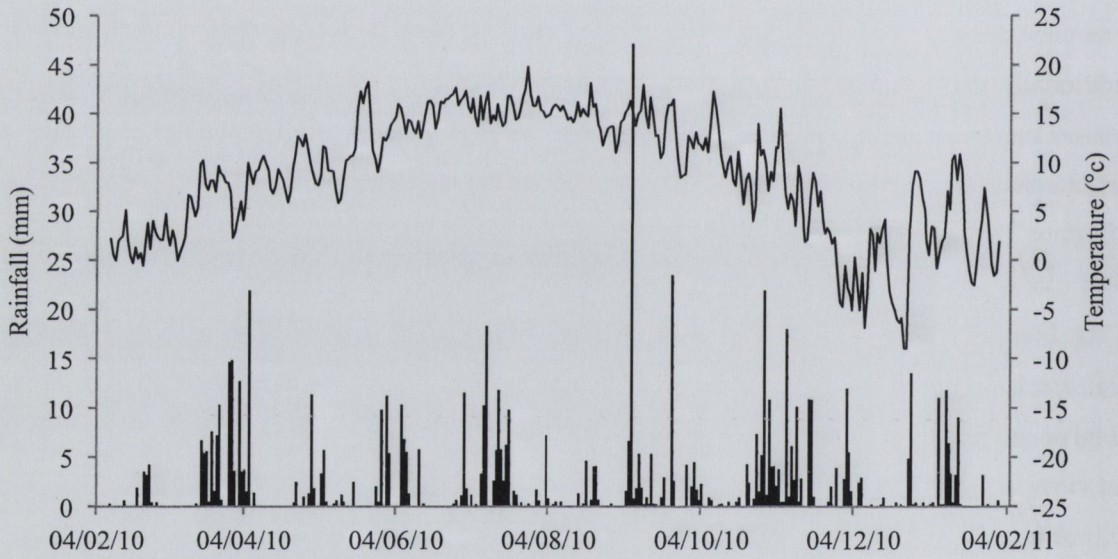


Figure 3.1 Temperature (line) and rainfall (bars) from February 2010 to February 2011 as recorded by Met Eireann at Oak Park, Co. Carlow, Ireland.

Measurements were taken over a one year period from February 2010 to February 2010, from a 15 year-old Miscanthus site subject to five rates of fertiliser application; 0, 37.6, 63, 90 and 125 kg-N ha^{-1}. These treatments were established in summer 2008. Only plots treated with 0, 63 and 125 kg-N ha^{-1} were used in this experiment. Fertiliser was applied in May 2009, and again on June 3rd 2010. Sulphur, phosphorus and potassium were also applied on these occasions at rates of 20 kg-S ha^{-1}, 30 kg-P ha^{-1} and 120 kg-K ha^{-1}, respectively. Prior to this the site had been unfertilised since 2002. The *Miscanthus* is harvested annually in March. The site was converted to *Miscanthus* from former arable land in 1994.

3.2.2 Experimental Design

A long established Miscanthus site was divided into 20 parallel plots measuring 2.5 x 5 m, surrounded by a buffer of 0.5 m (Figure 3.2). The plots were treated with fertiliser at rates of 0, 37.6, 63, 90 and 125 kg-N ha^{-1}. As previously mentioned only plots treated with 0 (0N), 63 (63N) and 125 (125N) kg-N ha^{-1} were used in this experiment. Each treatment consisted of four plots arranged in randomized blocks. On each sampling occasion two samples were taken from opposite ends of each plot.

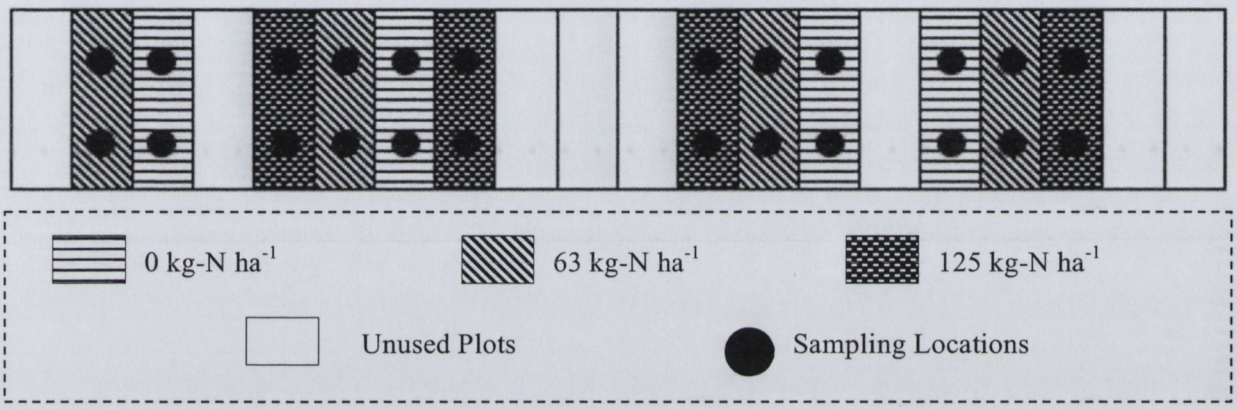


Figure 3.2 Diagram of experimental layout, (not to scale).

3.2.3 Chamber Design

Non-Flow-Through, Non-Steady-State (NFT-NSS) chambers were used to measure soil N_2O emissions. The chambers have been vented in order to minimize negative feedbacks caused by accumulating gas concentrations in the chamber (Hutchinson & Livingston, 2001, Davidson & Savage, 2002). The chambers have been designed to fit amongst the dense *Miscanthus* crop, with a surface area of 0.0314 m^2, a height of 0.194 m and a volume of 5 l. The vents attached to each chamber measure 0.2 m in length and have an internal volume of 25 ml, these measurements were chosen in order to minimize diffusive losses and to allow air to move in response to pressure changes inside the chamber (Hutchinson & Mosier, 1980), and also to have an internal volume greater than the volume of each sample removed from the chamber. Each chamber attaches firmly to a collar planted permanently in the soil. The collars measure 0.12 m in height, 0.1 m of which is buried below the soil surface, and have an internal surface area of 0.0314 m^2. These have been tested using the criteria set out by Rochette and Eriksen-Hamel (2008) scoring in the 'very good' category for chamber design.

3.2.4 N_2O Emissions

N_2O emissions were measured weekly using the chambers described above. Eight chambers per treatment were attached to collars positioned permanently in the soil. 22 ml gas samples were taken using a 60 ml syringe and stored in an evacuated 22 ml glass vial with a butyl rubber/PTFE septum. Samples were taken at 0.5 minutes after chamber deployment to allow for equilibration inside the chamber, again after 30.5 minutes, and finally after 60.5 minutes. Measurements were always taken between 10.30 and 13.30, in order to achieve the best approximation of daily average flux. The chambers were tested for linearity over five points at the start of the experiment, and then again over three points seasonally.

Samples were analysed for N_2O within one month of collection using a gas chromatograph fitted with an electron capture device (Perkin-Elmer Clarus 500 GC). The GC was calibrated every three months using standards concentrations of 0, 2, and 10 ppm N_2O. These standards were stored in evacuated 22 ml glass vials with butyl rubber/PTFE septa, filled using 60 ml syringes.

N_2O fluxes are calculated using the following formula derived from Hutchinson and Livingston (1993):

$$F = \left(\frac{\partial C}{\partial t}\right)\left(\frac{M_m}{V_m}\right)\left(\frac{V}{A}\right)$$

Where C is the N_2O concentration in the chamber (ppm), t is chamber deployment time, M_m is the molar mass of N_2O (g), V_m is the molar volume of N_2O (m^3), t is time (hours), V is the chamber volume (m^3) and A is the surface area covered by the chamber (m^2). Cumulative fluxes were calculated by plotting N_2O flux against time, interpolating linearly between the points and then integrating beneath the line (Dobbie & Smith, 2003b).

3.2.5 Soil Nitrate and Ammonium Content

Soil samples were collected monthly from a depth of 0-10 cm for analysis of nitrate and ammonium concentrations (n=4). Samples were collected from a 1 m^2 area surrounding the soil collars. Nitrate and ammonium were extracted from the soil samples based on the techniques described in Compton and Boone (2000). Samples were homogenized and manually sieved through a 2 mm mesh. A 10 g subsample was taken from each sample. This was then added to 100 ml of 2 M KCl and shaken for one hour a 135 rpm. The resulting extract was filtered through filter paper (Whatman no. 42), and the filtrate frozen until ready for analysis using a colorimeter (Lachat QuickChem QC8500 Automated Ion Analyzer).

3.2.6 Soil Temperature, Moisture, Bulk Density and WFPS

Soil temperature and volumetric water content (θ) were measured weekly (n=4), coinciding with N_2O measurements, using a WET sensor (Delta-T). Measurements were taken from a depth of 0-10 cm in a 0.25 m^2 area surrounding the soil collars. Mean bulk density (ρ) at 0-10 cm was measured once in March 2009. Eight 172 cm^3 soil cores were collected. Bulk density was calculated as the dry weight of soil (g) contained in a soil core of a known volume (cm^3). Soil porosity (*f*) was determined assuming a particle density of 2.65 as:

$$f = \left(1 - \frac{\rho}{2.65}\right)(100)$$

Water filled pore space (*WFPS*) was then calculated using the following equation (ref?):

$$WFPS = \frac{(\theta)(\rho)(100)}{f}$$

3.2.7 Biomass Yield

Above ground biomass was measured in November 2010 by the Teagasc Research Centre team (Finnan, 2010).

3.2.8 Economic Cost Analysis

The economic cost of increasing fertiliser application rate has been calculated both excluding and including the cost of N_2O emissions as CO_2 equivalents. In order to do this the cost of increasing fertiliser application rate from 0 kg-N ha^{-1} has been subtracted from the value of the resulting increase in yield for both the 63N and 125N treatments. Additionally the cost of consequential N_2O emissions has been subtracted from this value. The cost of fertiliser application has been calculated using the value of €161 ha^{-1} for a rate of 100:20:100 kg ha^{-1} N:P:K found in a study by Styles *et al.* (2008). The rate of nitrogen application was then adjusted using an Irish national average price for CAN of €32 t^{-1} (Hayes & Conway, 2011). *Miscanthus* has been assigned a price of €63 t^{-1} DM (Styles & Jones, 2008). The European Union benchmark (EUA) price for carbon as of 03/08/11, €11.05, was used to calculate the cost of N_2O emissions.

3.2.9 Statistical Analysis

Data was analysed using Prism 5 (Graph Pad) and Data Desk 6.2 (Data Description Inc.). The experiment was analysed as a randomised block design. All data were tested for normal Gaussian distribution, and transformed as appropriate. A repeated measures two-way ANOVA followed by a Bonferroni post-test was used in order to test the effects of plot and time on N_2O flux, WFPS, soil temperature, soil nitrate concentration and soil ammonium concentration. A best fit multiple regression analysis was carried out for N_2O flux versus soil temperature, soil moisture, WFPS, soil nitrate and ammonium. A one-way ANOVA followed by a Tukey's multiple comparison post-test was performed on above ground biomass data, and yearly cumulative N_2O emissions. All data were expressed as mean ± standard error values.

3.3 Results

3.3.1 Bulk Density & Water Filled Pore Space

WFPS was calculated using soil moisture and bulk density (0-10 cm). Bulk density at the site was found to be 0.87 ± 0.05 g cm^{-3} (n=8). Figure 3.3 shows mean WFPS and standard error (n=4) measured in each treatment from February 2010 to February 2011. WFPS ranged from 15.6 to 50.9 %, with the driest date occurring on 29/06/10 and the wettest on 13/12/10. Analysis by repeated measures two-way ANOVA revealed time and interaction to have a significant effect on WFPS but not treatment (Table 3.1). In December 2010 and January 2011 a trend towards higher WFPS in 0N can be seen, this is supported by the results of a Bonferroni post-test where differences were seen between 0N and 125N on 13/12/10 and 20/01/11. Despite this these differences did not affect the overall effect of treatment on WFPS as previously mentioned (Table 3.1)

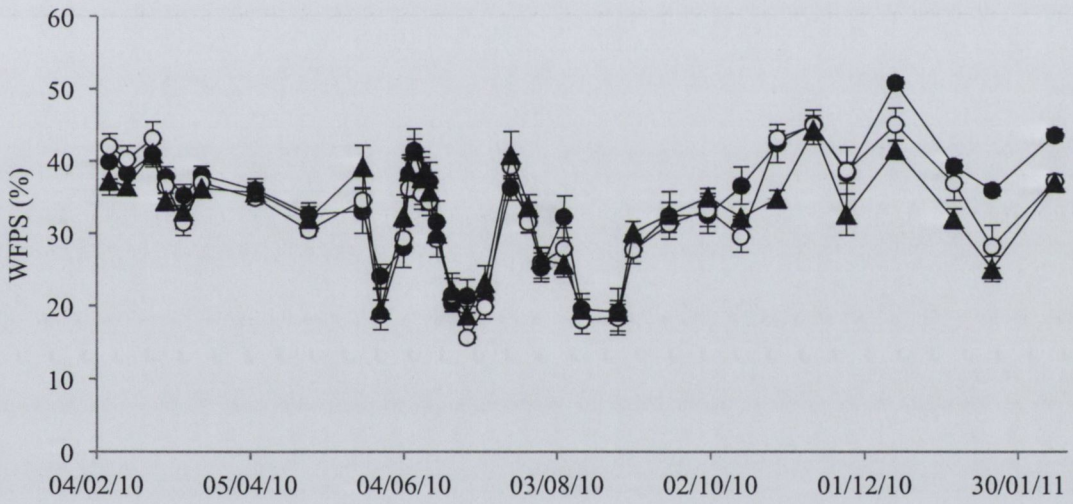


Figure 3.3 WFPS (%) from February 2010 to February 2011 from three treatments; 0 kg-N ha^{-1} (●), 63 kg-N ha^{-1} (○) and 125 kg-N ha^{-1} (▲).

Table 3.1 Summary of Repeated Measures Two-way ANOVA on WFPS data.

Source of Variation	Df	F	P	P Summary
Interaction	70	1.543	0.0040	**
Time	35	47.48	< 0.0001	***
Treatment	2	1.270	0.3015	ns

3.3.2 Soil Temperature

Soil temperature (0-10 cm) was recorded on each sampling date. Very little difference was observed between treatments as can be seen in Figure 3.4. The lowest temperature (2 °C) was recorded on 20/01/11 while the highest (22 °C) was observed on 04/06/10. Of time, treatment and interaction, analysis by repeated measures two-way ANOVA showed only time to have a significant effect on soil temperature (Table 3.2). As expected further analysis of treatment by a Bonferroni post-test shows no significant difference between any of the treatments.

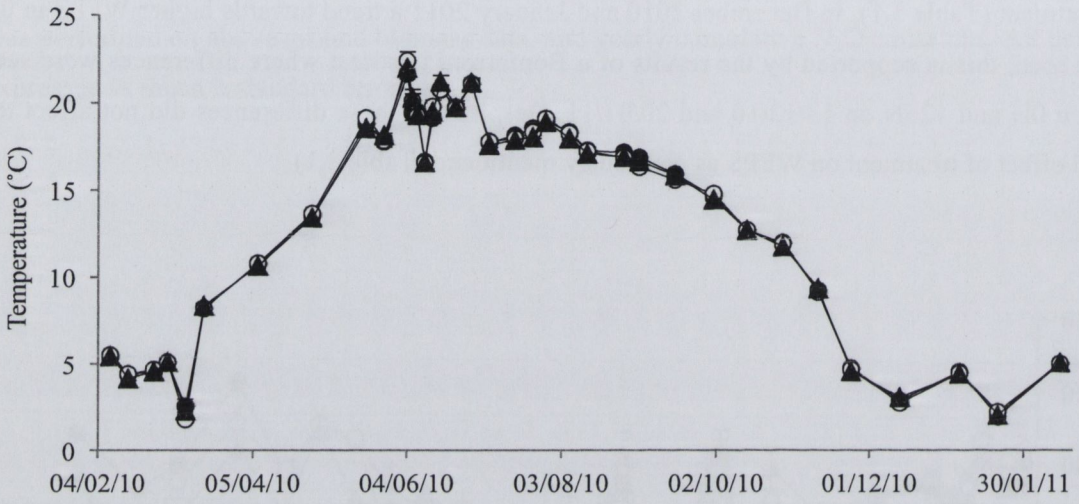


Figure 3.4 Mean soil temperature (°C) from February 2010 to February 2011 from three treatments; 0 kg-N ha^{-1} (●), 63 kg-N ha^{-1} (○) and 125 kg-N ha^{-1} (▲).

Table 3.2 Summary of Repeated Measures Two-way ANOVA on soil temperature data.

Source of Variation	Df	F	P	P Summary
Interaction	70	1.099	0.2784	ns
Time	35	3909	< 0.0001	***
Treatment	2	0.0536	0.9480	ns

3.3.3 Soil Nitrate & Ammonium

Figure 3.5 shows the variation in mean ± SE of soil NO_3^- concentrations (n=4) with time. Concentrations were very low until fertiliser was applied (03/06/10). A sharp peak can be seen in both the 63N and 125N treatments starting immediately after this date. This peak gradually decreases and a second peak can be seen in the 125N treatment almost four weeks later. NO_3^- concentrations return to background levels in the 63N treatment after 6 weeks having peaked at

22.17 mg kg^{-1}, and after around 9 weeks having peaked at 68.13 mg kg^{-1} in the 125N treatment. Analysis by repeated measure two-way ANOVA showed treatment, time and interaction to all have a significant effect on soil NO_3^- concentration (Table 3.3). Comparing each treatment individually by a Bonferroni post-test (Table 3.4) it is apparent that significant differences between the treatments only occurred during the six weeks proceeding fertiliser application, with generally larger differences seen when the 125N treatment is involved.

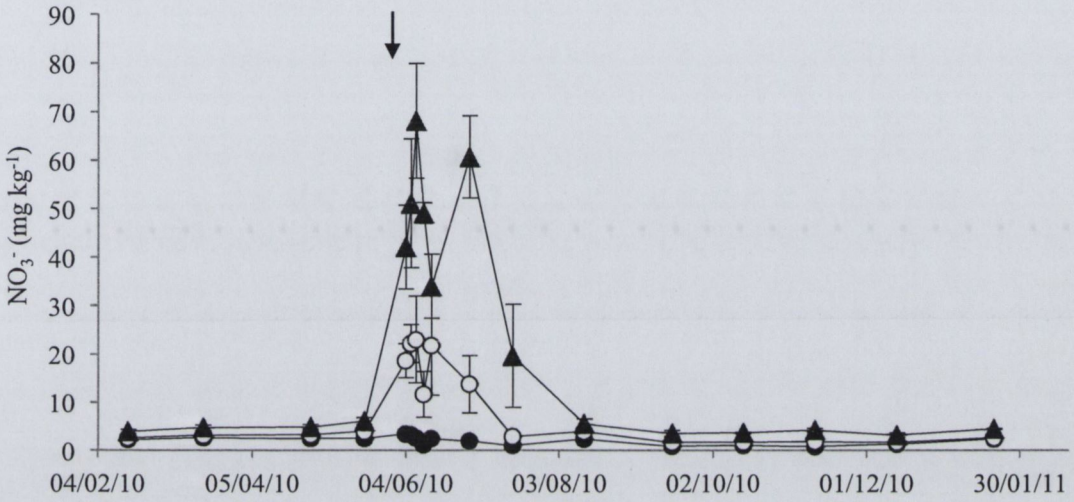


Figure 3.5 Soil NO_3. (mg kg^{-1}) from February 2010 to February 2011 from three treatments; 0 kg-N ha^{-1} (●), 63 kg-N ha^{-1} (○) and 125 kg-N ha^{-1} (▲). Arrow indicates date of fertiliser application (03/06/10).

Table 3.3 Summary of Repeated Measures Two-way ANOVA on NO_3^- data.

Source of Variation	Df	F	P	P Summary
Interaction	32	8.378	< 0.0001	***
Time	16	18.87	< 0.0001	***
Treatments	2	74.54	< 0.0001	***

Table 3.4 Summary of significant dates from a Bonferroni post-test on NO_3^- data.

Treatments	Date	Difference	t	P	P Summary
0N v 63N	06/06/10	18.89	3.163	< 0.05	*
0N v 63N	08/06/10	20.41	3.417	< 0.05	*
0N v 63N	14/06/10	19.37	3.244	< 0.05	*
0N v 125N	04/06/10	38.64	6.740	< 0.001	***

0N v 125N	06/06/10	47.83	8.009	< 0.001	***
0N v 125N	08/06/10	65.56	10.98	< 0.001	***
0N v 125N	11/06/10	47.90	8.021	< 0.001	***
0N v 125N	14/06/10	31.61	5.294	< 0.001	***
0N v 125N	29/06/10	58.77	9.840	< 0.001	***
0N v 125N	16/07/10	18.52	3.100	< 0.05	*
63N v 125N	04/06/10	23.51	3.937	< 0.01	**
63N v 125N	06/06/10	28.95	4.847	< 0.001	***
63N v 125N	08/06/10	45.15	7.560	< 0.001	***
63N v 125N	11/06/10	37.46	6.273	< 0.001	***
63N v 125N	29/06/10	46.95	7.861	< 0.001	***

Mean soil NH_4^+ concentrations also remained relatively low until fertiliser was applied although a far less dramatic peak was seen than with NO_3^-, peaking at 9.85 mg kg^{-1} in treatment 63N and 36.84 mg kg^{-1} in treatment 125N (Figure 3.6). The peaks also faded quickly with both treatments returning to background levels within 11 days. It should also be noted that a large amount of variation was seen among peak concentrations. A second peak was also seen in all treatments on 16/09/10, implying that this was not the result of fertiliser application. Analysis by repeated measure two-way ANOVA showed time and interaction – but not treatment – to have a significant effect on soil NH_4^+ concentration (Table 3.5). Comparing each plot individually by a Bonferroni post-test (Table 3.6) revealed that treatment did cause a difference between the 0N and 125N treatments, but only on three dates directly following fertiliser application. None of these differences where strongly significant and did not affect the overall effect of treatment.

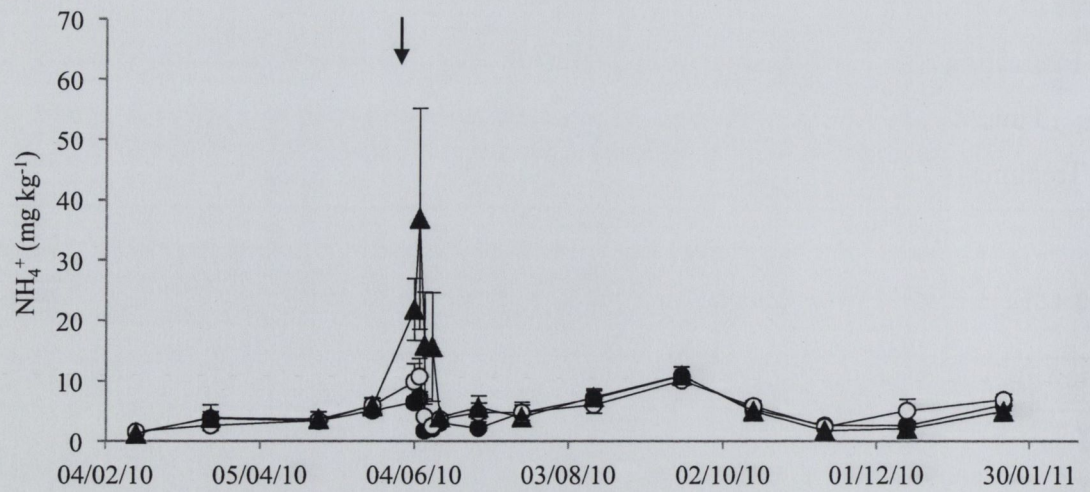


Figure 3.6 Mean soil NH_{4+} (mg kg^{-1}) from February 2010 to February 2011 from three treatments; 0 kg-N ha^{-1} (●), 63 kg-N ha^{-1} (○) and 125 kg-N ha^{-1} (▲). Arrow indicates date of fertiliser application (03/06/10).

Table 3.5 Summary of Repeated Measures Two-way ANOVA on soil NH_4^+ ($Ln(NH_4^+)$) data.

Source of Variation	Df	F	P	P Summary
Interaction	32	2.088	0.0018	**
Time	16	17.59	< 0.0001	***
Treatment	2	1.092	0.3762	ns

Table 3.6 Summary of significant dates from a Bonferroni post-test on soil NH_4^+ ($Ln(NH_4^+)$) data.

Treatments	Date	Difference	t	P	P Summary
0N v 125N	06/06/10	1.217	3.131	< 0.05	*
0N v 125N	08/06/10	1.601	4.121	< 0.01	**
0N v 125N	11/06/10	1.273	3.277	< 0.05	*

3.3.4 Nitrous Oxide Flux

Figure 3.7 shows the mean ± SE (n=8) hourly N_2O flux from treatments 0N, 63N and 125N. Fluxes remained at background levels until after fertiliser application. The first substantial increase in fluxes occurred three days later on 06/06/10. The largest peaks occurred on 08/06/10, 202.51 μg m^{-2} hr^{-1} in treatment 63N and 180.54 μg m^{-2} hr^{-1} in treatment 125N. N_2O fluxes returned to background levels by 29/06/10, but a second smaller peak was seen in both treatments 63N (30.09 μg-N m^{-2} hr^{-1}) and 125N (37.72 μg-N m^{-2} hr^{-1}) on 16/07/10. Analysing these data by repeated measures two-way ANOVA (Table 3.7) showed that 'treatment', 'time' and 'interaction' have a significant effect on N_2O flux. Further analysis of 'treatment' by a Bonferroni post-test (Table 3.8) shows highly significant differences were found between treatments 0N and 63N and 0N and 125N on several dates closely following fertiliser application, but not between treatments 63N and 125N.

Table 3.9 shows mean yearly cumulative N_2O flux and standard error (n=8). Fluxes were highest from the 125N treatment (1.05 ± 0.17 kg-N ha^{-1} yr^{-1}), and only marginally lower from treatment 63N (0.86 ± 0.12 kg-N ha^{-1} yr^{-1}). Meanwhile yearly cumulative N_2O flux from the 0N treatment was 0.46 ± 0.07 kg-N ha^{-1} yr^{-1}. Figure 3.8 illustrates how cumulative N_2O fluxes progressed throughout the year. It can be seen that a sharp increase in cumulative flux occurred after fertiliser application. This was apparent in all treatments including 0N. A one-way ANOVA of these data (Table 3.10) showed that treatment had a significant effect. A subsequent Tukey's multiple comparison test (Table 3.11) also showed a significant difference in yearly cumulative N_2O flux between treatments 0N and 125N, and 0N and 63N, but not 63N and 125N.

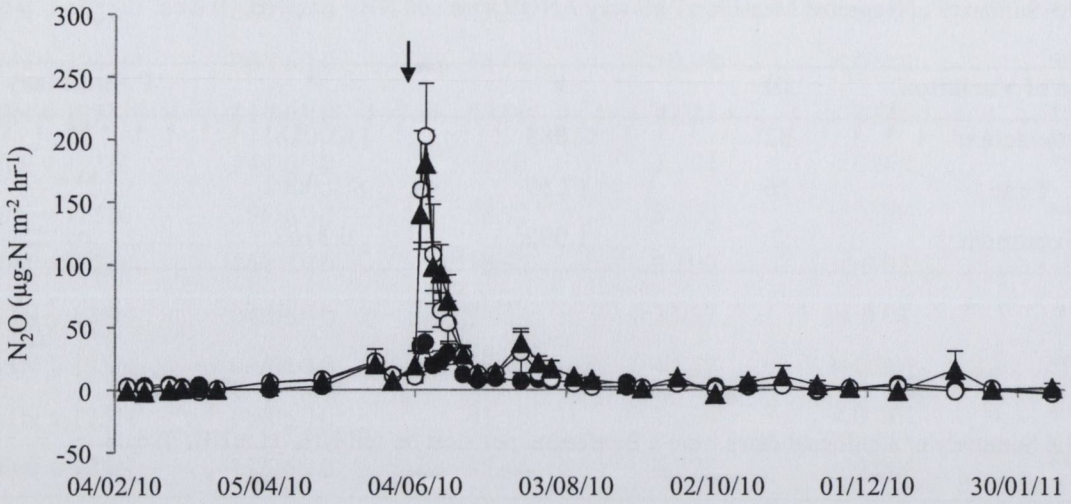


Figure 3.7 Mean N_2O Flux (µg-N m^{-2} hr^{-1}) from February 2010 to February 2011 from three treatments; 0 kg-N ha^{-1} (•), 63 kg-N ha^{-1} (○) and 125 kg-N ha^{-1} (▲). Arrow indicates date of fertiliser application (03/06/10).

Table 3.7 Summary of Repeated Measures Two-way ANOVA on N_2O flux ($1/(N_2O + 90)*1000$) data.

Source of Variation	Df	F	P	P Summary
Interaction	70	4.112	< 0.0001	***
Time	35	57.96	< 0.0001	***
Treatment	2	7.673	0.0032	**

Table 3.8 Summary of significant dates from a Bonferroni post-test on N_2O flux ($1/(N_2O + 90)*1000$) data.

Treatments	Date	Difference	t	P	P Summary
0N v 63N	06/06/10	-3.267	6.385	< 0.001	***
0N v 63N	08/06/10	-3.947	7.767	< 0.001	***
0N v 63N	11/06/10	-3.392	6.631	< 0.001	***
0N v 63N	14/06/10	-2.268	4.434	< 0.001	***
0N v 125N	06/06/10	-3.478	6.799	< 0.001	***
0N v 125N	08/06/10	-4.022	7.863	< 0.001	***
0N v 125N	11/06/10	-3.544	6.928	< 0.001	***
0N v 125N	14/06/10	-3.125	6.109	< 0.001	***
0N v 125N	17/06/10	-1.907	3.727	< 0.01	**

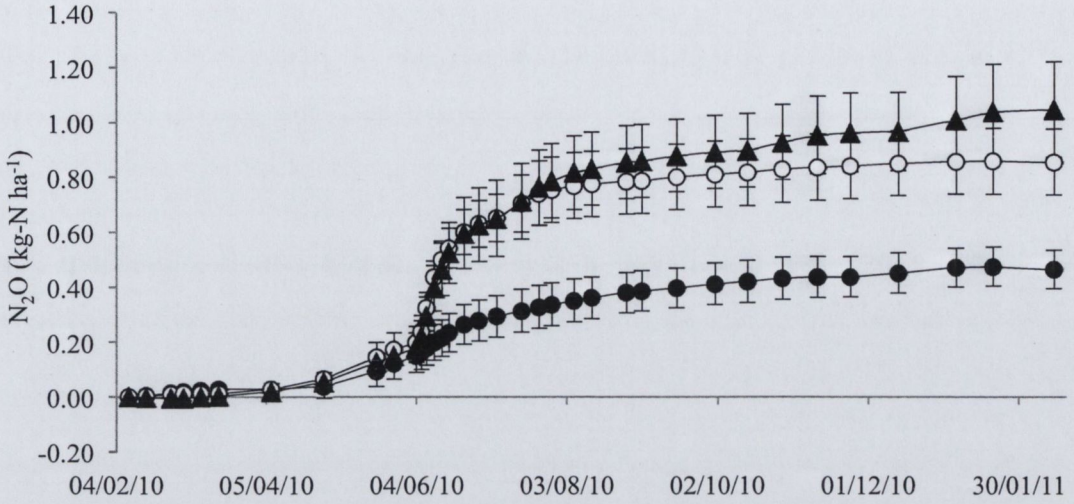


Figure 3.8 Cumulative N_2O Flux (kg-N ha^{-1}) over one year from February 2010 to February 2011 from three treatments; 0 kg-N ha^{-1} (●), 63 kg-N ha^{-1} (○) and 125 kg-N ha^{-1} (▲).

Table 3.9 Mean cumulative N_2O flux (kg-N ha^{-1} yr^{-1}) and standard error (n=8) for all treatments.

Treatment	0N	63N	125N
N_2O	0.46 ± 0.7	0.86 ± 0.12	1.05 ± 0.17

Table 3.10 Summary of one-way ANOVA of plot on cumulative yearly N_2O ($\sqrt{N_2O}$) data.

P	**P Summary**	**F**	**R^2**	**Groups**
0.0067	**	6.403	0.3788	3

Table 3.11 Summary of Tukey's Multiple Comparison Test after one-way ANOVA on cumulative yearly N_2O-N ($\sqrt{N_2O}$) data.

Treatments	**Mean Diff.**	**q**	**P < 0.05**	**Summary**
0N v 63N	-7.969	3.617	Yes	*
0N v 125N	-10.74	4.874	Yes	**
63N v 125N	-2.768	1.256	No	ns

A best fit multiple regression analysis was carried out on N_2O flux with soil temperature, WFPS, soil NO_3^-, soil NH_4^+ and their relative interactions (Table 3.12). Only data from dates when all variables were recorded have been used. The analysis was able to account for 60% of all observed N_2O fluxes. Several factors were found to have a significant controlling influence on N_2O flux. Soil NO_3^-, soil NH_4^+ and their interaction were all found to have a slightly significant influence (*), meanwhile a stronger influence (**) was exerted by the interaction between soil NH_4^+ and temperature and the interaction between soil NO_3^-, temperature and WFPS. The most important factors influencing N_2O flux here were found to be soil temperature and the interaction between soil temperature and WFPS (***).

Table 3.12 Summary of best fit multiple regression analysis of N_2O flux ($1/(N_2O+90)*1000$) data, with soil temperature (T^2), WFPS, soil NO_3^- ($1/\sqrt{NO_3^-}$) and soil NH_4^+ ($1/\sqrt{NH_4^+}$) (Adjusted $R^2 = 0.60$).

Source	**Sum of Square**	**df**	**Mean Square**	**F - ratio**
Regression	598.878	8	74.8598	37.7
Residual	381.675	192	1.98779	
Variable	**Coefficient**	**S.E. of Coefficient**	**t - ratio**	**Probability**
Constant	8.07261	1.224	6.59	≤ 0.0001 (***)
NO_3	3.68607	1.644	2.24	0.0261 (*)
NH_4	4.24823	1.732	2.45	0.0151 (*)
Temp	0.01331	0.0033	4.05	≤ 0.0001 (***)
NO_3*NH_4	-4.89438	2.194	-2.23	0.0269 (*)
NO_3*Temp	-0.00753	0.0052	-1.45	0.1484
NH_4*Temp	-0.00968	0.0036	-2.67	0.0083 (**)
Temp*WFPS	-0.00054	0.0001	-7.22	≤ 0.0001 (***)
NO_3*Temp*WFPS	-0.00041	0.0001	2.85	0.0049 (**)

Table 3.13 Emission factors for 63N and 125N treatments.

Treatment	63N	125N
Emission Factor	0.69 ± 0.21	0.52 ± 0.15

Table 3.14 Summary of two-tailed unpaired t-test on Emission Factor (Log(EF)) data.

Df	t	P	P Summary
14	0.7795	0.4487	ns

3.3.5 Emission Factors

Mean ± SE (n=8) N_2O Emission Factors (EF) were calculated for treatments 63N and 125N for the year from February 2010 to February 2011 (Table 3.13). EF's were 0.69 ± 0.21 from treatment 63N and 0.52 ± 0.15 from treatment 125N. Analysis by a two-tailed unpaired t-test (Table 3.14) showed no significant difference between EF's from both treatments. In this light an EF of 0.60 ± 0.13 can be applied for *Miscanthus* here.

3.3.6 Above Ground Biomass Yield

Table 3.15 shows above ground biomass yields measured in each of the three treatments. As expected the 0N treatment produced the lowest yield (14.84 ± 0.45 t DM ha^{-1} yr^{-1}), whilst the highest yield was found in the 125N treatment (17.63 ± 1.12 t DM ha^{-1} yr^{-1}). When analysed by one-way ANOVA, treatment was not found to have a significant effect on above ground biomass yield (Table 3.16), although a subsequent Tukey's multiple comparison post-test did find a significant difference (*) between treatments 0N and 125N (Table 3.17). Figure 3.9 shows yearly cumulative N_2O per tonne above ground biomass (N_2O/yield). Analysis by a one-way ANOVA shows treatment to have a significant effect (Table 3.18). Furthermore yearly cumulative N_2O-N and above ground biomass yield are presented as a percentage increase from the 0N treatment (Table 3.20). While increases of 10.58% and 18.80% are seen in yield in treatments 63N and 125N respectively, there are much greater percentage increases in N_2O; 84.41% in treatment 63N and 125.56% in 125N.

3.3.7 Economic Cost Analysis

Table 3.21 shows the costs of increasing nitrogen fertiliser application rate to 63 and 125 kg-N ha^{-1}, the resulting differences in yield, N_2O, and the total costs excluding and including N_2O emissions. Fertiliser was found to cost €115.51 t^{-1} and €191.75 t^{-1} for treatments 63N and 125N respectively. The resulting increase in yield was valued at €99.83 ± 63.06 t^{-1} and €178.78 ± 35.26 t^{-1} for each, while the increase in N_2O emissions were valued at €2.05 ± 0.63 t^{-1} and €3.04 ± 0.90 t^{-1} respectively. The total cost of increasing nitrogen fertiliser application rate was similar for both

treatments, €15.68 ± 63.06 t^{-1} (63N) and €12.97 ± 35.26 t^{-1} (125N) excluding the cost of N_2O emissions, €17.73 ± 63.68 t^{-1} (63N) and €16.01 ± 36.16 t^{-1} (125N) including the cost of N_2O emissions. Analysing the cost of increasing nitrogen fertiliser application rate using a two-tailed unpaired t-test showed no significant difference between an increase to 63 kg-N ha^{-1} or 125 kg-N ha^{-1} (Table 3.22).

Table 3.15 Above ground biomass yield (t DM ha^{-1} yr^{-1}) and standard error (n=4) for all treatments.

Treatment	0N	63N	125N
Yield	14.84 ± 0.45	16.41 ± 1.00	17.63 ± 1.12

Table 3.16 Summary of one-way ANOVA of treatment on above ground biomass yield data.

P	P Summary	F	R^2	Groups
0.0572	ns	4.000	0.4706	3

Table 3.17 Summary of Tukey's Multiple Comparison Test after one-way ANOVA on above ground biomass yield data.

Treatments	Mean Diff.	q	P < 0.05	Summary
0N v 63N	-1.585	2.229	No	ns
0N v 125N	-2.838	3.991	Yes	*
63N v 125N	-1.253	1.762	No	ns

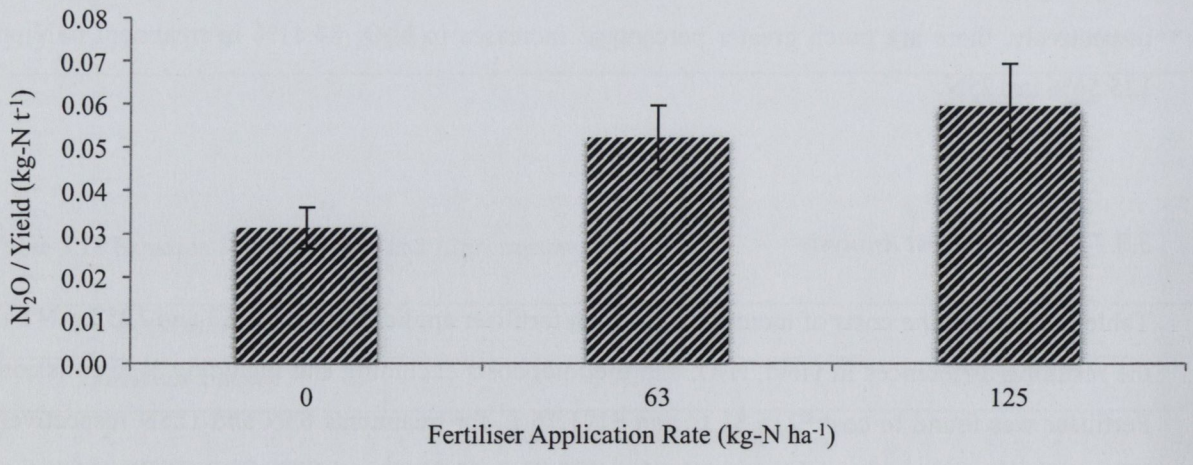


Figure 3.9 Yearly cumulative N_2O per tonne biomass yield at three rates of fertiliser application; 0 kg-N ha^{-1}, 63 kg-N ha^{-1} and 125 kg-N ha^{-1}.

Table 3.18 Summary of one-way ANOVA of treatment on N_2O/Yield ($\sqrt{(N_2O/Yield)}$) data.

P	P Summary	F	R^2	Groups
0.0283	*	4.247	0.2880	3

Table 3.19 Summary of Tukey's Multiple Comparison Test after one-way ANOVA on N_2O/Yield ($\sqrt{(N_2O/Yield)}$) data.

Treatments	Mean Diff.	q	P < 0.05	Summary
0N v 63N	-1.702	3.133	No	ns
0N v 125N	-2.110	3.886	Yes	*
63N v 125N	-0.4086	0.752	No	ns

Table 3.20 Percentage increase from the control in yearly cumulative N_2O and above ground biomass yield resulting from two rates of synthetic nitrogen fertiliser application; 63 kg-N ha^{-1} and 125 kg-N ha^{-1}.

Treatment	Increase in Yield (%)	Increase in N_2O (%)
63N	10.58	84.41
125N	18.80	125.56

Table 3.21 Economic costs of increasing fertiliser application rate (€ ha^{-1}) including standard error.

Treatment	Fertiliser	Yield	N_2O	Total (Ex. N_2O)	Total (Inc. N_2O)
63N	115.51	-99.83 ± 63.06	2.05 ± 0.63	15.68 ± 63.06	17.73 ± 63.68
125N	191.75	-178.78 ± 35.26	3.04 ± 0.90	12.97 ± 35.26	16.01 ± 36.16

Table 3.22 Summary of two-tailed unpaired t-test on total cost of increasing fertiliser application rate (including N_2O) data.

Df	t	P	P Summary
6	0.0239	0.9817	ns

3.4 Discussion

In the previous chapter background emissions of N_2O were calculated from a grassland, a newly planted, and an established *Miscanthus* stand receiving no fertiliser inputs. Here the proportional increase in N_2O emissions and yield of an established *Miscanthus* stand receiving nitrogen fertiliser is investigated.

It is important to note that this chapter is based on one year of field data. Nonetheless while it is acknowledged that a longer experimental period is preferable for drawing robust conclusions in agricultural studies, due to the current lack of knowledge in this area this study offers a valuable insight.

This experiment was designed to look at the relationship between fertiliser application and soil N_2O emissions beneath *Miscanthus x giganteus*. In order to do this N_2O flux, soil temperature, WFPS and soil NO_3^- and NH_4^+ concentrations were measured for twelve months. Additionally above ground biomass yield as affected by fertiliser addition was taken into account. As one would expect fertiliser treatment has not significantly affected WFPS or soil temperature, but has led to significant changes in soil nitrate concentrations. Interestingly fertiliser application did not significantly affect soil ammonium concentrations, which were found to significantly influence soil N_2O flux. Overall fertiliser application was found to have a significant effect on N_2O flux, but significant differences were only found between the 0N and 63N, and the 0N and 125N treatments. This is reflected by analysis of yearly cumulative N_2O emissions where fertiliser treatment was found to have a significant effect overall, but again no difference was found between the 63N and 125N treatments. While no significant difference was found between cumulative emissions from these treatments, neither was any found between their emission factors. This is likely due to the variation observed in yearly cumulative N_2O emissions. Overall above ground biomass yield has not been found to be significantly influenced by fertiliser treatment, although further analysis did reveal a significant difference between the 0N and 125N treatments.

It is known that N_2O emissions are affected in the main by substrate in the form of nitrate and ammonium, microbial biomass and soil parameters such as organic carbon, WFPS, temperature and texture. In this study soil NO_3^-, NH_4^+, WFPS and soil temperature and their relative interactions were evaluated in terms of their influence on N_2O flux by a best fit multiple regression analysis, results of which are given in Table 3.12. The variables and interactions listed in Table 3.12 account in total for 60% of the variation observed in hourly N_2O flux. There is no one clearly dominant N_2O production pathway apparent from this analysis. This is implied by the similar significance (*) of soil NO_3^-, soil NH_4^+ and their interaction. The interaction between NH_4^+ and temperature has been found to be significant (**) indicating that nitrification play's a key role here, while the interaction between soil NO_3^-, soil temperature and WFPS has also been found to be significant

(**) suggesting that N_2O production via denitrification is also likely to be important. In this case it is possible that both processes play an important role, multiple soil processes and microbial drivers have often been found to act simultaneously within the same soils (Stevens *et al.*, 1997), partially as it is possible for non-aerobic soil micro-sites to exist along side aerobic sites producing large amounts of N_2O through denitrification (Abbasi & Adams, 2000). Interestingly soil temperature and its interaction with WFPS were found to be highly significant (***) controlling factors of soil N_2O production. This may suggest that these were the limiting factors here as opposed to nitrate or ammonium availability. A saturation of soil inorganic N could have been influenced by Miscanthus' low nutritional requirements and efficient N use (Beale & Long, 1997). This in turn may explain the lack of difference seen in N_2O emissions between the 63N and 125N treatments. The influence of soil temperature and WFPS on N_2O production in the soil is well documented (Smith *et al.*, 1998, Rudaz *et al.*, 1999, Dobbie & Smith, 2003b). The relatively low WFPS' recorded throughout this experiment – mean WFPS ranged from 16% to 51% (Figure 3.3) - indicate that nitrification may have been a more common source of soil N_2O than denitrification. It is frequently reported that nitrification is the dominant N_2O production pathway at WFPS of 35-60% (Linn & Doran, 1984, Bateman & Baggs, 2005). However other aerobic N_2O production pathways cannot be discounted. Aerobic denitrification has been found to occur in soils when O_2 is not limiting (Patureau *et al.*, 2000). If as suggested an excess of inorganic N remains in the soil it must be lost to the environment through other pathways given that both soil NO_3^- and NH_4^+ concentrations return to background levels within eight weeks (Figuress. 3.5 & 3.6). Complete denitrification to N_2 is possible, but as nitrification appears to be more probable here large losses to ground water via leaching are highly likely. NO_3^- leaching is known to be higher in coarse textured soils – such as that found in this experiment – than fine textured soils (Geleta *et al.*, 1994, Sogbedji *et al.*, 2000).

Yearly cumulative N_2O fluxes recorded here (0.46 – 1.05 kg-N ha^{-1} yr^{-1}) are similar to those measured in 2009 at a nearby spring barley (*Hordeum vulgare*) crop grown in similar soil. Rueangritsarakul (2010) carried out a comparable experiment using fertiliser treatments of 0, 70 and 140 kg N ha^{-1}, finding cumulative fluxes of 0.66, 1.06 and 1.38 kg N_2O-N ha^{-1} in each respectively. These fluxes are also similar to a European mean derived by Freibauer and Kaltschmitt (2003) where 91 arable soil data sets were analysed and found to emit a mean of 1.8 kg N_2O-N ha^{-1} yr^{-1}. Meanwhile another similar experiment was carried out nearby in 2004 on a cut and grazed pasture (*Lolium/Trifolium* mixed sward), again on similar soil. Here fertiliser treatments of 0 and 200 kg N ha^{-1} were applied, resulting in cumulative emissions of 0.90 and 2.40 kg N_2O-N ha^{-1} respectively (Abdalla *et al.*, 2009a). Table 3.23 shows N_2O emissions measured over three years from a range of fertilised bioenergy crops grown in coarse textured soil (Hellebrand *et al.*, 2003).

Results from this chapter fall in the lower end of the range measured here with only Willow (1999) showing particularly lower emissions.

These comparisons are better served when examined as emission factors (EF), as they are then described as a function of fertiliser treatment. Despite there being no difference found between N_2O flux from treatments 65N and 125N, neither was any difference found between their emission factors, measuring 0.69 ± 0.21 % and 0.52 ± 0.15 % respectively. This is due to the variation observed in yearly cumulative N_2O flux. As no significant difference was found between treatments a mean EF of 0.60 ± 0.13 % may be applied here. These values – although slightly higher – are similar to EF's of 0.40 ± 0.14 % and 0.37 ± 0.38 % for fertiliser treatments of 70 and 140 kg N ha^{-1} respectively, measured in the aforementioned study by Rueangritsarakul (2010). Furthermore the study carried out by Abdalla *et al.* (2009a) on cut and grazed pasture found an EF of 0.83 ± 0.15 %. These emissions fall within the range of the IPCC default value, 1 ± 0.3-3 % (IPCC, 2007a). All of the emissions and emission factors compared here have been measured from free draining coarse textured soils. Soils of this type have been found to have lower N_2O emissions and resulting EF's than fine textured soils partially due to pore size distributions (Sogbedji *et al.*, 2000, Tan *et al.*, 2009), and higher levels of NO_3^- leaching (Geleta *et al.*, 1994). Nonetheless these comparisons show that despite lower N requirements than many conventional crops, excess nitrogen applied to *Miscanthus* is no more likely to be lost as N_2O in coarse textured soils.

Table 3.23 Accumulated annual emission of N_2O nitrogen for different crops cultivated on loamy–sandy soil. Fertilised at a rate of 150 kg N ha^{-1} yr^{-1} (Hellebrand *et al.*, 2003).

Crop	N_2O (kg-N ha^{-1} yr^{-1})		
	1999	2000	2001
Orchard Grass	1.20	1.16	1.37
Willow	0.55	0.92	1.67
Poplar	1.04	1.44	2.97[a]
Rye	1.12	-	2.06
Triticale	0.99	-	-
Hemp	-	1.48	-
Rape	-	3.89	-

[a] Fungi in the measuring ring

Fertiliser application was not found to significantly affect above ground biomass yield (Table 3.16). Similar results were found by Christian *et al.* (2008) during a study carried out over 14 years using fertilisation rates of 0, 60 and 120 kg-N ha^{-1} yr^{-1}. In order to look at the relationship between above ground biomass yield and N_2O emissions in relation to fertiliser application, yearly cumulative N_2O per tonne above ground biomass yield (N_2O/yield) has been calculated (Figure 3.9). Analysis of these data shows fertiliser treatment to have a significant effect (Table 3.19). Although similar to above ground biomass yield further analysis found this significance to only be present between treatments 0N and 125N (Table 3.21). This information indicates that as yield increases due to fertiliser application N_2O emissions increase correspondingly, but a significant increase may be avoided by limiting the amount of nitrogen fertiliser applied. To evaluate this association both N_2O and yield have been expressed as a percentage increase from the control treatment (Table 3.18). In each case a far greater percentage increase is seen in yield than N_2O, with an increase in yield of 10.58 % compared to an increase in N_2O of 84.41 % in the 63N treatment, and an increase in yield of 18.80 % compared to an increase in N_2O of 125.56 % in the 125N treatment. This large disparity in the scale of increase combined with the lack of a significant increase seen in above ground biomass yield brings into question the wisdom of applying nitrogen fertiliser to *Miscanthus* – particularly in large quantities – when the environmental impacts may outweigh the agronomic benefits.

To understand the real life costs to an operational farm of applying nitrogen fertiliser to *Miscanthus* it is useful to assess the economic costs. In order to do this a simple calculation has been carried out. The cost of increasing fertiliser application rate has been subtracted from the potential value of the resulting increase in yield. Furthermore the resulting value has also been adjusted to include the market cost of N_2O as carbon equivalents. Increasing fertiliser application rate from 0 to 63 kg-N ha^{-1} yr^{-1} led to a loss of €15.68 ± 63.06 ha^{-1} and a loss of €17.73 ± 63.68 ha^{-1} when adjusted to include the value of N_2O emissions. Increasing fertiliser to 125 kg-N ha^{-1} yr^{-1} resulted in similar losses of €12.97 ± 35.26 ha^{-1} and €16.01 ± 36.16 ha^{-1} (Table 3.21) when N_2O emissions where valued. The total cost of increasing the rate of fertiliser application shows no significant difference between the 63N and 125N treatments (Table 3.22) as any increase in yield is offset by the increased cost of nitrogen fertiliser. While these results may be unique to the study site and year examined, this loss per hectare offers very little incentive to apply nitrogen fertiliser at all. However it should be noted that a large amount of variation exists in these calculations and with the right conditions similar, albeit small, profits could be seen in the 63N and 125N treatments.

3.5 Conclusions

Soil temperature and the interaction between soil temperature and WFPS were found to be the main drivers of soil N_2O production here. This could indicate that these parameters were limiting factors, thus contributing to the lack of any significant difference in N_2O flux seen between the 63N and 125N treatments. There is evidence indicating that both nitrification and denitrification were active N_2O production pathways, with soil nitrate and ammonium concentrations each being found to have a significant influence on N_2O emissions. The relatively low WFPS's recorded throughout suggest that nitrification may have been dominant, nevertheless it is likely that the soil N_2O emissions measured throughout this experiment are the results of a number of drivers and pathways.

This study indicates that nitrous oxide emissions resulting from fertiliser addition are comparable in *Miscanthus* and other common agricultural crops grown in coarse textured soils. However *Miscanthus'* low nutrient requirements mean that little to no fertiliser is required for substantial growth. Reducing fertiliser application relative to other crops suggests *Miscanthus* can be environmentally beneficial in regards to N_2O emissions. Interestingly increasing the rate of fertiliser application did not significantly increase N_2O emissions – although a trend towards higher emissions was seen in the higher fertiliser treatment. This disproves the first hypothesis. The eventual fate of the excess inorganic nitrogen is unclear, and must be considered in future studies, as it is likely be lost through other environmentally damaging pathways such as nitrogen leaching.

Above ground biomass yield also failed to increase significantly due to nitrogen fertiliser application, but also exhibited a trend towards higher yields as the rate increased. Meaning that as N_2O emissions increased above ground biomass also increased proportionally, in turn disproving the second hypothesis. The rates of increase seen in each however differ greatly; with N_2O emissions increasing up to eight times more than above ground biomass yield. For this reason it is important that fertilisation rates are chosen carefully if fertiliser is to be applied at all. Only nitrogen that has been removed at harvest need be replaced as the environmental costs of excess inorganic fertiliser addition can potentially far outweigh the agronomic benefits.

Assessing this in economic terms revealed that in this case increasing the rate of nitrogen fertiliser application – to a medium or high rate – is likely to lead to a financial loss in the region of €15 ha^{-1}, verifying the third hypotheses, although with the caveat that this is derived from data collected over one year from one soil type. This loss is mainly driven by the cost of nitrogen fertiliser and was not particularly affected when the EU market price of nitrous oxide as carbon equivalents was included. This further supports the idea that the rate of nitrogen fertiliser application to *Miscanthus* must be carefully chosen and only applied when deemed necessary to maintain soil quality.

Chapter 4

DNDC as a tool for predicting N_2O emissions from Miscanthus x giganteus

4.1 Introduction

The previous chapter investigated N_2O emissions from *Miscanthus x giganteus* subject to varying rates of inorganic nitrogen fertiliser application. Published data of this type is limited at the moment, and as such there has been little opportunity to model N_2O emissions from *Miscanthus* due to a lack of suitable data for validation. This has led to the adoption of the IPCC default emission factor of 1 ± 0.3-3% of fertiliser N applied (IPCC, 2007a) for use in studies incorporating N_2O emissions from *Miscanthus* (Hoefnagels *et al.*, 2010, Popp *et al.*, 2011). However this approach has many limitations. Reported emission factors show large amounts of variation due to a large number of factors including soil type, climatic conditions, management practices, and importantly crop type (Hellebrand *et al.*, 2008, Kavdir *et al.*, 2008, Laville *et al.*, 2011). Furthermore Crutzen *et al.* (2008) suggest that default emissions factors may underestimate N_2O emissions by three to five fold.

Process based models offer a valuable tool to predict N_2O emissions under a large range of scenarios, and often offer a much more accurate view than simply applying default IPCC emission factors. Use of such models with *Miscanthus* has been rare due to the lack of field data to validate outputs.

The DNDC (DeNitrification-DeComposition) model is a process-based model that can be used to predict greenhouse gas emissions, crop growth and soil carbon and nitrogen dynamics (Li *et al.*, 2001). DNDC was first designed for use with agriculture soils in the USA (Li *et al.*, 1992a), but variations have been used with considerable success for a number of land-uses and climates, ranging from tropical rainforests (Kiese *et al.*, 2005), to temperate grasslands (Brown *et al.*, 2002). DNDC has been successfully used to simulate N_2O emissions from Irish agricultural soils on a number of occasions (Hsieh *et al.*, 2005, Abdalla *et al.*, 2009a, Rafique *et al.*, 2011). For this reason and also due to the availability of the required input parameters it has been chosen for use in this study.

This chapter aims to parameterise DNDC for use with *Miscanthus* using a range of measured and literature-derived values. Field observed measurements of above ground biomass yield, N_2O emissions, and soil NO_3^- and NH_4^+ concentrations are used to validate the model, whilst daily measurements of rainfall and minimum and maximum temperature provide the framework for each model run. Results are discussed with relation to assessing the suitability of DNDC as a predictive tool for N_2O emissions from *Miscanthus*.

4.2 Materials and Methods

4.2.1 Experimental Data

Field data used to validate DNDC and alter parameterisation for a better fit were collected over a one year period from February 2010 to February 2011 from the Teagasc Crop Research Centre at Oak Park, Co. Carlow, Ireland, as described in Chapter 3. N_2O fluxes, cumulative N_2O emissions, soil NO_3^- and NH_4^+ concentrations, and above ground biomass yields from Chapter 3 were used in this chapter.

4.2.2 DNDC

For this study DNDC 9.2 has been used. DNDC is a process based model capable of predicting N_2O emissions as well as CO_2, CH_4, Crop growth, and soil C and N dynamics. DNDC carries out these calculations based on the interactions of five sub-models. These are, thermal-hydraulic, aerobic decomposition, denitrification, fermentation, and plant growth (Giltrap *et al.*, 2010). The thermal hydraulic sub-model calculates soil conditions such as WFPS and temperature. The aerobic decomposition sub-model predicts rates of decomposition, CO_2 production, nitrification and ammonia volatilization. The denitrification sub-model simulates the various stages of the denitrification process, reducing NO_3^- to N_2. The fermentation sub-model simulates fermentation processes based on soil redox potential, whilst the crop growth sub-model predicts crop growth, partitioning and nutrient accumulation. DNDC can operate in a site specific mode or regional mode based on GIS databases. Site mode has been used for this study. Here DNDC is largely driven by measured climate data, soil parameters, and land management data. Further description of the model is available in Li *et al.* (2000) and Li (2007).

4.2.3 Model Input Data

The climatic data required for running DNDC has been collected automatically by a Teagasc Research Centre weather station in Oak Park, Co. Carlow located less than one kilometre from the experimental site. Daily precipitation, minimum and maximum temperatures have been used.

The necessary soil properties for running DNDC have been collected from a number of sources. Bulk density and initial NO_3^- and NH_4^+ concentrations were measured in a previous experiment as described in Chapter 3. Clay fraction, pH and initial soil organic carbon were recorded in a previous experiment and can be found in Dondini *et al.* (2009). Model default values have been

used for field capacity, wilting point, hydro-conductivity, porosity, depth of water retention layer, SOC partitioning, and microbial activity.

Farming management inputs are as described in Chapter 3. Due to limitations in the use of DNDC with perennial rhizomatous crops such as *Miscanthus* a planting date and harvest date have been applied to represent the start and end of the *Miscanthus* growing season. The dates 20/03/2010 and 20/11/2010 have been used for this purpose. Meanwhile the actual observed harvesting of the crop on 15/03/2010 has been described as a cutting. A summary of the inputs used can be seen in Table 4.1.

Table 4.1 Summary of input data used to run DNDC. Model default values are denoted by italics.

Site and Climate	
Latitude (°)	52.86
Mean Maximum Temperature (°C)	13.0
Mean Minimum Temperature (°C)	5.4
Total Precipitation (mm)	861
N Concentration in Rainfall (mg-N l^{-1})	0.001
Atmospheric CO_2 Concentration (ppm)	385
Soil	
Soil Texture	Loamy Sand
Bulk Density (g cm^{-3})	0.87
pH	6.8
Clay Fraction (0-1)	0.05
Field Capacity (0-1)	*0.25*
Wilting Point (0-1)	*0.13*
Hydro-Conductivity (m hr^{-1})	*0.563*
Porosity (0-1)	*0.411*
Soil Organic Carbon (kg-C kg^{-1})	0.044
Initial Soil NO_3^- (mg-N kg^{-1})	
- 0 kg-N ha^{-1}	2.26
- 63 kg-N ha^{-1}	2.62
- 125 kg-N ha^{-1}	3.88
Initial Soil NH_4^+ (mg-N kg^{-1})	
- 0 kg-N ha^{-1}	1.41
- 63 kg-N ha^{-1}	1.59
- 125 kg-N ha^{-1}	1.26
Microbial Activity Index (0-1)	*1*

4.2.4 Model Parameterisation

As *Miscanthus* is not available as a crop selection in DNDC, it was necessary to establish it as a new crop selection by inputting a range of required parameters. Input parameters were sourced from the literature, with three exceptions. Firstly, a number of parameters required for the physiological growth model were unavailable; here model default values for another common C_4 bioenergy crop – Maize – were selected. Secondly, DNDC's biomass growth model is heavily dependant on two crop parameters that are non-applicable to *Miscanthus* as it is a sterile cultivar; maximum grain production and the grain fraction of total biomass. A nominal value of 0.0001 was selected for the grain fraction of total biomass. The maximum grain production was then adjusted to match predicted above ground biomass yields with those observed in the field. A value of 6 kg ha^{-1} gave a good fit, with an overall relative deviation (calculated as described in Section 4.2.5) of 0.3% (Table 4.2). Finally, a method of incorporating nitrogen supply recycled from the rhizomes was required. In order to account for this and other sources of N, the N fixation index parameter was used. By treating all sources of N not coming directly from the soil N pool as fixation it is possible to include them in the model. Long and Beale (2001) reported only 36% of N found in *Miscanthus* to be provided directly from the soil, while 64% was provided by other sources. These other sources are likely to consist largely of nitrogen supplied by the rhizomes and nitrogen fixation, which has been found to be an important factor in *Miscanthus* nutrient supply (Eckert *et al.*, 2001, Davis *et al.*, 2010). Using these values an N fixation index of 2.8 has been applied. When parameterising the crop the option to define it as perennial grassland was not selected as it was found to seriously affect the timing of N_2O emissions, in particular peaks observed after fertilisation. Table 4.3 shows a complete list of the parameters used and their sources.

Table 4.2 Field observed and model simulated above ground biomass yield and their relative deviations.

Treatment	Observed (t ha^{-1})	Simulated (t ha^{-1})	Relative Deviation (%)
0N	14.84	13.75	7.9
63N	16.41	17.34	5.4
125N	17.63	17.96	1.8
All	16.29	16.35	0.3

4.2.5 Model Validation and Calibration

Model simulated N_2O emissions have been validated against field observed measurements recorded from February 2010 to February 2011. Annual cumulative N_2O emissions have been assessed by calculating the relative deviation (RD) of the simulated from observed emissions using the following equation:

$$\mathrm{RD} = \left(\frac{\sum x_s - \sum x_o}{\sum x_o}\right) 100$$

Where x_s are model simulated daily measurements and x_o are field observed. Model performance has been analysed with regards to daily simulations using three statistical criteria, coefficient of correlation (r), root mean square error (RMSE), and the value obtained assuming a deviation corresponding to the 95% confidence interval of the measurements ($RMSE_{95\%}$). An RMSE value lower than its corresponding $RMSE_{95\%}$ indicates that the model simulated values fall within the 95% confidence interval of the measurements (Smith *et al.*, 1997). These have been calculated according to the following equations:

$$r = \frac{n\sum x_o x_s - (\sum x_o)(\sum x_s)}{\sqrt{n(\sum x_o^2) - (\sum x_o)^2}\sqrt{n(\sum x_s^2) - (\sum x_s)^2}}$$

$$\mathrm{RMSE} = \frac{100}{\bar{x}_o}\sqrt{\frac{1}{n}\sum (x_s - x_o)^2}$$

$$\mathrm{RMSE}_{95\%} = \frac{100}{\bar{x}_o}\sqrt{\frac{1}{n}\sum \left(\left(t_{(n-2)95\%}\right)(S_x)\right)^2}$$

Where $t_{(n\text{-}2)95\%}$ is Student's t distribution with n - 2 degrees of freedom, and S_x is the standard error of the measurements.

A sensitivity analysis was carried out to assess which factors have the strongest effect on simulated N_2O emissions. Factors analysed were soil pH, bulk density, soil organic carbon, rainfall and temperature. Li *et al.* (2001) have highlighted SOC as the most sensitive factor controlling N_2O emissions from a range agricultural crops in DNDC, while Abdalla *et al.* (2009a) reported a high sensitivity to changes in bulk density beneath spring barley. Soil pH has been selected as it can

potentially affect N_2O emissions by affecting rates of nitrification and denitrification. Denitrification is known to be encouraged at low pH (Stevens *et al.*, 1998, Šimek & Cooper, 2002), while nitrification decreases as pH decreases (Bremner, 1997). The above parameters were adjusted individually to ± 20% of their original values, while all other parameters remained stable, similar to the approach seen in Abdalla *et al.* (2009b).

Further to the results of these analyses the model has been further parameterised to improve performance, similar to the approach seen in Rafique *et al.* (2011). Based on the results of the sensitivity analysis a parameter was chosen and adjusted within a 20% range. The results of these adjustments were re-analysed and contrasted with the earlier model predictions. From here on in the terms 'uncalibrated' and 'calibrated' will be used to distinguish between the initial model simulations and those carried out after the model was further parameterised to improve performance.

Table 4.3 Summary of *Miscanthus* crop parameters.

Parameter	
Maximum Grain Production (kg ha^{-1})	6
Grain Fraction of Total Biomass	0.0001
Leaf & Stem Fraction of Total Biomass	0.69[1]
Root Fraction of Total Biomass	0.3099[1]
Leaf & Stem C/N Ratio	61[2]
Root C/N Ratio	26[2]
N Fixation Index	2.8[2]
Water Requirement (kg kg-$Biomass^{-1}$)	295[2]
Maximum LAI	8[2]
Maximum Height (m)	3[2]
Accumulative Degree Days	1800[3]
Vascularity Index	0[4]
Initial Efficiency of Photosynthesis (kg-CO_2 ha^{-1} hr^{-1})/(J m^{-2} s^{-1})	0.4[4]
Maximum Photosynthesis Rate (kg-CO2 ha^{-1} hr^{-1})	60[4]
Growth Rate in Vegetative Stage (day^{-1})	0.012[4]
Growth Rate in Reproductive Stage (day^{-1})	0.025[4]

[1] Cadoux *et al.* (2011), [2] Long and Beale (2001), [3] Clifton-brown *et al.* (2004a), [4] Model default value (Maize)

4.3 Results

4.3.1 Uncalibrated Model Outputs: Nitrous Oxide

Table 4.4 illustrates the simulated and observed annual emissions of N_2O and their relative statistical criteria. Simulated annual N_2O emissions from the uncalibrated model ranged from 562 to 3105 g ha^{-1} across the three treatments, with a total annual N_2O emission from all treatments of 4890 g ha^{-1}. This is considerably more than the field observed total annual N_2O emissions, which measured 2367 g ha^{-1}. Significant correlation between simulated and observed data was seen for the 63N and 125N treatments (r = 0.74 & 0.65 respectively), whilst a very low correlation was observed for the 0N treatment (r = -0.16). In context of relative deviation the 0N treatment gave the best value of 21%, whilst 125N overestimated annual N_2O emissions by almost 197%. In terms of validating the model by RMSE, none of the three treatments produced RMSE values that were less than $RMSE_{95\%}$. Again this was most pronounced for the 125N treatment.

Figure 4.1 illustrates the temporal variation in observed and simulated daily emission of N_2O from February 2010 to February 2011. Times of the year at which simulated N_2O flux are overestimated are clearly visible in all three treatments, and relate in part to significant simulated fluxes in September and November 2010. In the case of the 63N and 125N treatments fertiliser induced peaks are reflected in the simulated results although again are higher than observed results, in particular for a second N_2O peak occurring in July 2010.

Figure 4.2 illustrates the temporal variation in cumulative annual N_2O emissions from the three treatments. The best fit in terms of pattern and final annual emissions was given for the 63N treatment. For the 0N treatment, although the final simulated and observed annual emissions were similar, the failure of the model to simulate summer emissions is clear. In the case of the 125N treatment significant overestimation of late summer and autumn fluxes is apparent.

Table 4.4 Summary of uncalibrated model simulated (x_s) and field observed (x_o) N_2O emissions (g-N ha^{-1} day^{-1}), and relative statistical criteria.

Treatment	Σx_o	Σx_s	RD (%)	r	RMSE (%)	$RMSE_{95}$ (%)
0N	464	562	21.0	-0.16	220.96	116.14
63N	856	1223	42.9	0.74	238.72	173.33
125N	1047	3105	196.6	0.65	524.86	114.58
All	2367	4890	106.6	0.64	436.85	152.98

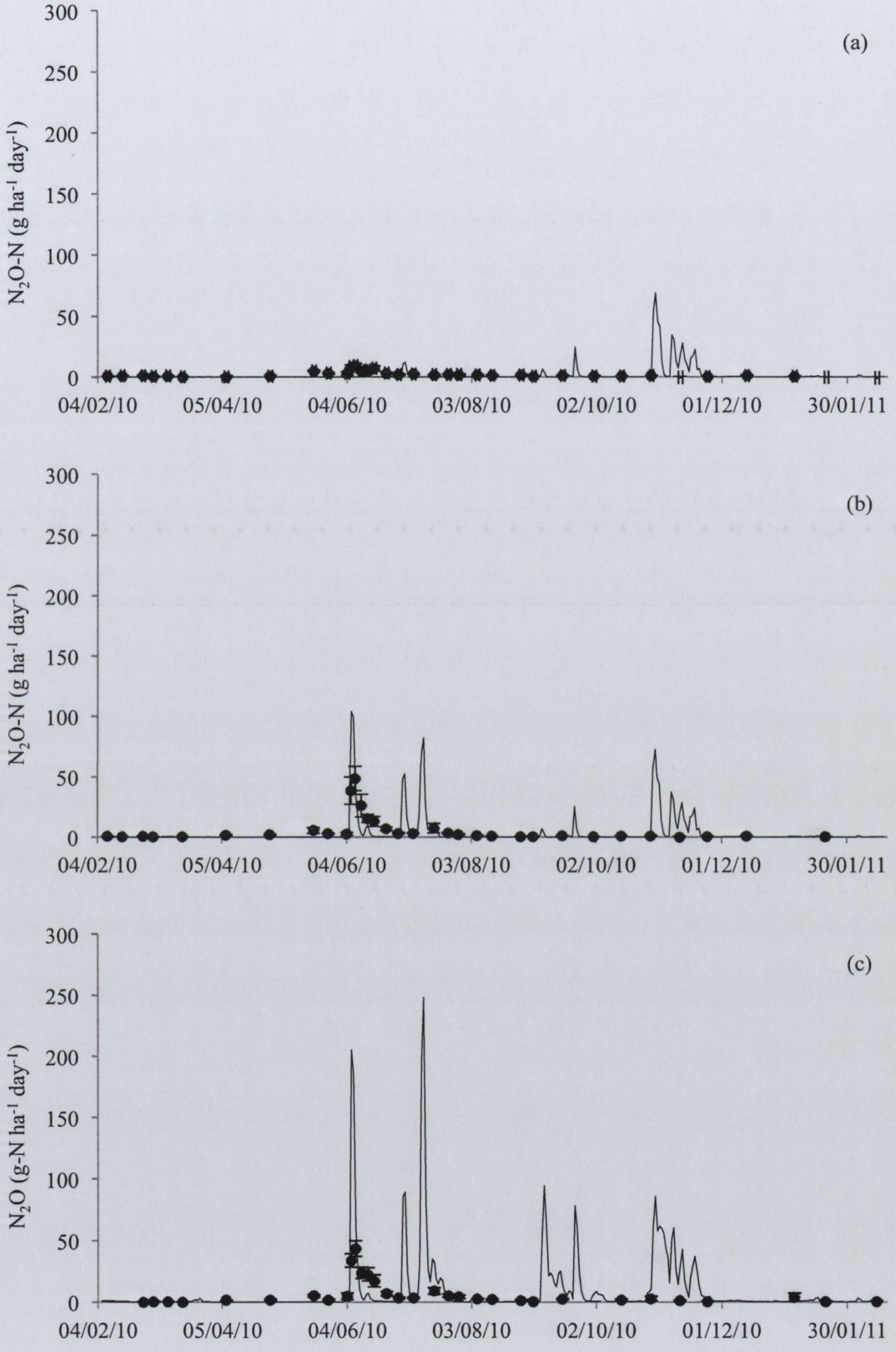


Figure 4.1 Comparison of uncalibrated model simulated (–) and field observed (●) N_2O Flux (g-N ha^{-1} day^{-1}) from three treatments; 0 kg-N ha^{-1} (a), 63 kg-N ha^{-1} (b) and 125 kg-N ha^{-1} (c).

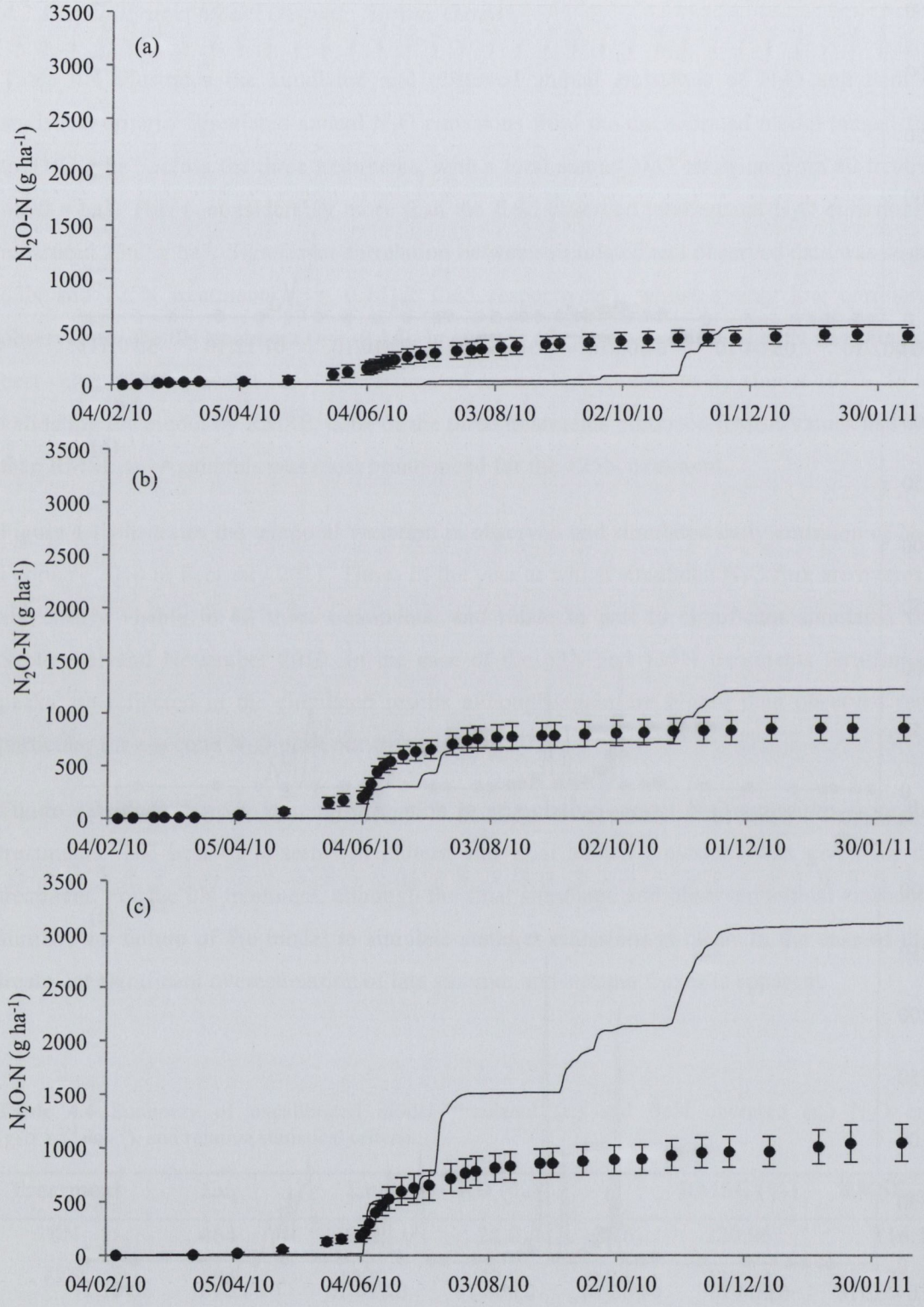


Figure 4.2 Comparison of uncalibrated model simulated (–) and field observed (•) annual cumulative N_2O emissions (g-N ha^{-1}) from three treatments; 0 kg-N ha^{-1} (a), 63 kg-N ha^{-1} (b) and 125 kg-N ha^{-1} (c).

4.3.3 Sensitivity Analysis

Table 4.5 shows the results of a sensitivity analysis for the model incorporating data from all three treatments. Here five key variables as identified in the literature were varied by ±20% and their effects on annual N_2O emissions quantified. Comparable results were generally observed across all treatments save for pH and temperature where the extent influence exerted when altering the variable appeared dependant on the amount of nitrogen fertiliser applied. A reduction in rainfall resulting in the lowest changes in all treatments, -11.4% when all treatments are considered together. Meanwhile increasing temperature led to the largest changes in each, 114.4% when all treatments are considered. Of the soil parameters analysed the greatest overall percentage change, both positive and negative, was caused by altering soil pH. Reducing pH led to an overall change of 38.9%, while increasing it resulted in a percentage change in N_2O emissions of -50.8%. However in the 125N treatment increasing soil organic carbon did lead to a greater percentage increase than decreasing pH – 36.8% compared to 19.0%. Reducing soil bulk density led to an overall change of -19.2%, while increasing it resulted in a change of 22.2%.

Table 4.5 Summary of sensitivity analysis. All values are percentage change in annual cumulative N_2O emissions.

Parameter	Change	0N	63N	125N	All
Bulk Density	+20	27.7	15.7	23.1	22.2
	-20	-25.4	-13.9	-18.3	-19.2
SOC	+20	47.3	28.0	36.8	37.3
	-20	-41.2	-23.6	-27.2	-30.6
pH	+20	-38.9	-46.6	-65.0	-50.8
	-20	60.3	37.6	19.0	38.9
Rainfall	+20	-0.5	30.0	12.2	13.9
	-20	-20.4	-2.0	-11.8	-11.4
Temperature	+20	127.4	176.3	39.4	114.4
	-20	-62.2	-70.0	-59.6	-63.9

4.3.4 Calibrated Model Outputs: Nitrous Oxide

The sensitivity analysis revealed, of the soil parameters analysed, pH to exert the greatest influence on annual N_2O emissions, and this influence to be directly related to nitrogen fertiliser input. Therefore to attempt to achieve a better fit of model outputs to observed values soil pH was adjusted across a range of values. A final soil pH of 8 was chosen after testing in the range of 6.8 to 8.2 (+20%). Table 4.6 illustrates how changing this single parameter to pH 8 – hereby called the calibrated model – has improved simulated annual emissions from all three treatments, resulting in an overall relative deviation of -1.3%.

Table 4.7 illustrates the new r, RMSE, and $RMSE_{95\%}$ values obtained for the calibrated model. For all treatments the correlation coefficient r has changed little from that for the uncalibrated model. As with the uncalibrated model RMSE values were greater than $RMSE_{95\%}$, except for the 63N treatment which revealed a good fit. In all cases the RMSE values were lower than those for the uncalibrated model indicating a significant improvement in model performance. This can be seen in the new cumulative N_2O emission graphs illustrated in Figure 4.3. There the best fit is now for the 125N treatment but all three simulated annual cumulative N_2O emissions are similar to observed values. However, the simulated model is still poor at predicting fluxes in the late summer and early autumn in treatments 0N and 63N. Also as with the uncalibrated model, there are several significant simulated peaks that were not measured in the observed data (Figure 4.3).

Table 4.6 Comparison of field observed, uncalibrated and calibrated model simulated cumulative annual N_2O emissions (g-N ha^{-1}), and relative deviation.

Treatment	Σx_o	Uncalibrated		Calibrated	
		Σx_s	RD (%)	Σx_s	RD (%)
0N	464	562	21.0	378	-18.6
63N	856	1223	42.9	716	-16.3
125N	1047	3105	196.6	1242	18.6
All	2367	4890	106.6	2336	-1.3

Table 4.7 Summary of r, RMSE, and $RMSE_{95\%}$ when comparing field observed and calibrated model simulated N_2O emissions (g-N ha^{-1}).

Treatment	r	RMSE (%)	$RMSE_{95}$ (%)
0N	-0.16	191.51	116.14
63N	0.74	149.47	173.33
125N	0.66	255.87	114.58
All	0.65	227.03	152.98

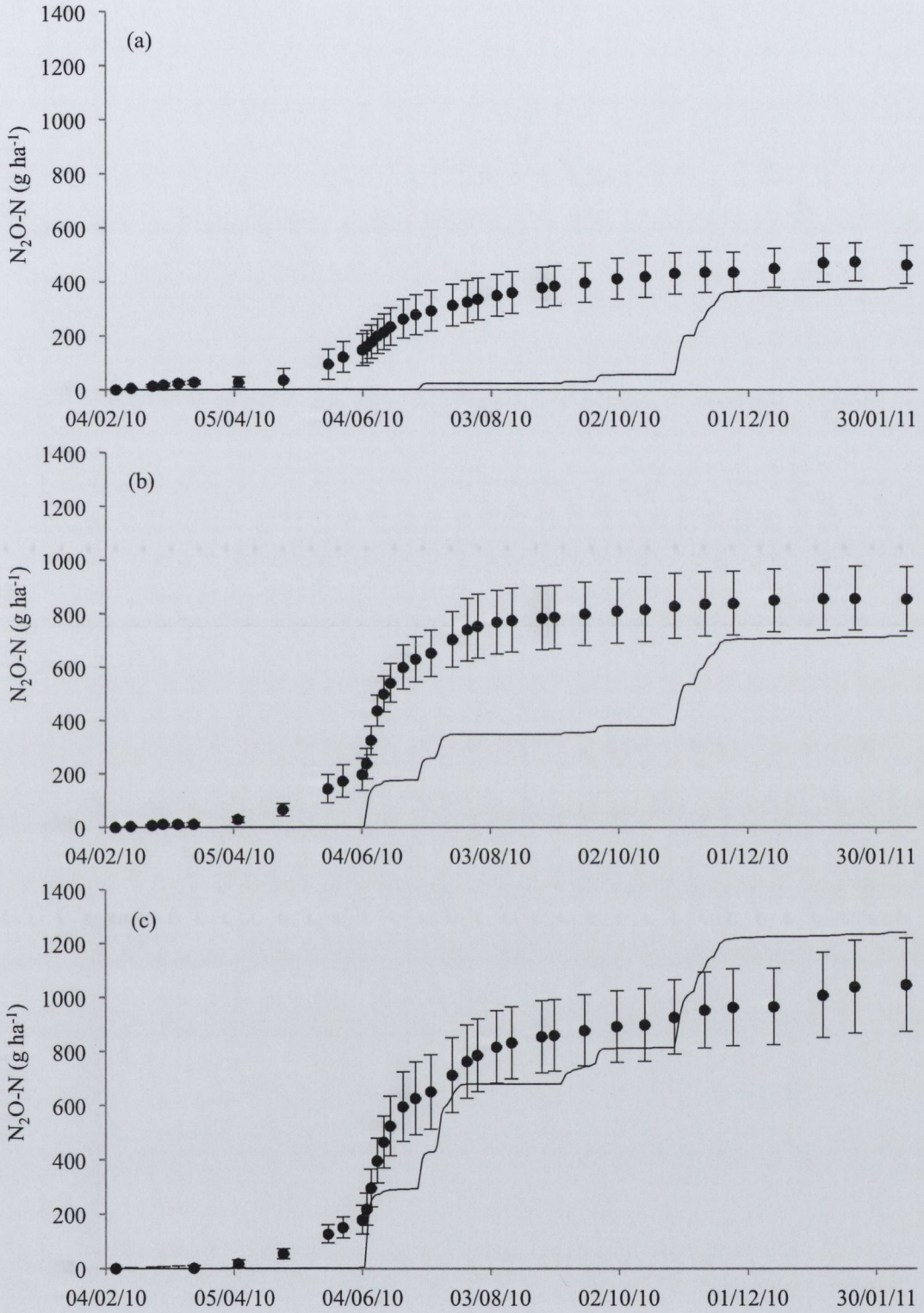


Figure 4.3 Comparison of calibrated model simulated (–) and field observed (•) annual cumulative N_2O emissions (g-N ha^{-1}) from three treatments; 0 kg-N ha^{-1} (a), 63 kg-N ha^{-1} (b) and 125 kg-N ha^{-1} (c).

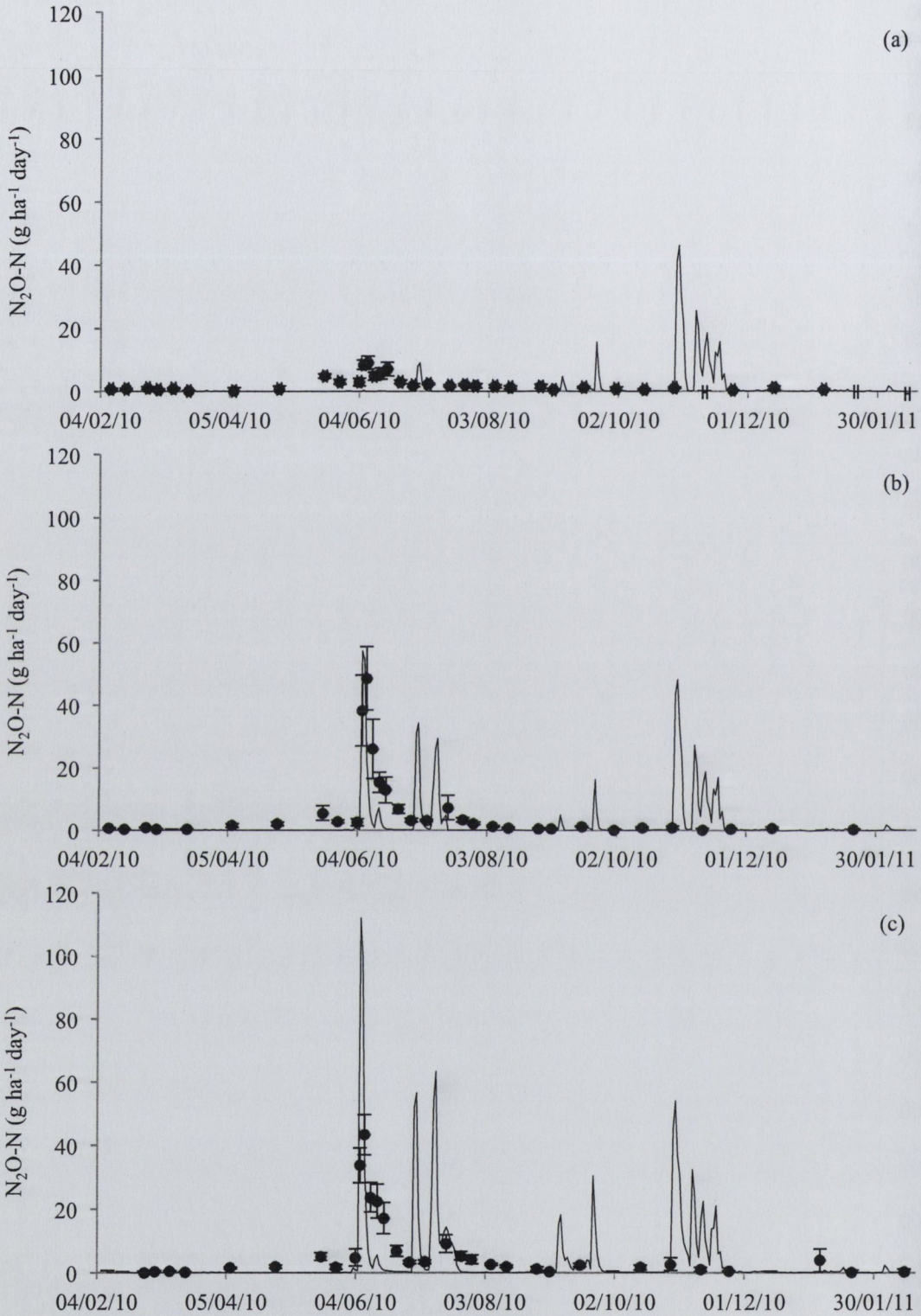


Figure 4.4 Comparison of calibrated model simulated (–) and field observed (•) N_2O Flux (g-N ha^{-1} day^{-1}) from three treatments; 0 kg-N ha^{-1} (a), 63 kg-N ha^{-1} (b) and 125 kg-N ha^{-1} (c).

4.3.5 Model Outputs: Inorganic Nitrogen

Tables 4.8 and 4.9 compare model outputs of soil nitrate concentrations from the uncalibrated and calibrated models of DNDC with field observations from all three treatments. In this case the overall means of daily values over the year have been compared and analysed by relative deviation, correlation coefficients, and root mean square errors.

Although calibration has improved the fit of the model with respect to the 63N and 125N treatments, relative deviation values remain high with modelled outputs significantly higher than measured mean values in the 63N and 125N treatments, and in contrast significantly lower than observed values for the 0N treatment. However RMSE values are favourable from the calibrated model where RMSE values are lower than $RMSE_{95\%}$ for treatments 63N and 125N and also when all treatment are considered together. Correlation coefficients calculated from the calibrated model did not change markedly from those for uncalibrated model outputs, and overall a good correlation was seen particularly for the 63N and 125N treatments (r = 0.92 and 0.95 respectively).

The reasonable fit of DNDC output values to observed results with regard to soil nitrate concentration can be seen in Figure 4.5 where both simulated and observed data are plotted against time. Only for the 0N treatment is poor agreement seen between simulated and observed values. The two other treatments show a reasonable fit between simulated and observed values, particularly in the case of the 125N treatment. For the 63N and 125N treatments the calibrated model gave a significantly better fit with field observed data.

Table 4.8 Summary of uncalibrated mean model simulated (x_s) and mean field observed (x_o) soil NO_3^- concentrations (mg-N kg^{-1}), and relative statistical criteria.

Treatment	$\bar{x}_o$	$\bar{x}_s$	RD (%)	r	RMSE (%)	$RMSE_{95}$ (%)
0N	2.03	0.38	-81.4	-0.54	101.47	64.06
63N	8.24	16.05	94.7	0.91	194.42	172.14
125N	21.68	39.16	80.7	0.93	115.31	98.67
All	10.65	18.53	74.0	0.93	161.38	139.35

Table 4.9 Summary of calibrated mean model simulated (x_s) and mean field observed (x_o) soil NO_3^- concentrations (mg-N kg^{-1}), and relative statistical criteria.

Treatment	$\bar{x}_o$	$\bar{x}_s$	RD (%)	r	RMSE (%)	$RMSE_{95}$ (%)
0N	2.03	0.27	-86.7	-0.56	101.80	64.06
63N	8.24	14.62	77.5	0.92	169.93	172.14
125N	21.68	30.72	41.7	0.95	89.36	98.67
All	10.65	15.20	42.8	0.95	129.83	139.35

Tables 4.10 and 4.11 compare model outputs of soil ammonium concentrations from the uncalibrated and calibrated models of DNDC with field observations from all three treatments. Again the overall means of daily values over the year have been compared and analysed by relative deviation, correlation coefficients, and root mean square errors.

Although calibration has improved the fit of the model with respect to the 63N and 125N treatments, relative deviation values remain high with modelled outputs significantly higher than measured mean values in the 63N and 125N treatments, and in contrast significantly lower than observed values for the 0N treatment. However RMSE values are favourable, measuring less than $RMSE_{95\%}$, in the 125N treatment and when all treatments are considered together. Correlation coefficients calculated from the calibrated model did not change markedly from those for uncalibrated model outputs, and overall a good correlation was seen for the 125N treatment ($r = 0.80$).

The reasonable fit of DNDC output values to observed results with regard to soil ammonium concentration can be seen in Figure 4.6 where both simulated and observed data are plotted against time. Only for the 0N treatment is poor agreement seen between simulated and observed values. The two other treatments show a reasonable fit between simulated and observed values, particularly in the case of the 125N treatment. For the 63N and 125N treatments the calibrated model gave a considerably better fit with field observed data.

Table 4.10 Summary of uncalibrated mean model simulated (x_s) and mean field observed (x_o) soil NH_4^+ concentrations (mg-N kg^{-1}), and relative statistical criteria.

Treatment	$\bar{x}_o$	$\bar{x}_s$	RD (%)	r	RMSE (%)	$RMSE_{95}$ (%)
0N	4.45	0.42	-90.5	-0.07	106.83	60.20
63N	5.31	9.47	78.2	0.28	243.05	99.87
125N	8.86	18.69	111.0	0.77	242.23	222.91
All	6.21	9.52	53.4	0.71	237.03	191.74

Table 4.11 Summary of calibrated mean model simulated (x_s) and mean field observed (x_o) soil NH_4^+ concentrations (mg-N kg^{-1}), and relative statistical criteria.

Treatment	$\bar{x}_o$	$\bar{x}_s$	RD (%)	r	RMSE (%)	$RMSE_{95}$ (%)
0N	4.45	0.31	-93.0	-0.08	106.56	60.20
63N	5.31	4.97	-6.4	0.35	122.35	99.87
125N	8.86	9.62	8.7	0.80	96.37	222.91
All	6.21	4.97	-19.9	0.73	109.45	191.74

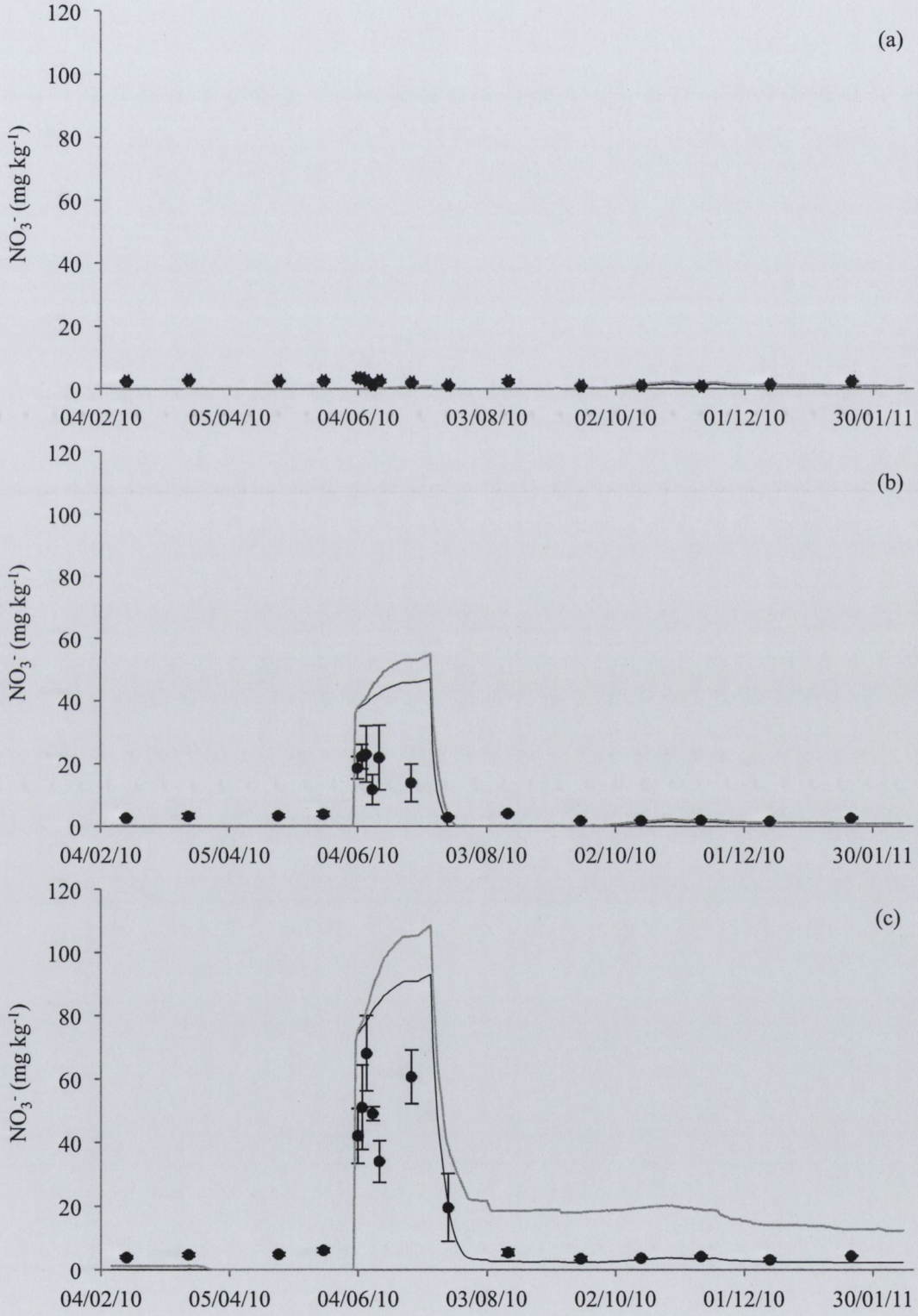


Figure 4.5 Comparison of uncalibrated (light line) and calibrated (dark line) model simulated, and field observed (•) mean soil NO_3^- concentrations (mg-N kg^{-1}) from three treatments; 0 kg-N ha^{-1} (a), 63 kg-N ha^{-1} (b) and 125 kg-N ha^{-1} (c).

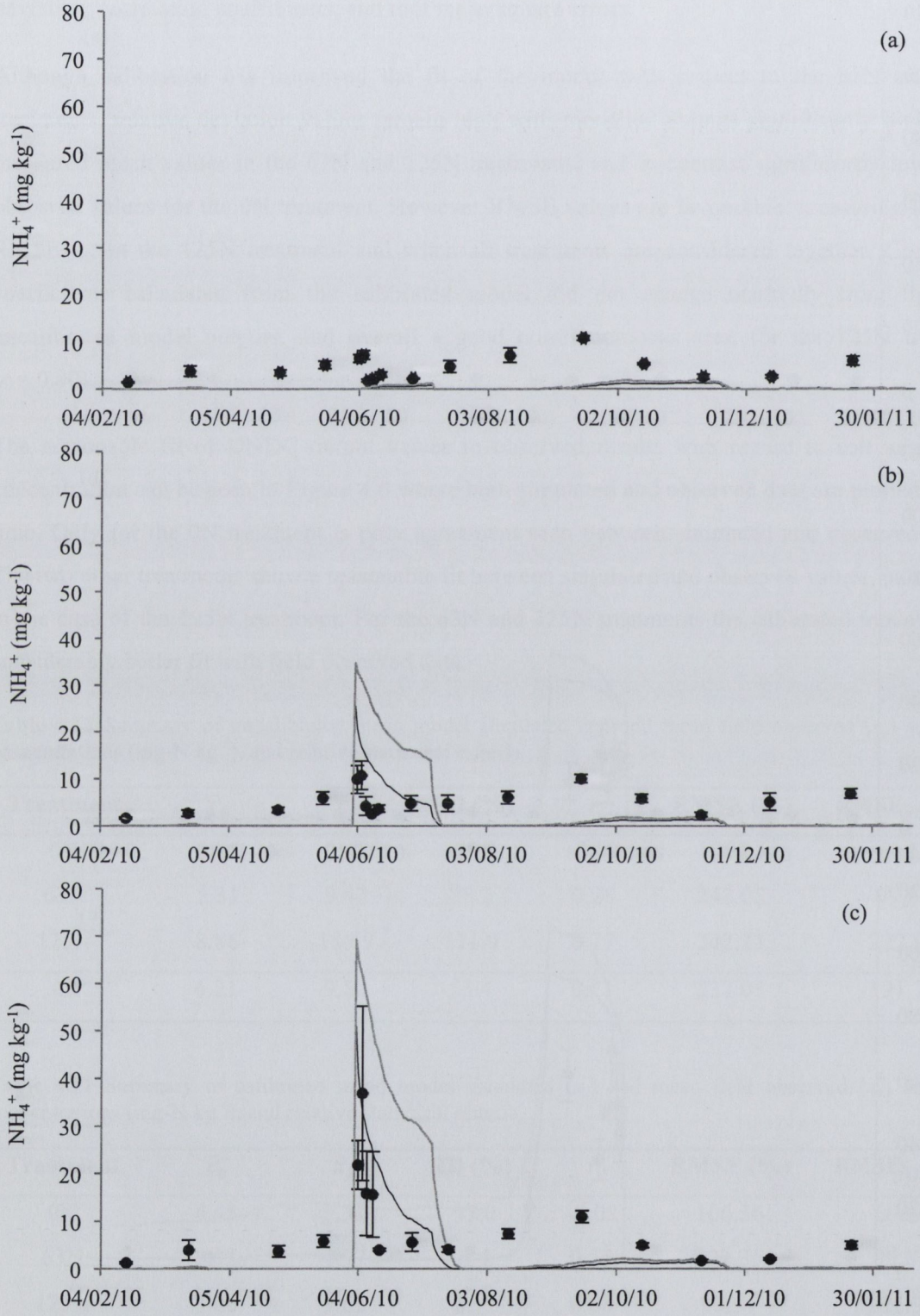


Figure 4.6 Comparison of uncalibrated (light line) and calibrated (dark line) model simulated, and field observed (•) mean soil NH_4^+ concentrations (mg-N kg^{-1}) from three treatments; 0 kg-N ha^{-1} (a), 63 kg-N ha^{-1} (b) and 125 kg-N ha^{-1} (c).

4.4 Discussion

The previous chapter investigated the effects of varying rates of inorganic nitrogen fertiliser application on soil N_2O emissions from *Miscanthus*. Data of this kind is currently uncommon and as such there has been little opportunity to validate model simulated N_2O emissions from *Miscanthus* with field observed data. Here the DNDC (DeNitrification-DeComposition) model has been adapted to simulate N_2O emissions from *Miscanthus* and validated with field observed measurements.

DNDC was initially designed for use with a number of common agricultural crops. The original validation study of the model found good agreements of simulated and observed N_2O emissions from a grassland, short-grass prairie, and winter wheat crops (Li *et al.*, 1992b). Since then DNDC has been adapted for use with a huge range of ecosystems from forests (Stange *et al.*, 2000) to rice paddies (Zheng *et al.*, 1999). However a number of limitations still exist within the model when applied to a crop such as *Miscanthus*. The crops mentioned all have the capacity to produce seed, as a sterile hybrid *Miscanthus* does not. This is an issue as the growth compartment of DNDC has a large dependence on maximum grain yield. Other issues stem from its C_4, rhizomatous, perennial characteristics and consequent nutrient use efficiencies. As a perennial rhizomatous crop *Miscanthus* only requires planting approximately once every 15 years (Price *et al.*, 2004), this causes an issue with DNDC as it is necessary to input a planting date every one to two years. Furthermore *Miscanthus*' rhizomatous nature dictates that above ground plant nutrients are relocated to the rhizomes at the end of each growing season. These make an important contribution *Miscanthus*' nutrient requirements the following growing season. Christian *et al.* (2006) found that after applying ^{15}N-labelled fertiliser to a three year old *Miscanthus* crop over 25% was found stored in the rhizomes at the end of the growing season, additionally this does not count for other sources of nitrogen relocated to the rhizomes. This cannot be accounted for directly in DNDC as options are only available to account for nutrient supply through soil stores, fertiliser application, and fixation. Nonetheless despite these limitations DNDC was chosen for use here due to its widespread use, and successful application to a huge range of ecosystems and crops across the globe as well as in Ireland. Its widespread use is evidenced by the fact that its website lists 71 publications incorporating the use of DNDC (University of New Hampshire, 2011). While in Ireland it has been used to successfully simulate N_2O emissions from a number of agricultural soils (Hsieh *et al.*, 2005, Abdalla *et al.*, 2009b, Rafique *et al.*, 2011). The strategies adopted to overcome these issues have been described in section 4.2.4.

This chapter lists a calibration procedure that has produced a reasonable fit between model simulated and field observed N_2O emissions and soil inorganic nitrogen concentrations. The applied methodology was to first run the model with a combination of model default and literature

derived crop parameters relative to *Miscanthus*, and analyse the resulting output for goodness of fit with field observations. Subsequent to this a sensitivity analysis was carried out testing major influencing variables. Based on the results of this analysis the model was calibrated by adjusting selected variables to improve fit. Unfortunately only one year of data from the nitrogen fertiliser experiment described in Chapter 3 was available. Ideally this chapter would be strengthened by the availability of further data with which to validate the calibrated model.

As previously mentioned a range of literature derived and model default values were used to parameterise DNDC for use with *Miscanthus*. These values are listed in Table 4.3. Unfortunately a single source was not available containing all the necessary information, however the majority of the values from the literature were derived from one source in order to try and keep consistency; a chapter by Long and Beale (2001) titled "Resource Capture by *Miscanthus*" from the book "*Miscanthus* for Energy and Fibre" (Jones & Walsh, 2001). Beyond this a recent review of published studies on the nutrient requirements of *Miscanthus* (Cadoux *et al.*, 2011) was used to find values for fractions of above and below ground biomass, this source was selected due to the comprehensive nature of the review and up to date publication. Only one source was discovered containing information about accumulated degree days for *Miscanthus* (Clifton-brown *et al.*, 2004a), and as such the value found there was applied. Information about rates of photosynthesis and growth, as well as vascularity proved difficult to obtain. Default model values for another common bioenergy crop utilising a C_4 photosynthetic pathway – Maize – were chosen for use in this case.

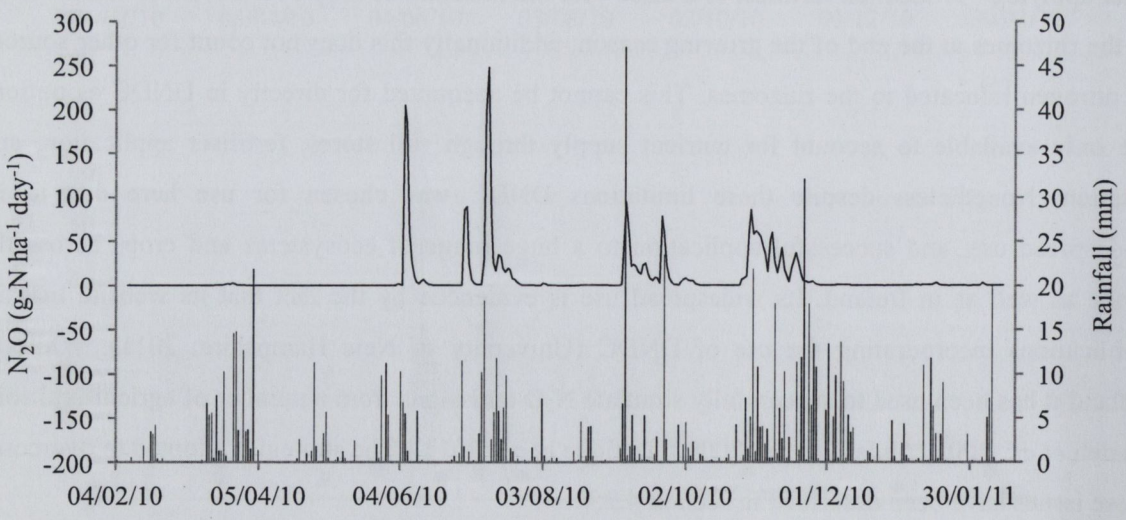


Figure 4.7 Comparison of uncalibrated model simulated N_2O Flux (g-N ha^{-1} day^{-1}) at a N fertiliser application rate of 125 kg-N ha^{-1} (line) and observed rainfall (mm) (bars).

The parameterised model was run using a combination of observed and default inputs. Simulated annual cumulative N_2O emissions were overestimated for all treatments, particularly 125N were a relative deviation of 196.6% was found (Table 4.4). This overestimation is apparent in Figure 4.1 where simulated peaks in daily N_2O flux either outreach their corresponding observed peak or occur when no observed peak was measured. To investigate these non-corresponding simulated peaks – seen similarly in all treatments – simulated daily N_2O emissions from the 125N treatment have been plotted against rainfall and temperature (Figures 4.7 & 4.8). Comparing simulated N_2O with rainfall it can be seen that most peaks correspond with a rainfall event (Figure 4.7). A similar relationship can also be seen with temperature (Figure 4.8). All simulated peaks occur on days with above average temperatures (Mean annual temperature = 8.5 °C). A sensitivity analysis revealed temperature to be the most sensitive of the factors analysed suggesting that it highly likely to be a major controlling factor of simulated N_2O emissions. Soil microbial responses and soil nitrate and moisture are heavily dependant on temperature due to its positive relationship with microbial activity. (Bramley & White, 1990). The importance of soil temperature in soil N_2O production is also demonstrated by Q_{10} values which are often particularly high when compared to other biochemical processes (Smith *et al.*, 2003). Q_{10} values of up to 50 for soil N_2O emissions have been found in arable soils (Dobbie & Smith, 2001). A similar sensitivity was found by Abdalla *et al.* (2009b), where DNDC was found to be more sensitive to temperature than a number of other factors when predicting N_2O emissions from an arable site with a similar soil type.

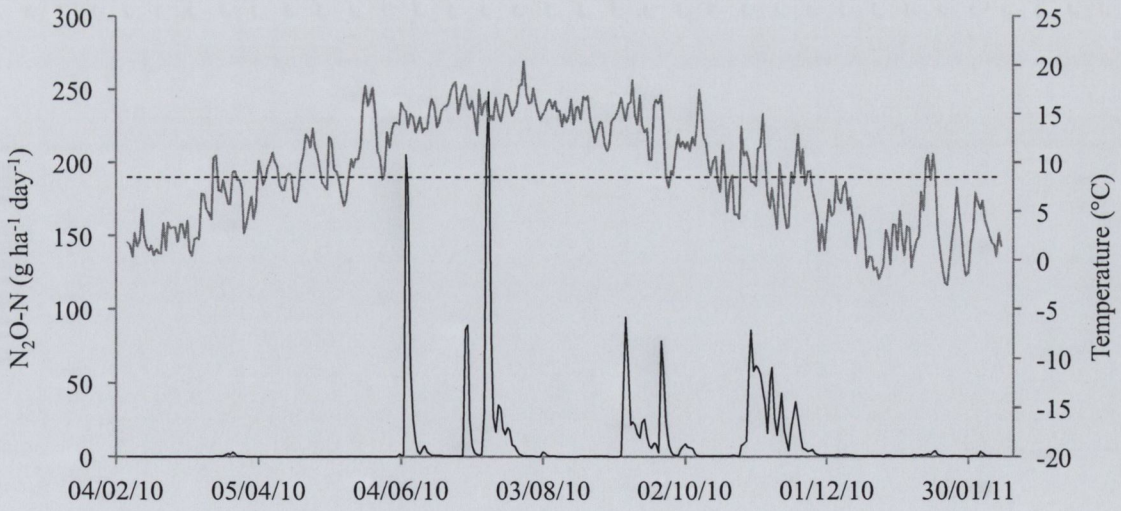


Figure 4.8 Comparison of uncalibrated model simulated N_2O-N Flux (g ha^{-1} day^{-1}) at a N fertiliser application rate of 125 kg-N ha^{-1} (dark line), observed mean daily temperature (°C) (light line), and mean annual temperature (°C) (dashed line).

Another possible explanation for this severe overestimation lies with the techniques used to measure field observed N_2O flux. Many of the un-corresponding simulated N_2O fluxes occur on days when no field measurements were taken. It is common practice when using manual closed chambers to only take field measurements weekly, hence creating opportunities for peaks in N_2O emissions to be missed (Yao *et al.*, 2009). However the use of manual closed chambers is generally accepted. A study carried out by Christensen *et al.* (1996) comparing manual and automated closed chamber techniques, and micro-meteorological techniques showing little significant difference.

Unlike temperature, adjusting rainfall had a little impact on N_2O emissions, with a reduction of 20% leading to a reduction in annual cumulative N_2O emissions of -11.4% across all plots, while similarly an increase of 20% lead to an increase in N_2O emissions of 13.9%. This is likely to be due to saturation in simulated WFPS caused by the low default field capacity value of 0.25 used when running the model. This can be seen clearly in Figure 4.9, which shows model simulated and field observed WFPS from the 125N treatment. The model was tested with increased field capacity but did not improve performance. As actual measured data was unavailable a decision was made to use default values.

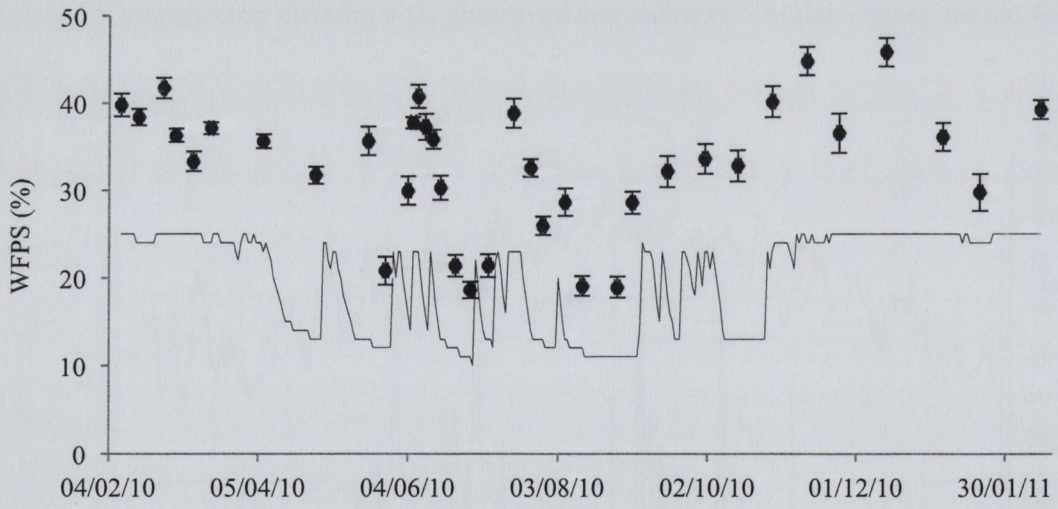


Figure 4.9 Comparison of uncalibrated model simulated (–) and field observed (•) WFPS (%) as a mean from all three treatments.

Expressing the results of the sensitivity analysis as the rate of change in N_2O emissions as determined by linear regression (Table 4.12) shows clearly the effects of the tested parameters on simulated N_2O emissions. As previously discussed it is clear that temperature has had the greatest

effect in all treatments, while rainfall has had the least, except in the 63N treatment where bulk density has had a similarly weak impact. Looking at the soil parameters tested it can be seen that the model is highly sensitive to soil pH and soil organic carbon. Although SOC has been cited as the most sensitive factor controlling agricultural N_2O emissions in DNDC (Li *et al.*, 2001) soil pH was found to have a greater effect in this case. This is likely to be caused by its effect on the rates of N_2O production by nitrification and denitrification, as influenced by its effects on the size, diversity, and activity of soil microbial communities (Fierer & Jackson, 2006), and its impact on the N_2O product ratios of both processes (Mørkved *et al.*, 2007). For these reasons soil pH was selected to calibrate the model.

Table 4.12 Summary of rates of change as determined by linear regression of a number of parameters tested in a sensitivity analysis.

Treatment	Bulk Density	SOC	pH	Rainfall	Temperature
0N	1.33	2.21	-2.48	0.50	4.47
63N	0.74	1.29	-2.11	0.80	6.16
125N	1.04	1.60	-2.10	0.60	2.47
All	1.03	1.70	-2.24	0.63	4.46

Results from Chapter 3 indicate that nitrification was the dominant source of field observed N_2O emissions. By increasing pH it is likely to decrease the simulated rate of denitrification, allowing a greater dominance of nitrification (Stevens *et al.*, 1998, Šimek & Cooper, 2002). Therefore to calibrate the model to achieve a better model input pH was adjusted from 6.8 to 8.0. By adjusting only the pH value a considerably improved fit of model outputs of N_2O emissions and soil inorganic nitrogen concentrations with field observations was found.

Table 4.13 shows mean annual simulated NH_4^+ and NO_3^- concentrations from the uncalibrated and calibrated models. Simulated NH_4^+ concentrations from the calibrated model are reduced considerably when compared to the uncalibrated model, suggesting a possible increase in simulated nitrification. Simulated NO_3^- concentrations also decrease - albeit to a lesser extent – implying that simulated denitrification rates have not declined. However simulated N_2O production by denitrification may still have declined due to a reduction in the N_2O/N_2 ratio caused by increasing pH (Dannenmann *et al.*, 2008). Looking at Figure 4.5a it is clear that nitrate concentrations in the 0N treatment are poorly simulated for both the uncalibrated and calibrated model, this is reinforced by the fact that the weakest correlation between simulated and observed concentrations are seen in 0N (uncalibrated -0.54, calibrated -0.56) (Tables 4.8 & 4.9). Conversely in treatment 125N there is a particularly good correlation (uncalibrated 0.93, calibrated 0.95). Calibrated model simulated

NO_3^- from the 63N and 125N treatments can also be seen to be a good fit with field observations due to the fact RMSE values are less than $RMSE_{95\%}$. When all treatments were considered together comparing RMSE and $RMSE_{95\%}$ revealed a good fit between model simulated NO_3^- and field observations. Soil NH_4^+ concentrations display similar trends (Figure 4.6). There is particularly poor correlation between simulated and observed concentration in treatment 0N (uncalibrated -0.07, calibrated -0.08), and the best correlation is seen in treatment 125N (uncalibrated 0.77, calibrated 0.80). In this case comparing RMSE and $RMSE_{95\%}$ revealed a good fit in the 125N treatment, but also when all treatments are considered together. Overall simulated soil NO_3^- and NH_4^+ concentrations are clearly improved by calibrating the model. This is demonstrated by decreases in RMSE observed across the board (Tables 4.8, 4.9, 4.10 & 4.11), with the exception of soil NH_4^+ in treatment 0N were only a slight decrease was seen.

Table 4.13 Comparison of uncalibrated and calibrated model simulated mean annual soil NO_3^- concentrations (mg kg^{-1}).

	NO_3^-		NH_4^+	
Treatment	**Uncalibrated**	**Calibrated**	**Uncalibrated**	**Calibrated**
0N	0.37	0.27	0.42	0.31
63N	16.05	14.62	9.47	4.97
125N	39.16	30.72	18.69	9.62
All	18.53	15.20	9.53	4.97

The improved performance by the calibrated model seen in relation to soil NO_3^- and NH_4^+ concentrations can, importantly, also be seen in relation to N_2O emissions. RMSE has been reduced in each of the treatments, with overall RMSE falling from 437% to 227% (Tables 4.4 & 4.7). RMSE calculated from the 0N and 125N treatments are greater than $RMSE_{95\%}$ values, however the opposite has been found in the 63N treatment indicating that simulated N_2O emissions the 63N treatment are a good fit with field observations, this is also supported by the *r* value of 0.74 recorded here, the highest seen in any of the treatments. Nonetheless it is apparent from Figure 4.3 that the calibrated model suffers from many of the same issues as the uncalibrated version, in particular the presence of simulated peaks at times when no field observed measurements were taken. Smith *et al.* (2008b) have also found DNDC to have difficulty predicting daily fluxes despite simulating annual N_2O emissions with reasonable accuracy. Additionally the generally statistically poor relationship between model simulated and field measured daily N_2O flux is likely to be compounded by a general underestimation of background emissions. Zero emissions were

predicted on an average 56% of all days across all three treatments, whereas in the field zero emissions were only observed once and positive emissions accounted for 83% of all measurements.

Annual cumulative N_2O emissions have been predicted with a good accuracy. Relative deviations of -18.6%, -16.3%, and 18.6% were calculated for treatments 0N, 63N, and 125N respectively. The overall relative deviation across all three treatments is particularly small at -1.3%. The improvement in accuracy seen in annual N_2O emissions between the uncalibrated and calibrated models is likely to be related to the previously discussed relationship between pH and rates of N_2O production by nitrification and denitrification. The results here indicate that although DNDC performed poorly in some cases when predicting daily N_2O emissions it can be a useful tool for predicting annual N_2O emissions from *Miscanthus*. However in order to fully assess it's worth in this regard further validation is desirable. As it was necessary to calibrate the model based on one year of available field N_2O data, further data collected from a range of meteorological conditions and soil types would greatly benefit this assessment.

4.5 Conclusions

When parameterising DNDC to include *Miscanthus* it was necessary to include nominal values for grain production. Additionally all sources of non-derived nitrogen needed to be classified as nitrogen fixation. These consisted largely of nitrogen recycled annually from the rhizome, however Long & Beale (2001) reported a large unknown source of nitrogen supply to *Miscanthus*. Further work by Davis *et al.* (2010) propose biological N fixation to have a considerable contribution to nitrogen cycling in *Miscanthus*, and found including a source of N fixation resulted in an accurate simulation of the nitrogen balance in *Miscanthus*, when modelled with the process based model DAY-CENT. After including grain production and nitrogen fixation in the models parameter set for *Miscanthus* above ground yield was successfully simulated.

Model simulations of daily N_2O emissions were generally poor largely due to an overestimation in peak size and frequency. Despite this reasonable correlations were seen with field observed values in the treatments amended with inorganic nitrogen fertiliser. Similar results were seen when looking at model simulated soil inorganic nitrogen concentrations.

A sensitivity analysis revealed N_2O emissions to be extremely sensitive to temperature. This is interesting as it may have serious impacts under expected climate change scenarios. N_2O emissions were also found to be very sensitive to soil pH. The model was calibrated by increasing soil pH. This theoretically should decrease N_2O production by denitrification and increase the importance of nitrification, mirroring the findings of Chapter 3.

Table 4.14 Summary of calibrated model performance for N_2O emissions and soil inorganic nitrogen concentrations: Relative deviation = 0-10% (***), 11-40% (**), 41-100% (*), 100% + (ns).

Treatment	N_2O	NO_3^-	NH_4^+
0N	**	*	*
63N	**	*	***
125N	**	*	***
All	***	*	**

The calibrated model showed a considerable improvement when predicting N_2O emissions and soil inorganic nitrogen concentrations, and was shown by comparing RMSE and $RMSE_{95\%}$ to accurately simulate N_2O emissions from the mid-level fertiliser treatment. Simulated soil inorganic nitrogen concentrations from the calibrated model improved for the 63N and 125N treatments, no improvement was seen in the 0N treatment, and performed well when all treatments were considered together. Table 4.14 illustrates the performance of the calibrated model when simulating annual cumulative N_2O and mean annual soil NO_3^- and NH_4^+ concentrations in terms of relative deviation from field observations. The calibrated model performed very well overall when simulating cumulative annual N_2O emissions, while annual emissions from the individual treatments were predicted with reasonable accuracy. Soil NH_4^+ concentration was predicted extremely well in the 63N and 125N treatments, whilst relative deviations for simulated soil NO_3^- concentrations were only passable from all treatments.

These results suggest that DNDC can be a useful tool for predicting soil inorganic nitrogen concentrations and N_2O emissions from *Miscanthus*. The calibration applied here has significantly improved model performance, but further improvements are desirable when predicting daily N_2O fluxes. These improvements could possibly be made by fully integrating perennial rhizomatous crops into DNDC, allowing for crops that with multi-year life spans post-planting to be included, and allowing for rhizomatous nutrient translocation. Additionally the inclusion of an option for sterile non-seed bearing crops may improve simulations from *Miscanthus*. Ideally further data is required to validate the calibrated model. To fully assess DNDC's potential in regards to N_2O emissions from *Miscanthus* a greater range of data is necessary including a variety of meteorological conditions and soil types.

Chapter 5

General Discussion

Agriculture represents the largest global source of N_2O emissions, accounting for 60% of all anthropogenic emissions in 2005 (IPCC, 2007b). Land-use and management are important influences on rates of agricultural N_2O emissions, and as such are an important component of global greenhouse gas budgets. The use of bioenergy crops as fossil fuel replacements has expanded in the recent past (Tao & Aden, 2009) and is likely to continue to grow in the near future as countries attempt to meet greenhouse gas reduction targets outlined in various policies. For example the EU have targeted 10% of all vehicle fuel to be provided by biofuels by 2020 (Antizar-Ladislao & Turrion-Gomez, 2008). This will drive land-use change to bioenergy crops. *Miscanthus* is a promising bioenergy crop due to a number of advantageous physiological characteristics, however little is known about its relationship with soil N_2O emissions.

This study was designed to investigate the effects of land-use change from grassland to *Miscanthus,* as well as nitrogen fertiliser management, on soil N_2O emissions. Additionally the suitability of the process based model DNDC for simulating N_2O emissions from *Miscanthus* was assessed. The key questions asked during this study have been:

- *Does land use change from grassland to Miscanthus significantly affect soil N_2O emissions?*

- *Does increasing the rate of nitrogen fertiliser application significantly affect N_2O emissions from Miscanthus?*

- *Is any increase in N_2O emissions related to nitrogen fertiliser application reflected by a proportional increase in above ground biomass?*

- *Is the application of nitrogen fertiliser to Miscanthus economically viable when the cost of N_2O as CO_2 equivalents is taken into account?*

- *Is DNDC a suitable tool to simulate N_2O emissions from Miscanthus?*

Results from Chapter 2 found no significant difference in N_2O fluxes measured from a grassland, newly planted *Miscanthus* crop, and long established *Miscanthus* crop, all receiving no nitrogen fertiliser additions. However a trend towards higher emissions from the newly planted *Miscanthus* stand was seen with mean emissions almost three times greater than those seen in the grassland. These results indicate that land-use change from grassland to *Miscanthus* is unlikely to negatively affect medium to long term N_2O emissions, but there may be a slight if insignificant increase in the

first few years after establishment. This is likely to be compounded by a short-term peak in emissions caused when previously stable grassland is tilled prior to establishment.

Chapter 3 found that while nitrogen fertiliser application had a significant effect on N_2O flux increasing the rate of fertiliser from 63 to 125 kg-N ha^{-1} did not. Additionally nitrogen fertiliser application was not found to significantly affect above ground biomass, but showed a similar pattern to N_2O emissions. This is reflected by the fact that nitrogen fertiliser was found to significantly affect the N_2O/yield ratio. However this proportional relationship is subject to vastly different rates of increase, with N_2O emissions rising up to eight times more than the corresponding increase in yield. An economic analysis of the data presented here found that adding nitrogen fertiliser led to losses of around €15 ha^{-1} when taking into consideration the market prices of *Miscanthus* and synthetic nitrogen fertiliser. It should be noted that there was large variation within this cost due to highly variable yields. Including the cost of N_2O using the EU benchmark price for carbon did not especially affect this cost. This chapter shows that due to potentially damaging environmental and economic costs very careful consideration must be taken when adding nitrogen fertiliser to *Miscanthus*, with application only occurring when soil nutrients are found to have been significantly depleted.

In Chapter 4 data from the literature and model default values were used to parameterise DNDC for use with *Miscanthus*. The parameterised model was then run and validated using soil, crop, and management data collected during Chapters 2 & 3 in combination with data from a previous experiment run at the same site and model defaults. Model performance was initially poor. A sensitivity analyses revealed soil pH to be an important determining factor of simulated N_2O emissions. As analyses from Chapter 3 suggested that nitrification may have been the dominant N_2O production pathway in that case, the model was calibrated by increasing soil pH to favour nitrification. This calibration procedure significantly improved model performance with an overall relative deviation of -1.3% (as opposed to 106.6% from the uncalibrated model) found when model simulated and field observed yearly cumulative N_2O emissions from all three fertiliser treatments were compared. This indicates that DNDC can be a useful tool for predicting soil N_2O emissions from *Miscanthus*.

Figure 5.1 illustrates yearly cumulative N_2O emissions measured during field trials in Chapters 2 & 3, alongside those simulated by DNDC after calibration in Chapter 4. Although in Chapter 2 the grassland, newly planted *Miscanthus* crop, and long established *Miscanthus* crop were referred to as plots, they will be referred to as treatments from here on in for ease of comparison. Mean N_2O emissions varied considerably ranging from 216 g-N ha^{-1} yr^{-1} in the grassland to 1242 g-N ha^{-1} yr^{-1} in the model simulated 125 kg-N ha^{-1} treatment, however some similarities were seen.

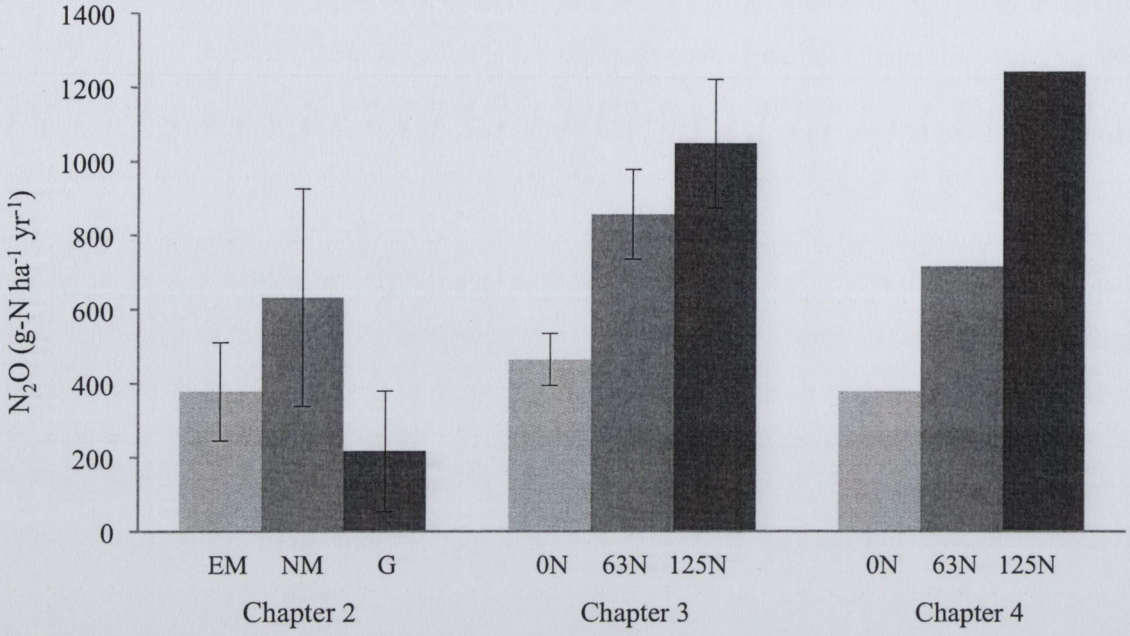


Figure 5.1 Comparison of cumulative annual N_2O emissions (g-N ha^{-1} yr^{-1}) from a grassland (G), newly planted *Miscanthus* (NM), long established *Miscanthus* (EM) (Chapter 2), and field observed (Chapter 3) and calibrated model simulated (Chapter 4) emissions from an established grassland treated with nitrogen fertiliser application rates of 0 kg-N ha^{-1} (0N), 63 kg-N ha^{-1} (63N), and 125 kg-N ha^{-1} (125N).

The long established *Miscanthus* (Chapter 2) and 0 kg-N ha^{-1} treatments (Chapters 3 & 4) are comparable as they have been subject to the same management – albeit in different years. Cumulative annual N_2O emissions from all three are remarkably similar, in particular those from the long established *Miscanthus* and the calibrated model which both measure 378 g-N ha^{-1} yr^{-1}. Another noteworthy similarity can also be seen when these are compared to one of the only analogous studies in the literature. Drewer *et al.* (2011) measured soil N_2O emissions from an unfertilised *Miscanthus* crop over two years finding mean annual fluxes of 324 g-N ha^{-1} yr^{-1}. As previously mentioned the grassland showed the lowest emissions, but when variation is taken into account they too fall into the same range as the unfertilised established *Miscanthus* treatments. The highest mean annual emissions were unsurprisingly seen in the treatments subject to nitrogen fertiliser application, but again when variation is taken into account N_2O emissions from the newly planted *Miscanthus* are comparable with those from the 63 kg-N ha^{-1} treatments.

In general the amount of variation seen within treatments was greatest in Chapter 2. This is likely to be due to the large amounts of N_2O uptake seen throughout the year. Soil N_2O uptake was seen in over 39% of all daily N_2O fluxes measured in Chapter 2. Treatments showed little variation with uptake found in 42%, 36%, and 40% of all measurements in the grassland, newly planted *Miscanthus*, and long established *Miscanthus* respectively. The frequency of N_2O uptake was

considerably lower in Chapter 3 where uptake was recorded in only 16% of measurements. Again little variation was seen between treatments with uptake found in 15%, 17%, and 17% of all measurements in the 0, 63, and 125 kg-N ha^{-1} treatments respectively.

Low soil NO_3^- concentrations are reported to be linked to high rates of soil N_2O consumption, as it promotes the use of N_2O as an electron acceptor during denitrification (Ryden, 1983, Khalil *et al.*, 2002). Figures 5.2 & 5.3 show soil N_2O emissions and NO_3^- concentrations from the comparable unfertilised established *Miscanthus* treatments from each Chapter (EM & 0N) plotted against each other. This has been done in order to examine how the relationship between N_2O consumption and soil NO_3^- concentration compare and contrast in these two treatments. No particular pattern can be seen in either case, although it is clear that not only the frequency, but also the magnitude, of N_2O uptake was greater in the EM treatment than 0N. This is also apparent in Table 5.1. Table 5.1 shows mean N_2O emissions, soil NO_3^- and NH_4^+ concentrations, WFPS, and soil temperature from all treatments in Chapters 2 and 3 measured only on occasions when N_2O uptake was observed. While soil NO_3^- concentrations are relatively low compared to nitrogen fertilised soils, again when contrasting the comparable EM and 0N treatments it can be seen that mean soil NO_3^- concentrations were lower in the 0N treatment (1.92 mg-N kg^{-1}) compared to the EM treatment (4.66 mg-N kg^{-1}), somewhat contradicting the assertion that low soil NO_3^- concentrations promote uptake. Warmer soils have also been found to encourage N_2O uptake due to increased microbial activity (Chapuis-Lardy *et al.*, 2007). It is apparent from Table 5.1 that soil temperature was higher across the board on occasions when N_2O uptake was observed in Chapter 2 than in Chapter 3. This combined with overall relatively low soil inorganic nitrogen concentrations may help explain the large amount of N_2O uptake seen in Chapter 2.

Table 5.1 Summary of mean N_2O emissions (µg-N m^{-2} hr^{-1}), annual soil NO_3^- and NH_4^+ concentrations (mg-N kg^{-1}), WFPS (%), and soil temperature (°C), measured from all treatments in Chapters 2 & 3 on occasions when N_2O uptake was observed.

Chapter	Treatment	N_2O	NO_3^-	NH_4^+	WFPS	Temp
	G	-48.52	2.63	4.42	34.40	12.58
2	NM	-38.53	4.94	3.29	36.00	14.15
	EM	-41.54	4.66	3.00	47.73	11.54
	0N	-12.85	1.92	4.88	35.08	9.22
3	63N	-2.20	4.58	3.56	37.43	10.3
	125N	-3.97	4.05	3.78	34.77	4.66

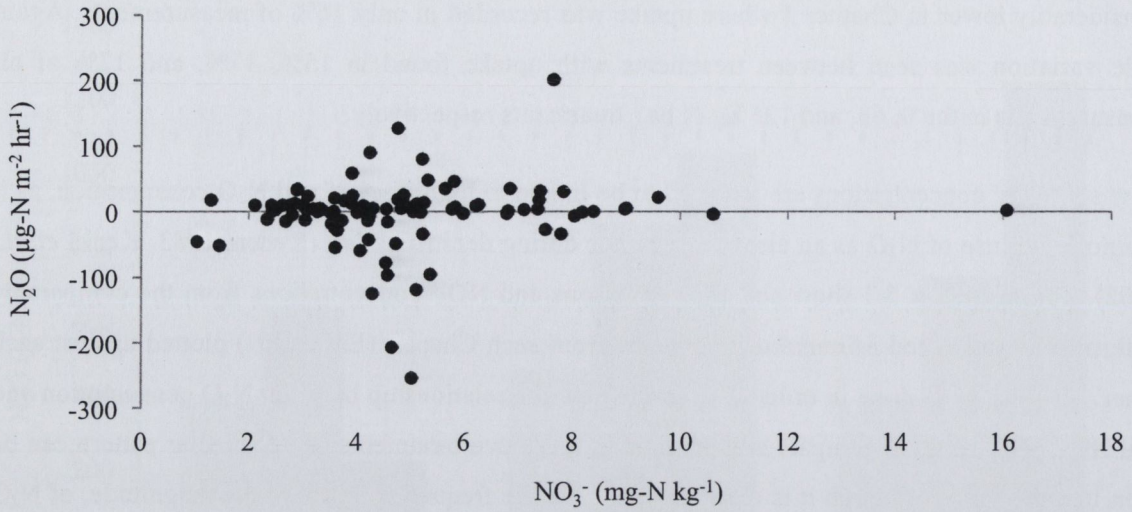


Figure 5.2 The relationship between soil N_2O emissions (g-N ha^{-1} yr^{-1}) and soil NO_3^- concentrations from the long established *Miscanthus* treatment (EM) (Chapter 2).

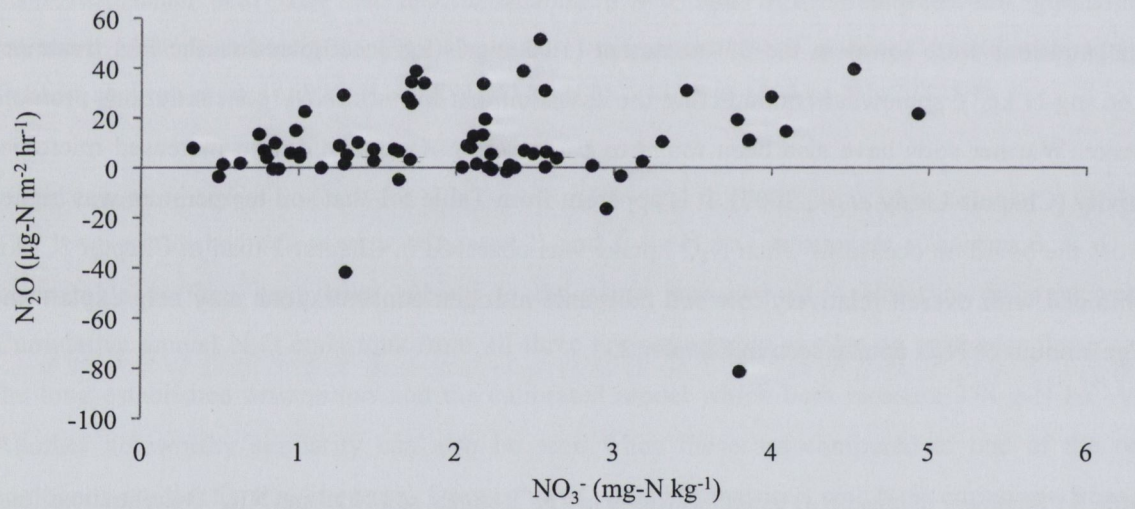


Figure 5.3 The relationship between soil N_2O emissions (g-N ha^{-1} yr^{-1}) and soil NO_3^- concentrations from the unfertilised *Miscanthus* treatment (0N) (Chapter 3).

The economic cost analysis carried out in Chapter 3 found that Nitrogen fertiliser application led to a loss per hectare in the region €15. It is of considerable interest to investigate how this will be affected by future changes in climate. Here a further economic cost analysis has been carried out using DNDC simulated N_2O emissions and above ground biomass yields dictated by a potential future climate scenario. The A1B scenario from the IPCC Special Report on Emission Scenarios (SRES) has been chosen for this purpose. The A1 family of scenarios project a future with a modest rate of population growth, rapid economic growth, and a rapid introduction of new and

efficient technologies. A1B specifically focuses on a future energy supply balanced between fossil fuel based and renewable energy sources (IPCC, 2007a). The calibrated model as described in Chapter 4 has been used to simulate *Miscanthus* biomass yields and N_2O emissions under future high (HS) and low (LS) temperature sensitive conditions as predicted by the SRES A1B scenario. High and low temperature sensitive scenarios are used in order to account for uncertainty in future projections (Collins, 2006). The model has been run for a 30 year period using predictions of temperature and precipitation from 2061-2090 and a baseline period of 1961-1990, similar to the approach seen in Abdalla *et al.* (2009b). The inputs described in Chapter 4 have been used to run the model, with the exception of climate data and initial atmospheric CO_2 concentrations. For the baseline the concentration of 370 ppm has been used, while for the future projections a concentration of 700 ppm – as predicted by the A1B scenario – was utilised (IPCC, 2007a). Analysis has been carried out using mean values calculated over the 30 year period.

Table 5.2 illustrates theses results. Interestingly the LS scenario has led to decreases in mean N_2O emissions from both fertiliser treatments when compared to the baseline, while the HS scenario has led to an increase in all cases. Both future scenarios have resulted in considerable increases in yield from all treatments, with slightly greater increases in the LS scenario. The LS scenario is characterised by a lesser increase in mean annual temperature than the HS scenario when compared to the baseline. The large increase in N_2O caused by the HS scenario is unsurprising considering temperature was found to have the largest impact on simulated N_2O emissions during a sensitivity analysis carried out in Chapter 4 (Table 4.5). That simulated above ground biomass yield has been found to be greater in the LS scenario suggests that peak conditions for growth are met here, beyond which increasing temperatures begin to have a negative effect.

Table 5.2 Summary of mean soil N_2O emissions (kg-N ha^{-1} yr^{-1}) and above ground biomass yield (t ha^{-1} yr^{-1}) simulated by the calibrated version of DNDC using SRES A1B climate scenarios for the period 2061 - 2090.

Source	Treatment	N_2O	Yield
Baseline	0N	0.31	13.85
	63N	0.76	16.71
	125N	1.53	17.62
Low Temperature Sensitivity	0N	0.47	23.10
	63N	0.72	27.27
	125N	1.10	29.28
High Temperature Sensitivity	0N	1.19	22.48
	63N	1.64	26.11
	125N	2.15	28.65

Table 5.3 Mean economic costs of fertiliser application (€ ha^{-1}) calculated using field observations and data simulated by DNDC based on SRES A1B climate scenarios for the period 2061 – 2090. Negative values indicate profit.

Source	Treatment	Cost (Ex. N_2O)	Cost (Inc. N_2O)
Field Observed	63N	15.68	17.73
	125N	12.97	16.01
Baseline	63N	-64.04	-61.71
	125N	-45.76	-39.45
Low Temperature Sensitivity	63N	-147.20	-145.91
	125N	-197.59	-194.33
High Temperature Sensitivity	63N	-112.55	-110.27
	125N	-196.96	-191.99

The simulated N_2O emissions and yields presented in Table 5.2 have been then used to repeat the economic cost analysis both excluding and including the cost N_2O as CO_2 equivalents. These data are presented in Table 5.3 and compared with those based on field observations as calculated in Chapter 3. While Chapter 3 found fertiliser application to *Miscanthus* to lead to a loss at the farm gate, here each of the simulated scenarios predict considerable profits. This may reflect poorly on the performance of DNDC when predicting *Miscanthus* yields. Nonetheless comparing the baseline to the LS and HS scenarios still provides an appreciable insight into the relative impacts of climate change. Both LS and HS scenarios lead to a significant increase in profits compared to the baseline under all conditions. Similar maximum increases to profits are seen in both future scenarios – over 300% in the 125N treatments. This is due to the considerable increase in above ground biomass yield presented in Table 5.2. As seen in Chapter 3 N_2O emissions have had little effect on overall profits, but did have a greater impact in the HS scenario due to higher emissions. Overall these results suggests that under the conditions outlined in the SRES A1B climate scenario the profits achieved by fertiliser application to *Miscanthus* may increase considerably in the future. Interestingly in the LS scenario this is also accompanied by a slight decrease in N_2O emissions. It must be noted that these profits will also be heavily influenced by future prices of nitrogen fertiliser, *Miscanthus,* and carbon, which have not been considered here. Additionally this analysis is subject to a number of limitations encountered in Chapter 4, in particular the need for further validation. This is further discussed later.

In a broader context the work presented in this thesis presents a valuable opportunity for further integrated research. The experimental site used throughout this thesis has been host to a number of *Miscanthus* associated carbon and nitrogen budget related studies. These include examinations of

soil profile CO_2 fluxes (Albanito *et al.*, 2009), CO_2 fluxes by eddy-covariance (Carroll, unpublished PhD thesis), soil carbon sequestration (Dondini *et al.*, 2009), and soil profile dissolved carbon and nitrogen concentrations (Smyth, unpublished PhD thesis). It is recommended therefore that these data be used to compile a complete carbon and nitrogen budget for this *Miscanthus* site, and hence take an important step towards a greater understanding of the impacts of land-use change to *Miscanthus*. In this context, this study indicates that N_2O emissions will not have a negative effect and may contribute positively. N_2O emissions are unlikely to differ significantly, however lower fertiliser requirements than many conventional arable crops do provide an opportunity to reduce emissions. Therefore the extent of these reductions are likely to depend heavily on former and future management practices, with savings of 40% to >99% suggested by Don *et al.* (2011). Proportionally the contribution of N_2O to a complete carbon budget will be relatively low, but not unimportant. As a crude example the previously mentioned study by Dondini *et al.* (2009) found the established *Miscanthus* site to sequester *Miscanthus* derived carbon at a rate of 3.2 Mg C ha^{-1} yr^{-1}, comparable N_2O emissions from this site as measured in treatments EM and 0N were 0.05 and 0.06 Mg C ha^{-1} yr^{-1} respectively when presented as carbon equivalents. Studies compiling complete carbon and nitrogen budgets at agricultural sites are limited. One such study investigating intensively and extensively managed grasslands has found N_2O and NO fluxes combined to contribute to less than 5% of the total budget. However the same study also notes that although small, N_2O emissions in this context still hold importance due to their role in atmospheric chemistry and strong radiative forcing (Ammann *et al.*, 2009).

This importance is amplified when considering that large-scale future land-use change from conventional agricultural food crops to bio-energy crops is probable, particularly in Europe. As discussed in Chapter 1 this is motivated in large by government biofuel targets (Don *et al.*, 2011), and changes in food demand and crop production efficiency (Rounsevell *et al.*, 2006). Given that reducing fossil fuel derived greenhouse gas emissions is one of the most important drivers behind the establishment of biofuel production targets, it is vital to understand not only the full implications of the use of *Miscanthus* as a bio-energy crop on N_2O emissions, but also how land-use change from conventional crops will affect this balance. This study provides a foundation for future work of this kind.

Various limitations to the work presented in this thesis have been highlighted in previous chapters. In Chapter 2 it was necessary to use a split-plot experimental design. A randomised block experimental layout was not possible due to the need to use long established *Miscanthus* and grassland plots. A comparable study has been carried out using the same design at the site in question (Dondini *et al.*, 2009), justifying its use here. Also in Chapter 2 there was an issue with poor establishment of the new *Miscanthus* as illustrated in Figure 2.13. This was caused by cold damage during the winter. However this is a common issue faced during land-use change to

Miscanthus (Clifton-Brown & Lewandowski, 2000) and as such should not be discounted when examining its impacts. Several complications were also encountered when parameterising DNDC for use with *Miscanthus* in Chapter 4. These were due to DNDC's inability to account for the importance of rhizomes in *Miscanthus*' nutrient use and related soil nutrient cycling, as well as the reliance of its crop growth component on seed yield – which was inapplicable here as *Miscanthus* is a sterile cultivar. The approaches taken to overcome these issues are documented in Chapter 4.

There are also some limitations that are applicable across chapters. Foremost of these has been the length of the individual field trials. Twelve months periods were chosen for each due to time and labour constraints. Ideally, in order to draw robust conclusions, agricultural experiments would run for a number of years so as to account for variation and anomalies in influencing factors such as climate and atmospheric deposition. Large inter-annual variations in N_2O emissions driven by climatic variation have been found on numerous occasions. In a review of agricultural N_2O emissions Skiba & Smith (2000) found emission factors ranging from 0.1 to 7 %, citing inter-annual variations in rainfall timing and intensity as one of the key driving factors. Meanwhile Du *et al.* (2006) have found the distribution of rainfall intensity to be the key influence on inter-annual N_2O emissions measured over eight years from a Mongolian grassland. Nonetheless it is common for many field trials monitoring agricultural N_2O emissions to occur over a one-year period. Jungkunst *et al.* (2006) conducted a review of N_2O emissions from German agricultural soil utilising all available data from studies at least one year in length. Of the 27 studies included, 10 consisted of just one year of measurements. Further issue with the length of the field trials were found in Chapter 4. As it was necessary to calibrate DNDC based on the one year of available data from the fertiliser experiment, further data would be desirable in order to provide a more robust validation. However, in addition to factors discussed earlier in this chapter the lack of published research currently available detailing N_2O emissions from *Miscanthus* justifies the work presented here and underlines its value.

N_2O emissions simulated by DNDC in Chapter 4 highlight a potential issue in the previous chapters; that of sampling techniques. A number of peaks in N_2O emissions were simulated by both the uncalibrated and calibrated models that were not observed during field measurements. It can be seen in Figures 4.1 & 4.4 that many of these occur on days when sampling did not occur, suggesting that some peaks may have been potentially overlooked. Throughout the trials discussed in Chapters 2 & 3 N_2O emissions were sampled either weekly, bi-monthly, or several times a week in the period directly after fertiliser application. Closed chambers were used for a deployment time of 40 (Chapter 2) or 60 minutes (Chapter 3). It is common practice when using this technique to sample at frequencies such as those used here, however this creates opportunities for rapid but potentially important peaks in N_2O emissions occurring on non-measurement days to be missed (Yao *et al.*, 2009). It is also possible to monitor soil N_2O fluxes using automated closed chambers

or micrometeorological techniques, which can account for greater temporal variability. However the use of manual closed chambers is generally accepted and widespread, with the majority of field based N_2O measurements are made using this approach (Bouwman *et al.*, 2002). Christensen *et al.* (1996) carried out a study comparing manual closed chambers, automated closed chambers, and micrometeorological techniques finding little significant difference.

Based on the work presented in this thesis land-use change from grassland to *Miscanthus* is unlikely to have a negative effect on medium to long term N_2O emissions. There may be a short-term increase due to a slight increase in emissions in the first few years as well as emissions related to tilling previously undisturbed land prior to establishment. However any negative effects this may have on greenhouse budgets are likely to be counteracted by savings made by the replacement of fossil fuels. Estimates of greenhouse savings caused by the replacement of coal with *Miscanthus* during energy production are as high as 90% (Lewandowski *et al.*, 1995). Additionally lower fertiliser requirements compared to most common agricultural crops imply that land-use change to *Miscanthus* will have a positive effect on N_2O emissions in many scenarios. This is supported by the findings here that nitrogen fertiliser application did not significantly affect yield but did have a significant impact on N_2O emissions. This is further reinforced by the economic cost analysis that showed inappropriate nitrogen fertiliser application to *Miscanthus* could lead to financial losses at the farm gate. This highlights the importance of proper management, only adding fertiliser when it is required to maintain good soil quality. Nevertheless evidence has been presented that under certain potential climate change scenarios nitrogen fertiliser application could lead to considerable profits at the farm gate, due largely to climate induced increases in yield. This has been done using the model DNDC which has been shown to have potential as a useful tool for predicting soil N_2O emissions from *Miscanthus*. However these conclusions must be taken with the caveat that they may be unique to the conditions encountered here, as only one year of data were used in each case. Therefore a continuation of this work, ideally carried out over a range of climate and soil types, is recommended. Further monitoring of N_2O emissions for several years after the initial establishment of *Miscanthus* would be beneficial. This would help to ascertain how long it may take emissions from newly planted *Miscanthus* to subside to levels seen in long established *Miscanthus*. Additionally work studying both CO_2 and CH_4 fluxes from *Miscanthus* is recommended in order to achieve a complete greenhouse gas budget. It is also recommended that the data presented in this thesis be used in conjunction with other carbon and nitrogen related studies carried out at the same site in order to compile a more detailed carbon and nitrogen budget. These works would offer an important component of a more thorough life-cycle analysis of the complete environmental and economic farm-level costs of the establishment and production of *Miscanthus* as a biofuel.

Annex 2.1 Summary of significant dates from a Bonferroni post-test on WFPS data.

Plot	Date	Difference	t	P	P Summary
G v NM	16/12/08	7.772	3.386	< 0.05	*
G v NM	11/03/09	10.08	4.393	< 0.001	***
G v NM	24/06/09	8.088	3.523	< 0.05	*
G v EM	25/03/09	9.778	4.259	< 0.001	***
G v EM	01/04/09	8.217	3.579	< 0.05	*
G v EM	22/04/09	11.27	4.908	< 0.001	***
G v EM	06/05/09	8.488	3.698	< 0.05	*
G v EM	14/05/09	12.15	5.293	< 0.001	***
G v EM	20/05/09	9.710	4.230	< 0.01	**
G v EM	27/05/09	8.119	3.537	< 0.05	*
G v EM	02/06/09	19.12	8.329	< 0.001	***
G v EM	10/06/09	16.76	7.301	< 0.001	***
G v EM	18/06/09	14.95	6.514	< 0.001	***
G v EM	24/06/09	21.46	9.350	< 0.001	***
G v EM	09/07/09	13.68	5.960	< 0.001	***
G v EM	22/07/09	16.01	6.974	< 0.001	***
G v EM	30/07/09	8.823	3.844	< 0.01	**
G v EM	06/08/09	9.684	4.219	< 0.01	**
G v EM	14/08/09	10.31	4.489	< 0.001	***
G v EM	25/08/09	9.023	3.930	< 0.01	**
G v EM	22/09/09	13.11	5.711	< 0.001	***
G v EM	29/10/09	9.102	3.965	< 0.01	**
NM v EM	04/03/09	7.567	3.296	< 0.05	*
NM v EM	18/03/09	8.079	3.519	< 0.05	*
NM v EM	25/03/09	14.37	6.262	< 0.001	***
NM v EM	01/04/09	10.42	4.541	< 0.001	***
NM v EM	16/04/09	11.01	4.796	< 0.001	***
NM v EM	22/04/09	12.26	5.342	< 0.001	***
NM v EM	29/04/09	8.688	3.785	< 0.01	**
NM v EM	06/05/09	14.01	6.103	< 0.001	***
NM v EM	14/05/09	15.17	6.608	< 0.001	***

NM v EM	20/05/09	15.36	6.689	< 0.001	***
NM v EM	27/05/09	12.25	5.335	< 0.001	***
NM v EM	02/06/09	16.27	7.086	< 0.001	***
NM v EM	10/06/09	12.87	5.607	< 0.001	***
NM v EM	18/06/09	15.60	6.797	< 0.001	***
NM v EM	24/06/09	13.38	5.827	< 0.001	***
NM v EM	09/07/09	8.766	3.819	< 0.01	**
NM v EM	22/07/09	14.41	6.277	< 0.001	***
NM v EM	30/07/09	9.093	3.961	< 0.01	**
NM v EM	06/08/09	8.238	3.589	< 0.05	*
NM v EM	14/08/09	8.201	3.573	< 0.05	*
NM v EM	10/09/09	8.360	3.642	< 0.05	*
NM v EM	22/09/09	9.469	4.125	< 0.01	**

Annex 2.2 Summary of significant dates from a Bonferroni post-test on soil temperature data.

Plot	Date	Difference	t	P	P Summary
G v NM	04/02/09	5.563	17.49	< 0.001	***
G v NM	12/02/09	-1.050	3.301	< 0.05	*
G v NM	18/03/09	2.375	7.466	< 0.001	***
G v NM	01/04/09	1.075	3.379	< 0.05	*
G v NM	08/04/09	1.912	6.012	< 0.001	***
G v NM	16/04/09	1.175	3.694	< 0.05	*
G v NM	22/04/09	1.938	6.091	< 0.001	***
G v NM	29/04/09	2.113	6.641	< 0.001	***
G v NM	06/05/09	1.163	3.654	< 0.05	*
G v NM	14/05/09	1.237	3.890	< 0.01	**
G v NM	20/05/09	2.063	6.484	< 0.001	***
G v NM	27/05/09	1.275	4.008	< 0.01	**
G v NM	02/06/09	5.300	16.66	< 0.001	***
G v NM	10/06/09	2.063	6.484	< 0.001	***
G v NM	24/06/09	7.633	24.09	< 0.001	***
G v NM	09/07/09	2.188	6.877	< 0.001	***
G v NM	16/07/09	1.563	4.912	< 0.001	***

G v NM	22/07/09	1.650	5.187	< 0.001	***
G v NM	30/07/09	2.350	7.387	< 0.001	***
G v NM	06/08/09	1.988	6.248	< 0.001	***
G v NM	14/08/09	1.200	3.772	< 0.01	**
G v NM	25/08/09	1.087	3.419	< 0.05	*
G v EM	04/02/09	7.863	24.72	< 0.001	***
G v EM	12/02/09	-1.075	3.379	< 0.05	*
G v EM	25/02/09	-1.200	3.772	< 0.01	**
G v EM	18/03/09	-1.087	3.419	< 0.05	*
G v EM	20/05/09	1.675	5.265	< 0.001	***
G v EM	16/07/09	-1.213	3.812	< 0.01	**
G v EM	22/07/09	-2.225	6.994	< 0.001	***
G v EM	30/07/09	-1.900	5.973	< 0.001	***
G v EM	06/08/09	-2.275	7.152	< 0.001	***
G v EM	14/08/09	-1.188	3.733	< 0.01	**
G v EM	25/08/09	-2.150	6.759	< 0.001	***
G v EM	02/09/09	-1.937	6.091	< 0.001	***
G v EM	16/09/09	-2.037	6.405	< 0.001	***
G v EM	22/09/09	-1.188	3.733	< 0.01	**
G v EM	08/10/09	-1.775	5.580	< 0.001	***
NM v EM	13/01/09	-1.125	3.536	< 0.05	*
NM v EM	04/02/09	2.300	7.230	< 0.001	***
NM v EM	25/02/09	-2.162	6.798	< 0.001	***
NM v EM	11/03/09	-1.825	5.737	< 0.001	***
NM v EM	18/03/09	-3.463	10.88	< 0.001	***
NM v EM	25/03/09	-1.413	4.440	< 0.001	***
NM v EM	01/04/09	-1.463	4.597	< 0.001	***
NM v EM	08/04/09	-2.350	7.387	< 0.001	***
NM v EM	16/04/09	-1.138	3.576	< 0.05	*
NM v EM	22/04/09	-1.300	4.087	< 0.01	**
NM v EM	29/04/09	-1.388	4.362	< 0.001	***
NM v EM	14/05/09	-1.325	4.165	< 0.01	**
NM v EM	02/06/09	-5.450	17.13	< 0.001	***
NM v EM	10/06/09	-2.200	6.916	< 0.001	***
NM v EM	24/06/09	-7.550	23.73	<0.001	***
NM v EM	09/07/09	-2.125	6.680	< 0.001	***

NM v EM	16/07/09	-2.775	8.723	< 0.001	***
NM v EM	22/07/09	-3.875	12.18	< 0.001	***
NM v EM	30/07/09	-4.250	13.36	< 0.001	***
NM v EM	06/08/09	-4.263	13.40	< 0.001	***
NM v EM	14/08/09	-2.387	7.505	< 0.001	***
NM v EM	25/08/09	-3.237	10.18	< 0.001	***
NM v EM	02/09/09	-2.812	8.841	< 0.001	***
NM v EM	16/09/09	-1.962	6.169	< 0.001	***
NM v EM	22/09/09	-1.363	4.283	< 0.001	***
NM v EM	08/10/09	-1.175	3.694	< 0.05	*

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