Geoderma 181–182 (2012) 36–44

Contents lists available at SciVerse ScienceDirect

Geoderma

journal homepage: www.elsevier.com/locate/geoderma

What are the impacts of grazing and cutting events on the N_2O dynamics in humid temperate grassland?

Rashad Rafique [a,b,*], Robert Anex [a], Deirdre Hennessy [c], Gerard Kiely [b]

[a] *Department of Biological System Engineering, University of Wisconsin-Madison, USA*
[b] *Centre for Hydrology, Micrometeorology and Climate Change, Department of Civil and Environmental Engineering, University College Cork, Ireland*
[c] *Departments of Animals &, Grassland Science Research, Teagasc-Moorpark, Fermoy, Ireland*

ARTICLE INFO

Article history:
Received 29 June 2011
Received in revised form 21 February 2012
Accepted 8 March 2012
Available online xxxx

Keywords:
Nitrous oxide
Grassland
NH_4–N
NO_3–N
Grass cutting
Animal grazing

ABSTRACT

Atmospheric concentrations of nitrous oxide (N_2O) have been increasing over the last century with much of this increase from agricultural soils, fertilized with nitrogen. To understand the N_2O emissions from terrestrial ecosystems (e.g. grassland soils) it is necessary to understand the processes leading to N_2O production. From February to August in 2010, we conducted a field study to measure the N_2O fluxes using the chamber technique, at a grazed and cut grassland site in South West Ireland. The objectives of the study were: 1) to understand the temporal variation of the N_2O fluxes during the spring and summer periods; 2) to examine the separate effects of grazing and cutting events on N_2O fluxes; and 3) to examine the relationship of soil ammonium (NH_4+-N) and soil nitrate (NO_3–N) with N_2O fluxes. We found the highest peak of daily N_2O flux occurred at the start of spring; however the total of summer fluxes (June, July and August) of 1.81 ± 0.7 kg N_2O–N ha^{-1} were higher than those of the spring (March, April and May) fluxes of 1.51 ± 0.6 kg N_2O–N ha^{-1}. The soil NH_4+-N concentration was higher than the soil NO_3–N concentration over the study period and elevated N_2O fluxes coincided with elevated soil NH_4–N concentrations. There were two short (2 day duration) cattle grazing events; one on April 26/27 and the second on June 27/28. There were two grass cutting (for silage) events: on May 30 and on August 4. After the two grazing and two cutting events, the N_2O fluxes increased markedly. After both grazing events, there was an immediate step increase of ~200 μg N_2O–Nm^{-2} h^{-1}, after which the fluxes decreased over the next few weeks. After both cutting events, there was a gradual increase in N_2O fluxes over the next several weeks. We found that the N_2O flux increases post grazing, were due to grazing only, since the other variables (soil temperature, WFPS, N application) did not change. However, the flux increases post cutting could not ascribed to cutting only, as other flux favouring variables of: changes in soil temperature and WFPS also occurred at this time. The N_2O fluxes correlated better with soil NH_4–N concentration ($r^2 = 0.73$ ($p = < 0.05$)) than with NO_3–N ($r^2 = 0.25$ (p = not significant)). The occurrence of elevated NH_4–N in conjunction with elevated WFPS, frequently in the range of 50–60% suggests that nitrification rather than denitrification was likely the dominant process involved in the production of N_2O at this site.

1. Introduction

Nitrous oxide (N_2O) is an important and highly effective green house gas (GHG) which also plays an important role in the destruction of the ozone layer (Sowers and Rice, 2001). N_2O is a key component of the global nitrogen (N) cycle and is estimated to be increasing at a rate of 0.25% per year (IPCC, 2001). Estimates of global N_2O emissions are uncertain and the global budget remains unconstrained. Soils are a dominant source of N_2O emissions, (especially nitrogen fertilized agricultural soils) and uncertainty remains in estimating the emissions. This is due to the episodic nature of N_2O fluxes over time and space (Mishurov and Kiely, 2010). In agricultural ecosystems this spatial and temporal variability can result from small scale differences in soil ammonium and soil nitrate, and soil organic carbon (SOC) (Hu et al., 2011; Rover et al., 1999; Yanai et al., 2003). The heterogeneity and differences in soil properties that control N_2O emissions along with environmental variables (soil temperature and water filled pore space, WFPS) make it a challenge to reliably estimate N_2O fluxes.

In soils, N_2O is primarily produced by two processes: denitrification and nitrification. These processes, as well the physical transport of N_2O through soil diffusion, are regulated by a number of environmental and edaphic factors. At regional scale, these factors include soil type and climate (Cantarel et al., 2011; Matson, 1997), while at a local scale, soil moisture and temperature, soil organic matter, and agricultural management practices contribute to variability in N_2O

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* Corresponding author at: Department of Biological System Engineering, University of Wisconsin-Madison, 53706, USA. Tel.: +1 6087704236.
E-mail address: rashidbao@gmail.com (R. Rafique).

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doi:10.1016/j.geoderma.2012.03.006

emissions (Bouwman, 1990; Robertson, 1989; Rover et al., 1999; Yanai et al., 2003). Grass cutting (also known as silage making or harvesting) has been reported as both a source of N_2O fluxes (Neftel et al., 2000) as well as a sink of N_2O fluxes (Chen et al., 1999; Glatzel and Stahr, 2001). While livestock grazing has been reported to cause increased N_2O emissions (Rafique et al., 2011; Saggar et al., 2007), there is limited information on the immediate effect of grazing events at different times of the year.

Until the late 1970's, denitrification was believed to be the principle source of microbially produced N_2O fluxes, but laboratory and field studies since then have demonstrated that N_2O can also be a product of nitrification (Carter, 2007). The key factors influencing the rate of nitrification are the concentration of NH_4+-N and of free oxygen (O_2), by being substrates for the process (Firestone and Davidson, 1989). Nitrate produced in nitrification can be subject to: assimilation by plants or microorganisms, leaching, dissimilatory reduction to NH_4+-N, or reduction to NO, N_2O or N_2 via denitrification (Tiedje, 1988). The requirements for denitrification are: the presence of bacteria having a metabolic pathway; the availability of suitable reductants such as carbon; a low level of oxygen; and the supply of NO_3–N or other nitrogen oxides (Firestone and Davidson, 1989). The presence of O_2 is often the limiting factor for denitrification, followed by NO_3–N availability.

Differentiating nitrification and denitrification sources, and understanding how much N_2O emissions are influenced by changes in management practices, is important for the accurate understanding, estimation and prediction of N_2O fluxes from soils (Matson, 1997). After fertilizer application in the field, it is generally believed that the observed N_2O flux is derived from microbial processes in the top soil layer where most of the applied mineral N remains (Granli and Bøckman, 1994). However, to fully understand the dynamics of N_2O emissions from soils to the atmosphere, it is necessary to identify the mechanisms and factors responsible for the N_2O production and consumption within the soil profile. It is assumed that frequent measurements of N_2O fluxes will largely improve the estimate of N_2O emissions in the grass growing season at Irish grasslands compared to the infrequent N_2O measurements of prior studies (e.g. Abdalla et al., 2009; Hsieh et al., 2005; Hyde et al., 2006; Rafique et al., 2011). It is further considered that the silage cutting and immediate effect of grazing events might enhance the N_2O emissions and this has not been efficiently studied in Irish grassland ecosystems. Furthermore, it is hypothesized that in Irish grazed grasslands the dominant N_2O production process in the spring and summer season is likely to be nitrification. Specifically, the objectives of this study were: 1) to examine the temporal variation of N_2O over the period, February to August using relatively frequent chamber measurements; 2) to evaluate the effect of grass cutting and cattle grazing events on N_2O fluxes; and 3) to evaluate the relationships between N_2O fluxes with soil concentrations of NH_4+-N and NO_3–N.

2. Materials and methods

2.1. Site description

In February 2010, a site was selected in the South of Ireland for N_2O fluxes and inorganic N (NH_4-N and NO_3-N) measurements. The experimental site is a grassland pasture located at the Animal and Grassland Research and Innovation Centre, Moorepark (Teagasc) in Fermoy, Co. Cork, Ireland (52°07 N, 08°16 W). The site has an elevation of 52 m a.s.l; a mean annual rainfall 1040 mm; and a mean annual air temperature of ~9 °C. The soil is freely draining, derived from mixed sandstone-limestone glacial till. The soil texture is sandy loam and the soil depth varies from 0 to 4.5 m with bedrock commonly occurring at 2.0 to 3.0 m below the ground surface. The bulk density of the soil was 0.89 g cm^{-3} and the pH value was 6.0. The C:N ratio was 8.61. The pasture has been in permanent grassland for at least the last 35 years with perennial ryegrass (Lolium perenne) swards. This site is an active pasture, regularly cattle grazed and fertilized with N fertilizers. Grass (silage) cutting took place twice during the experimental period; firstly, on 30 May 2010 and secondly, on 4 August 2010. A cattle grazing was carried out on two short periods; a 2 day period from April 26/27 and a 2 day period June 27/28. The two week N_2O fluxes (two measurements per week) were measured before and after each cutting and grazing event. This time interval was chosen because the N_2O flux peaks (if to occur) are known to takes about two weeks return to background levels. The stocking rate was 2.9 cattle per hectare (livestock unit ha^{-1}(LSU)). Nitrogen in the form of urea was applied at a rate of 122 kg N ha^{-1} in three applications of 50, 37.5 and 34.5 kg N ha^{-1} on the 14th March, 23 rd April and 24th August 2010, respectively. Similarly, N in the form calcium ammonium nitrate (CAN) was also applied at a rate of 75 kg N ha^{-1} in three different applications of 37.5, 12.5 and 25 kg N ha^{-1} on 7 May, 11 June and 7 July 2010 respectively. There was no organic animal slurry application over the experimental period. The annual total N was therefore 197 kg N ha^{-1}. The management data (number of grazing animals, application of fertilizer, silage cutting and grazing events) was collected regularly from the farm manager.

2.2. Soil sampling and laboratory analysis

Soil samples for the measurement of soil NO_3–N and NH_4+-N were collected on a regular basis (13 measurements total) throughout the experimental exercise. Soil samples were taken from the top 5 cm, using rings (5 cm × 8 cm, Eijkenkamp Agrisearch Equipment BV, The Netherlands) at 5 points within the site: at the corners and at the centre of the square plot (size 10 × 10 m).

For laboratory analysis of NH_4-N and NO_3-N concentrations, 30 g of the field moist soil was extracted with 2 M KCL (Merck KGaA, Frankfurter StraBe 250, 64293 Darmdtadt, Germany) solution by shaking for 2 h using the methodology described by Zaman et al. (1999). The extractant was filtered and frozen until analysis to determine NH_4-N and NO_3-N concentrations. The NH_4+-N and NO_3–N concentrations of the extractant were measured using an Aquakem 600 Discrete Analyser (Thermo Scientific, Ratastie 2, P.O. Box 100, FI-01621 Vantaa, Finland). Other Soil parameters including bulk density (BD), and SOC were calculated using the methodology described in Rafique et al. (2011). Soil pH was measured by the method in Kalra (1995).

2.3. Environmental measurements

A meteorological station was set up at the study site to measure the time series of rainfall, soil temperature and soil moisture. A soil temperature probe (Campbell Scientific, UK) and soil moisture time domain reflectometry (TDR) probe (Campbell Scientific, UK) were installed at a depth of 5 cm. The soil temperature and soil moistures were also recorded on each sampling occasion using a hand held digital thermometer (Hanna, THV-240-020W, UK) and soil moisture meter (Delta-T Devices, HH2, UK). A tipping bucket rain gauge (Young Transverse MI 52203, USA) was used to measure rainfall with a resolution of 0.1 mm. Soil temperature, soil moisture and rainfall were logged at half hour intervals on a CR 200 data logger (Campbell Scientific, UK). The water filled pore space (WFPS) was determined as the ratio of volumetric water content and soil porosity. Soil porosity was estimated as [1-(bulk density/particle density)] × 100, using a particle density of 2.65 g cm^{-3} (Barton et al., 2008; Rafique et al., 2011).

2.4. Nitrous oxide flux measurement techniques

Nitrous oxide emissions were measured using the closed chamber technique (Skiba et al., 1998). The chambers were made of a cylinder of Polyvinyl Chloride (PVC) with a volume of 0.028 m^3

(height = 45 cm; diameter = 28.2 cm). The chamber had a vent tube (length = 10 cm) and thermocouple (ELE International, UK) for internal air temperature. Additionally, each chamber had an aluminium ring which was used to insert on the collar during sampling. The site sampling points (eight chamber points) were fixed and located along two grid lines, 4 m apart. In order to maintain experimental consistency, the same sampling points were used throughout the sampling period. Four gas samples, each of 12 ml volume were taken at 20 min intervals over a 1 h period. At each interval the chamber inner temperature was also recorded. The measurements were carried out twice a week from mid February to August 2010.

The rate of increase of the N_2O concentration in the headspace of the chamber gives a direct estimation of the N_2O flux between the soil and the atmosphere (Flechard et al., 2007). The N_2O concentration in each sample was analysed using a gas chromatograph (GC 3800, Varian, USA) fitted with a packed column (Porapak QS 80–100 MESH, Sigma Aldrich, USA) using an electron capture detector at 300 °C. Gases of known N_2O concentrations were used as reference points for the chromatography system. This system was attached to a Combi Pal automatic sampler (CTC analytics, Switzerland) which extracted a sample of 750 μl from the sampling vial and injected it into the GC. The analysis time of each sample was approximately 9 min. The areas under the N_2O curve peaks were integrated using a Star Chromatography Work Station Version 6.2 (Varian, USA) to estimate the N_2O concentration (Hyde, 2004).

Hourly N_2O emissions (μg N_2O–N $m^{-2} h^{-1}$) were calculated from the slope of the linear increase in N_2O concentration during the chamber lid closure period (Holland et al., 1999). The daily N_2O flux at each site was estimated using the arithmetic mean of the fluxes from the individual chambers (Barton et al., 2008; Dobbie and Smith, 2003). The daily N_2O emission values as g N_2O-N $ha^{-1} d^{-1}$ were estimated from the concentration measured in the chambers over a measurement period of 1 hour converted to those over a period of 24 h. Annual emission rates were estimated by integrating hourly rates using linear interpolation (Flechard et al., 2007).

2.5. Grass cutting and grazing events

To examine the N_2O fluxes under grass cutting events, the N_2O fluxes were measured for two weeks before and for two weeks after the event and were then compared and analysed statistically. The same methodology and time frame was used to study the effect of grazing on N_2O fluxes. During the study period, the field site was cut twice and grazed twice. The climatic parameters i.e. soil temperature and WFPS on the specific days of N_2O measurements were used to check the effect of these parameters on the N_2O fluxes.

2.6. Statistical analysis

The Pearson correlation and *t*-test was used to find the correlation and significant differences in N_2O fluxes for both cutting and grazing events. Similarly the Pearson correlation coefficients and regression analysis were used to examine the relationship of N_2O fluxes with soil NH_4-N and NO_3-N concentrations. Differences and the correlations were considered significant at the $P<0.05$ level. Calculations, statistical analysis and graphical outputs were determined using MATLAB (Math works USA, 7.6.0, R2008a).

3. Results

3.1. Climatic characteristics

The rainfall, soil moisture (WFPS) and soil temperature during the observation period (Figure 1) were typical of late winter, spring and summer conditions in this region. The maximum daily rainfall was 24 mm. The spring period with 212 mm rainfall was drier than the summer with 262 mm rainfall. We define spring as March, April and May (MAM) and summer as June, July and August (JJA). The WFPS was directly related to the pattern of rainfall events. In the 200 day observation period, 56% of the days had a WFPS < 60%; 33% of days were 60–80% WFPS and only 11% of days were > 80% WFPS. The selection of the arbitrary 60% WFPS is based on previous work where we found that the range of 60–80% WFPS resulted in the highest N_2O fluxes (Rafique et al., 2011). The daily soil temperature ranged from 4 to 22 °C. More than 66% of the days had a soil temperature above 10 °C. As grass requires a minimum temperature > 6 °C for growth, this indicates that there was grass growth for most of the study period.

3.2. N_2O fluxes variability

The hourly N_2O fluxes from late winter to the end of summer 2010 are shown in Fig. 1(D). The fluxes were episodic in nature throughout the study period. Elevated N_2O emissions are noted, shortly after N applications and coinciding with elevated WFPS, and high soil temperature. The hourly N_2O fluxes were in the range of -174 ± 46 to 684.11 ± 247.16 μg N_2O-N $m^{-2} h^{-1}$. The range noted on each measurement day is due to variation among the eight different chamber measurement sites. The latter magnitude is about three and half times higher than the 2nd highest peak. The highest peak N_2O emission was observed on 17th March 2010, shortly after an N fertilizer application when the soil temperature was 11 °C and WFPS was 53%. Negative or uptake N_2O fluxes were observed on only two occasions in February (Figure 1(D)). The maximum negative flux was observed on 21st February, 2010 when the soil temperature was 9 °C and WFPS was 92%. The monthly fluxes ranged from -29.03 ± 10.01 to 685.44 ± 31.13 g N_2O-N ha^{-1} (Figure 2). The maximum monthly flux was observed in April followed by July and August. However, the seasonal flux was found to be higher in summer than in spring which was 1.81 ± 1.1 and 1.51 ± 0.9 kg N_2O-N ha^{-1} respectively (see inlet Figure 2). However, the total N_2O-N loss as a factor of applied N was higher in spring (i.e. 2.0%) than in summer (i.e. 1.5%).

3.3. Seasonal dynamics of NH_4-N and NO_3-N concentration in soil

The temporal variations in the soil NH_4+-N and NO_3-N contents are shown in the Fig. 1(E) and (F), respectively. The NH_4+-N concentrations were in the range of 0.59 ± 0.25 to 14.84 ± 1.4 mg N kg^{-1} of dry soil and the NO_3–N concentrations ranged from 0.75 ± 0.66 to 16.80 ± 1.36 mg N kg^{-1} of dry soil. The peak value of NH_4+-N was observed on the 30th April (JD 120) just after an N application. At the same time the NO_3–N values was found to be low at 1.61 ± 0.09 mg N kg^{-1} of dry soil. The peak NO_3–N value was observed on 30th June (JD 181) coincident with the second highest value of NH_4-N, and also after an N application event.

The NH_4+-N concentrations were observed to fluctuate more than the NO_3–N concentrations. The NO_3-N concentrations remained almost constant up to the beginning of July. The maximum fluctuation in both NH_4+-N and NO_3–N concentrations were observed from May to July during which frequent rainfall events occurred and the soil temperature ranged between 17 and 22 °C. Such environmental conditions are suitable for mineralization. After July, both NH_4-N and NO_3-N concentrations fell to their lowest values of the study period (Figure 1(E) and (F)).

3.4. Effect of grass cutting on N_2O fluxes

Fluxes of N_2O (approximately 2 weeks ±2 days), soil temperature, WFPS and NH_4+-N and NO_3–N concentrations are shown in Fig. 3, for the weeks leading up to and after the two cutting events. Post these two cutting events, the fluxes of N_2O increase gradually

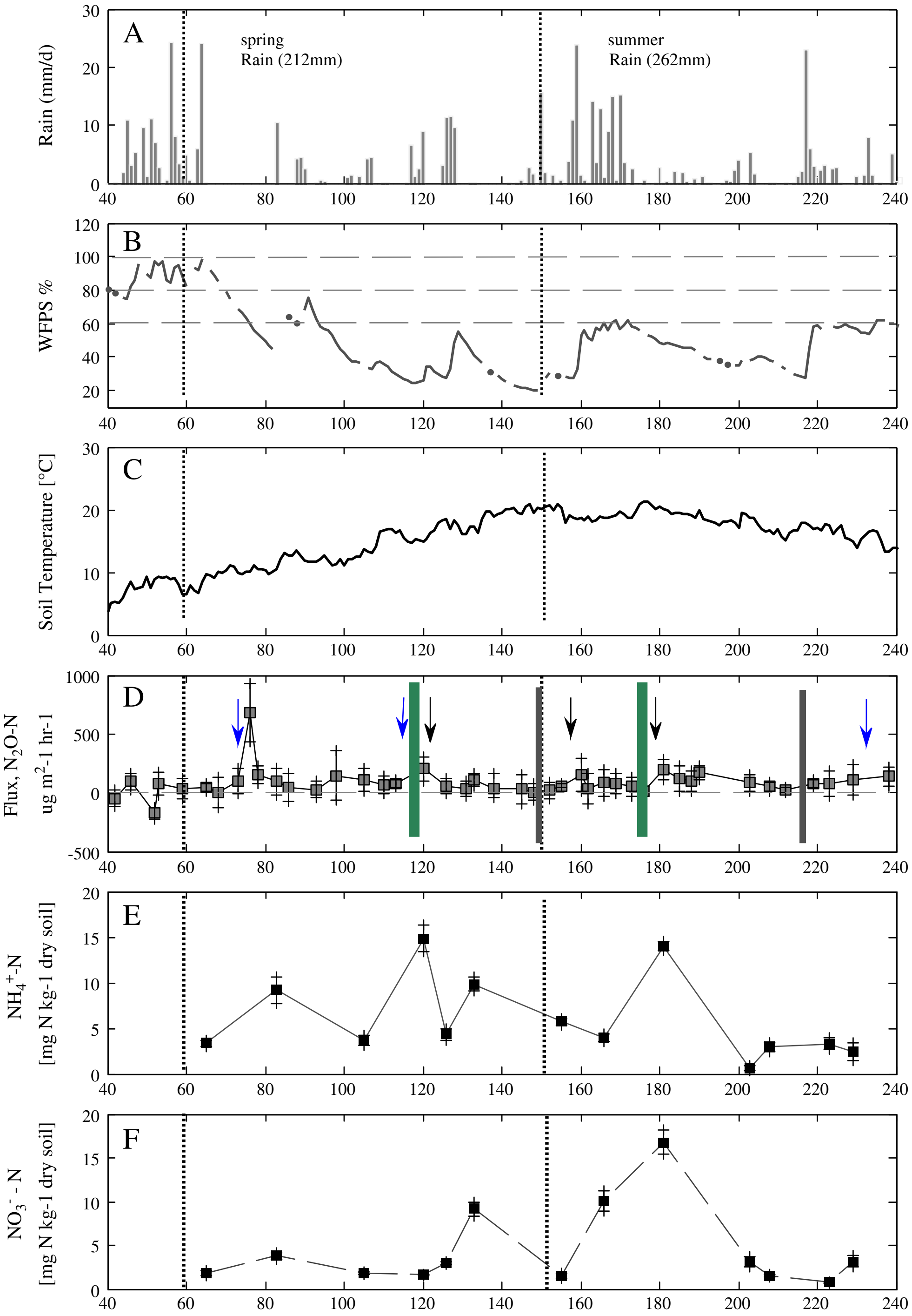

Fig. 1. (A) Daily rain in mm, (B) Daily soil WFPS % at 5 cm soil depth, (C) Daily soil temperature measured at 5 cm, (D) N_2O flux time series (± standard deviation). Arrows (blue for urea and black for calcium ammonium nitrate (CAN)) indicate timing of inorganic fertilizer application. Green lines show timing of the two grazing events while grey shades show the timing of the two cutting events. (E) NH_4+-N soil contents (± standard deviation) temporal variation. (F) NO_3–N soil contents (± standard deviation) temporal variation.

over the following few weeks, as does the WFPS, while soil temperature remains unchanged. There was no N application at this time. While there appears to be no correlation between the increasing N_2O fluxes and temperature (post cutting events), there is some correlation ($P = 0.05$) between the flux increases at this time with WFPS.

The concentration of NH_4+-N and NO_3–N decreased after the first cutting event (Figure 2(D)). After the second cutting event, the NH_4+-N increased slightly, while the NO_3–N concentration decreased.

3.5. Effect of grazing events on N_2O fluxes

The grazing events in the study period (each of 2 day duration) along with the N_2O flux (approximately 2 weeks ± 2 days) before and after grazing is shown in Fig. 5(A). The corresponding soil temperature and WFPS are shown in Fig. 5(B) and (C). Within 2 days after both grazing events, there was a spike in N_2O emissions. For those same two days there was little change in soil temperature and WFPS. However at that time there were N applications (see Figure 1(D). Immediately after the short term spike in N_2O (post the

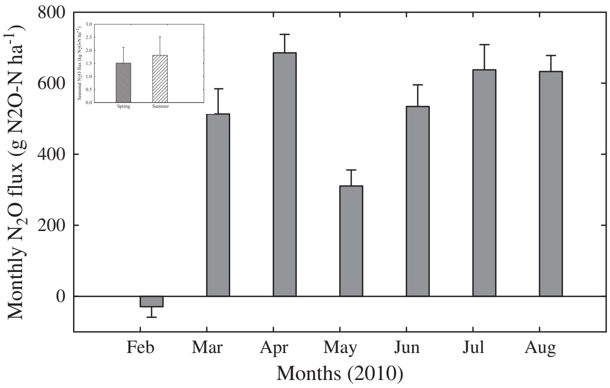

Fig. 2. Monthly N_2O fluxes (± standard deviation). Inlet fig shows the seasonal N_2O fluxes of spring and summer (2010).

grazing events), the N_2O emissions decrease rapidly for a period of about two weeks. There was a sharp increase of NH_4-N concentration in the soil just after the cessation of both grazing events. There was a significant relation ($P = 0.001$) of N_2O fluxes with soil temperature during the first grazing event. There was no significant relation was observed between N_2O fluxes and soil temperature and WFPS parameters during the second grazing event. The only significant difference between "before" and "after" an event (cut or grazing), based on the error bars, Fig. 5(A), is for the second grazing event. For all the other paired flux integrals, the event effect cannot be seen as statistically significant.

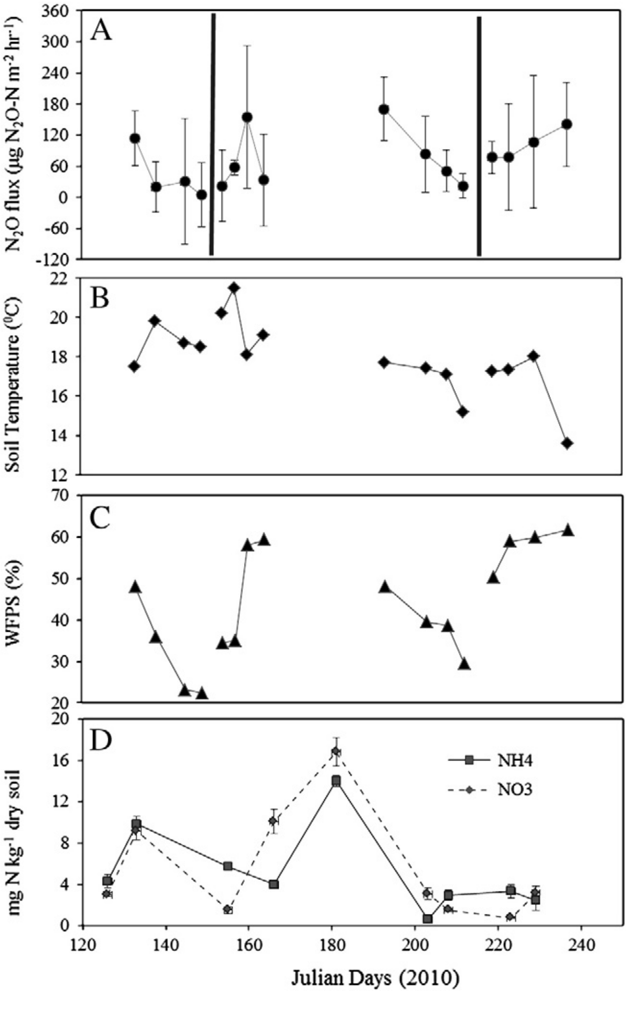

Fig. 3. The N_2O fluxes, Soil temperature, WFPS and soil nitrate and ammonium for two weeks before and two weeks after the cutting events. The dates of the cutting events were 30th May 2010 and 4th August 2010. (A) N_2O flux dynamic before and after grass cutting events. (B) Soil temperature variability before and after cutting events (C) WFPS variability before and after cutting events (D) NH_4+-N and NO_3–N variability before and after cutting events. The vertical lines show cutting events.

3.6. Relationship of NH_4-N and NO_3-N with N_2O emissions

The N_2O fluxes followed the pattern of soil NH_4-N and NO_3-N contents. As illustrated in Fig. 1, elevated soil NH_4-N values corresponded to high N_2O fluxes. A reasonable dependency of N_2O fluxes on NH_4+-N was found with $r^2 = 0.73$ (Figure 6). A comparatively weak linear regression was found between NO_3–N and N_2O fluxes (Figure 7). Correlation analysis showed that correlation exists between soil NH_4-N content ($r = 0.86$ and $P = < 0.05$) and N_2O emissions. The NO_3–N and N_2O correlation was not significant ($r^2 = 0.25$, Figure 7). This suggests that the N_2O fluxes for most of the time during the spring and summer periods may result from the nitrification process which depends on the NH_4+-N concentration and free available oxygen. The statistical analyses for these parameters are given in Table 1.

4. Discussion

4.1. N_2O fluxes

The hourly N_2O fluxes had a large temporal variation dependent on climatic conditions (WFPS and soil temperature) and management practices (N applications, cutting and grazing). Higher emissions were associated with N applications, grass cutting events and grazing events. The range of N_2O fluxes found in this study are similar to those observed by Hyde et al. (2006), Flechard et al. (2007) and Rafique et al. (2011). The highest peak in mid March occurred after a fertilizer application. As grass growth (productivity) is still low in March, suitable environmental conditions (WFPS and temperature) associated with maximum N_2O emissions can lead to high N_2O fluxes (and NO_3-N leaching) when fertilizer N is applied early in the growing season. According to Murphy et al. (1986) the highest dry matter (DM) yield in Irish grasslands (in the presence of N application) occurs in late May/June. O'Donovan et al. (2004) also found that the N application in early spring and late summer can result in increased N_2O emissions instead of grass growth because of more N available for nitrification or denitrification processes. The negative fluxes were observed only twice in the late winter period which is an indication that the soil is acting as a small sink especially when the soil moisture is very high (>80% WFPS). The same observation was made by Flechard et al. (2005). One of the properties of N_2O is that it readily dissolves in water, and so when soil is wet it may be out gassed through drainage water and become a source of soil and water pollution (as NO_{3-}) (Beauchamp, 1997).

Rafique et al. (2011) found that the typical annual N_2O flux from Irish grasslands were found to be in the range 2.0 to 11.0 kg $N_2O-N\,ha^{-1}yr^{-1}$ (average 6.5 kg $N_2O-N\,ha^{-1}yr^{-1}$) and the sum of the N_2O flux from spring and summer period was 3.32 ± 1.0 kg $N_2O-N\,ha^{-1}yr^{-1}$ with most from the summer season. Rafique et al. (2011) also observed the temperature range of 5 °C to 17 °C to be suitable for N_2O emissions with the higher end temperature enhancing the microbial activity causing higher N_2O fluxes (Scanlon and Kiely, 2003). Similarly, Rafique et al. (2011) also observed that the WFPS range of 40% to 60% resulted in approximately three times more N_2O fluxes than those fluxes associated with a WFPS below 40%. In the current study, the 40–60% WFPS range, prevailed during the summer period (see Figure 1(B)). However, Neftel et al. (2000) argued that the N_2O production process occurs primarily in the top 1–2 cm layer of soil

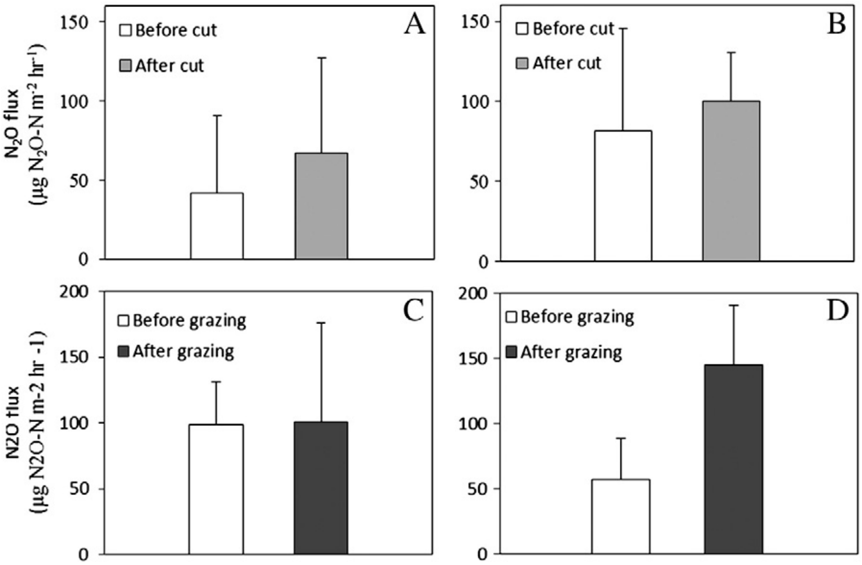

Fig. 4. (A) N_2O fluxes differences occurred before and after first cutting event (B) N_2O fluxes differences occurred before and after second cutting event (C) N_2O fluxes differences occurred before and after first grazing event (D) N_2O fluxes differences occurred before and after second grazing event.

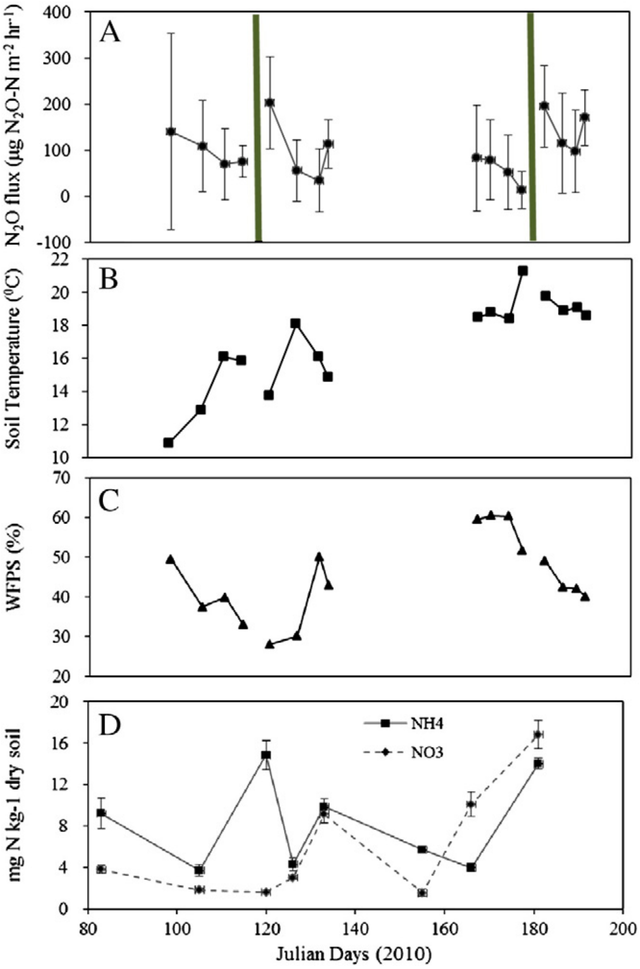

Fig. 5. The N_2O fluxes, Soil temperature, WFPS and soil nitrate and ammonium for two weeks before and two weeks after the grazing events. The dates of the grazing events were 26/27 April 2010 and 27/28 June 2010. (A) N_2O flux dynamic before and after grazing events. (B) Soil temperature variability before and after grazing events (C) WFPS variability before and after grazing events and (D) NH_4+ and NO_{3-} variability before and after cutting events. The vertical lines show grazing events.

and thus the WFPS measurements of the top (1–2 cm) layer instead of the top 5 cm soil layer may have an effect on the flux magnitudes.

4.2. The impact of cutting events on N_2O fluxes

There were two cutting events; May 30 (Julian day, 150), and August 4 (Julian day, 216). We observed the N_2O fluxes to increase gradually for about two weeks immediately following both cutting events (Figure 3(A)). After the first cutting event (May 3), both NH_4+-N and NO_3–N concentrations increased while after the second cutting event both NH_4+-N and NO_3–N concentrations remained unchanged. The larger N_2O flux increase was observed after the first cutting event. The first cutting event coincided with a low WFPS (~30%), a high soil temperature (~20 °C) and an N application on June 7 (8 days after cutting). The second cutting event coincided with a higher WFPS (~60%), a higher soil temperature, (~18 °C) and no N application. Considering our two cutting events above, the three drivers of an N_2O pulse were present: increasing soil temperature (Figure 3B); increasing WFPS (Figure 3C); and cutting events. This suggests that cutting may have enabled the pulses, but other favourable conditions were also present.

Some studies support the thesis that reduced N_2O fluxes occur after cutting grass (Chen et al., 1999; Kammann et al., 1998) while

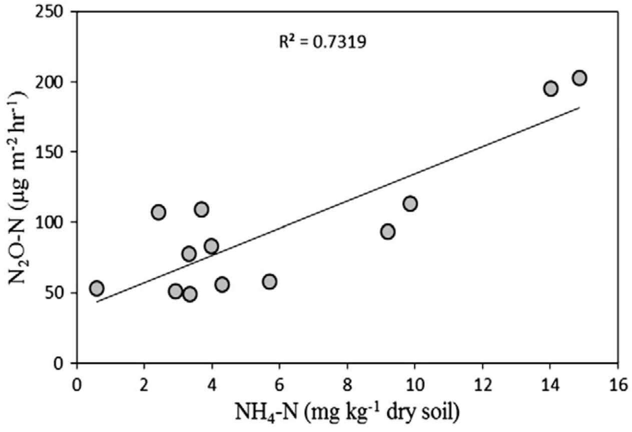

Fig. 6. The linear relationship between the daily N_2O flux and NH_4-N soil contents at study site. The relationship is linear with an r^2 of 0.73.

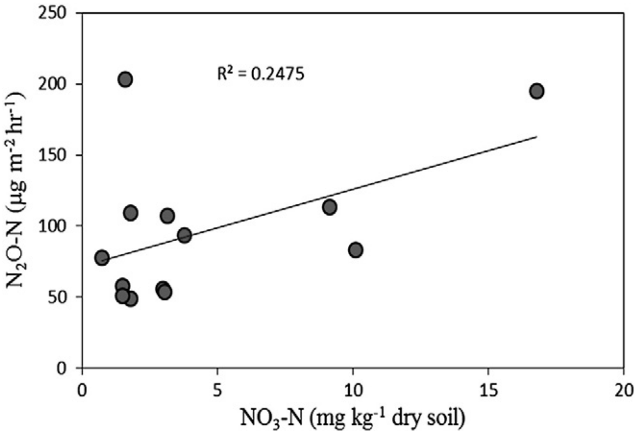

Fig. 7. The linear relationship between the daily N_2O flux and NO_3-N soil contents at study site. The relationship is linear with an r^2 of 0.25.

others (Neftel et al., 2000) reported increased N_2O fluxes. Neftel et al. (2000) reported that after cutting, the plants cease to take up the $NH_{4+}-N$ and $NO_{3-}-N$ and the inorganic N becomes available for nitrification and denitrification. Another explanation for increased N_2O emissions might be an increased amount of C obtained from the decomposition of dead roots of the plants (Kaiser et al., 1996) which is an important substrate for the N_2O production process. Opposite to these findings, Kammann et al. (1998) argues that the more frequently plants are cut the greater their ability to compete for inorganic N, thereby withholding it from denitrifiers, resulting in low N_2O emissions. Chen et al. (1999) explained that lower N_2O emissions from cut grasslands were due to a reduced transport of water-dissolved N_2O in the transpiration stream of the plant. However, the literature still contains contradictory results which require process oriented research to explain the dynamics of N_2O emissions. We also found that the first (Spring) cutting event resulted in higher N_2O fluxes compared to the fluxes of the summer cutting event. This suggests that spring may be more favourable for mineralization resulting in an increase in concentrations of NH_4+-N and NO_3–N (Figure 3D) compared to the Summer. This suggests that the timing of cutting events may impact on the magnitude of N_2O fluxes. Analysis by Peichl et al. (2011) and Jaksic et al. (2006) of eddy covariance CO_2 data in Irish grasslands, found that the timing of cutting events had a greater impact on the net ecosystem exchange (NEE) of CO_2, than environmental parameters (such as temperature, WFPS and vapour pressure deficit).

4.3. The impact of grazing events on N_2O fluxes

Immediately after the two grazing events, each of two day duration, April 26/27 (JD day, 116/117) and June 27/28 (JD, 178/179), there was a step increase in the N_2O fluxes. For the first grazing event, the WFPS was ~30% and the soil temperature was ~16 °C and there was an N application on April 23 (3 days before the grazing event). For the second grazing event, the WFPS was ~50% and the soil temperature was ~18 °C and there was an N application on June 11 (14 days before the grazing event). The step increase in N_2O fluxes immediately after both of the grazing events coincided with increased $NH4+-N$ concentrations (Figure 5(D)). While the N application 3 days before the first grazing event may have impacted the magnitude of its N_2O pulse, it is unlikely that the N application 14 days prior to the second grazing event contributed to the N_2O pulse at that time of the second grazing event. Neither does favourable soil temperature nor favourable WFPS ensure an N_2O pulse on their own without an external input of say – grazing (or cutting or an N application). Immediately after these two grazing events, there were significant pulses of N_2O fluxes, and it is very likely that, neither N applications, nor WFPS nor soil temperatures changes caused these pulses. We suggest therefore, that the main cause of the N_2O pulses were the grazing events themselves.

Increased N_2O fluxes after grazing events have been reported in other studies (Rafique et al., 2011; Saggar et al., 2007; Velthof and Oenema, 1995). The presence of dung or urine patches during and after grazing is known to lead to the accumulation of NO_2- and the emissions of N_2O (Velthof and Oenema, 1995). Clayton et al. (1997) argued that the compaction of soil by animal treading may increase the N_2O fluxes. In this study the N_2O emissions after the first grazing event in April was not as high as the spike after the second grazing in June.

4.4. Nitrification or dentrification

The strong association of soil NH_4+-N concentrations with N_2O emissions can be seen by comparing N_2O emission levels with soil NH_4+-N contents ($r^2 = 0.73$, Figure 6). Throughout the study period, short term peaks in N_2O emissions levels coincided with short term peaks in soil NH_4+-N concentrations which occurred after fertilizer N applications or cutting or grazing events, after which both soil NH_4+-N and N_2O returned to background level. Compared to NH_4+-N, the NO_3–N concentrations generally (except for 4 out of 13 measurements) remained low throughout the study period, indicating uptake of the plant-available NO_3 by the grass or as a result of the transformation of urinary N after grazing (Hyde et al., 2006; Merino et al., 2001). The poor association of soil $NO_{3-}-N$ concentrations with N_2O emissions is noted by comparing N_2O emission level with soil $NO_{3-}-N$ contents ($r^2 = 0.25$, Figure 7).

The soil moisture (WFPS) was observed to have the same temporal trend as NH_4+-N. This suggests that soil moisture may affect the flux of N_2O through its interaction with soil NH_4-N, affecting the availability of this ion for nitrification. Koops et al. (1997) found that N_2O from dry sites is mainly via nitrification; however in moist soils denitrification was reported to be the dominant source (De Klein and Van Logtestijn, 1994).

The key factors influencing the rate of nitrification are the concentrations of NH_4+-N and of free oxygen (O_2) as substrates for the process (Firestone and Davidson, 1989). The WFPS determines the movement of O_2 in soil and hence determines the aerobic and anaerobic conditions within the soil. Grundmann et al. (1995) found that in a sandy loam soil, maximum nitrification occurred at ~50% WFPS. In the present study, about 56% of the study days had a WFPS less than 60% which is considered suitable nitrification. The same findings were observed by Carter (2007) where he noted nitrification was the dominant process when WFPS was <60%. He also found that the rate of nitrification decreased with the increase of water content above 45% WFPS. However, in some other studies, denitrification was considered to be the main process contributing to the N_2O production (Eckard et al., 2010). Carter (2007), noted that denitrification is favoured with a WFPS in the range of 60- 80%. In the present study only 33% of days showed WFPS in this range. This suggests that nitrification conditions (from a WFPS perspective) were more frequently

Table 1
Statistics for the data shown in Figs. 3, 4 and 5. ns stands for non significant.

	Regression equation	Number of measurements	Coefficient of determination (r^2)	Coefficient of correlation (r)	Significance (p)
NH_4-N vs. N_2O	$y = 9.86x + 37.81$	13	0.73	0.86	<0.05
NO_3-N vs. N_2O	$y = 5.42x + 71.87$	13	0.25	0.50	ns

experienced at this site than denitrification conditions. However this arbitrary division of WFPS cannot be confirmed with this current data set. Furthermore, the absolute values of WFPS at which nitrification and denitrification dominate with respect to N_2O production may be site specific (because of soil texture). Compared to other factors (e.g. NH_4-N concentration and WFPS), soil temperature is considered a less important factor controlling N_2O fluxes (Firestone and Davidson (1989)oil pH is also an important factor controlling N_2O emissions. For denitrification the soil pH should be less than 6.0 (Schmidt, 1982); however in present study the soil pH was 6.0 suggestive of possibly nitrification. The nitrification process can be controlled through the application of nitrification inhibitors (Eckard et al., 2010). Nitrification inhibitors reduce the oxidation of NH_4+-N to NO_3–N and thus reduce N_2O emissions from NH_4+-N based fertilizers and from urine (Di and Cameron, 2002). The most widely used nitrification inhibitors are nitrapyrin and dicyandiamide (DCD) (de Klein and Eckard, 2008). Nitrification inhibitor coated fertilizers have been shown to be effective in reducing nitrification and N_2O emissions by up to ~80% as noted by De Klein et al. (2000). If nitrification inhibitors are applied as spray, they can significantly reduce N_2O emissions from animal urine (Di et al., 2007; Smith et al., 2008).

5. Conclusion

We studied the temporal patterns of N_2O fluxes from February to August in 2010 and conducted an analysis to evaluate impact of cutting and grazing events on N_2O fluxes from grasslands. This study also examined the relationship of NH_4+-N and NO_3–N with N_2O fluxes. The N_2O study shows that the N_2O fluxes were higher in the early spring and late summer, when grass productivity as measured by dry matter (DM) is lowest, enabling a larger fraction of N application at these times to be lost to the atmosphere as N_2O. The highest N_2O peak of 684.11 ± 247.16 μg $N_2O-N\ m^{-2}\,h^{-1}$ was observed just after heavy fertilizer N application in early spring. However, the cumulative summer N_2O flux of 1.81 ± 0.7 kg $N_2O-N\ ha^{-1}$ was higher than the cumulative spring N_2O flux of 1.51 ± 0.6 kg $N_2O-N\ ha^{-1}$ which was due to constant elevated N_2O fluxes in the summer period.

We found that post grazing events, that grazing only caused the increased pulses in N_2O fluxes. However, post cutting events, we found that increases in soil temperature and WFPS also contributed to the N_2O pulses. The correlation of NH_4-N with N_2O fluxes was significantly higher ($r^2 = 0.73$, $r = 0.86$, $p = <0.05$) than the relation between NO_3-N and N_2O fluxes ($r^2 = 0.25$, $r = 0.50$, $p = $ns).

This and the more frequent occurrence of WFPS at <56% suggest that nitrification was likely more dominant than dentirification at this site. N_2O fluxes can be reduced by reducing the amount or changing the time of N application in early spring. We conclude that N_2O fluxes can be reduced by changing the timing of grass cutting and grazing in spring and summer. We recommend a further study for the quantification of immediate effects of grass cutting and grazing events in grasslands, on sites of different soils and environmental conditions. We also recommend a further study to check the influence of nitrification inhibitor which can be used to reduce or stop the nitrification process.

Acknowledgement

This project is funded by the Department of Agriculture, Fisheries and Food of the Irish Government under the Research Stimulus Fund Programme (RSF 06 372) and the Science, Technology, Research and Innovation for the Environment (STRIVE) programme 2007–2013 (Grant No. 2008-CCRP-1.1A). Special thanks to Dr. Paul Leahy for his continuous assistance and valuable comments. We also would like acknowledge Jimmy Casey and Nelius Foley for their support in data collection and maintenance of instrumentation.

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