Science of the Total Environment 781 (2021) 146515

Contents lists available at ScienceDirect

Science of the Total Environment

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

Source partitioning using N_2O isotopomers and soil WFPS to establish dominant N_2O production pathways from different pasture sward compositions

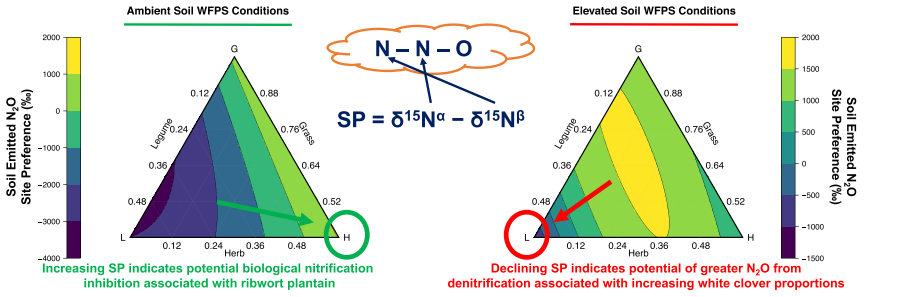
Conor J. Bracken [a,b,c], Gary J. Lanigan [c], Karl G. Richards [c], Christoph Müller [b,d,e], Saoirse R. Tracy [a,b], James Grant [f], Dominika J. Krol [c], Helen Sheridan [a,g], Mary Bridget Lynch [a,g], Cornelia Grace [a,g], Rochelle Fritch [a,g], Paul N.C. Murphy [a,b,*]

[a] UCD School of Agriculture and Food Science, University College Dublin, Belfield, Dublin 4, Ireland
[b] UCD Earth Institute, University College Dublin, Belfield, Dublin 4, Ireland
[c] Teagasc, Environmental Research Center, Johnstown Castle, Wexford, Ireland
[d] Institute of Plant Ecology, Justus-Liebig University, Heinrich-Buff-Ring 26, 35392 Giessen, Germany
[e] UCD School of Biology and Environmental Science, University College Dublin, Belfield, Dublin 4, Ireland
[f] Teagasc Food Research Center Ashtown, Dublin 15, Ireland
[g] UCD Lyons Farm, Lyons Estate, Celbridge, Naas, Co. Kildare, Ireland

HIGHLIGHTS

- N_2O samples from static chambers (<20 ml) analysed using novel CRDS technique.
- Site preference (‰) and soil WFPS (%) used to determine N_2O production pathways.
- Daily N_2O fluxes attributed to 34.2% nitrification and 29.0% denitrification.
- $\delta^{15}N^{bulk}$ and SP (drier soil) indicate ribwort plantain may inhibit nitrification.
- SP drop with increasing WC (wet soil) implies stimulation of denitrification.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 29 October 2020
Received in revised form 11 March 2021
Accepted 11 March 2021
Available online 17 March 2021

Editor: Jay Gan

Keywords:
Nitrous oxide
Site preference
Soil WFPS
Isotopomer
Perennial ryegrass (*Lolium perenne*)

ABSTRACT

Nitrous oxide (N_2O) is a potent greenhouse gas (GHG) emitted from agricultural soils and is influenced by nitrogen (N) fertiliser management and weather and soil conditions. Source partitioning N_2O emissions related to management practices and soil conditions could suggest effective mitigation strategies. Multispecies swards can maintain herbage yields at reduced N fertiliser rates compared to grass monocultures and may reduce N losses to the wider environment. A restricted-simplex centroid experiment was used to measure daily N_2O fluxes and associated isotopomers from eight experimental plots (7.8 m^2) post a urea-N fertiliser application (40 kg N ha^{-1}). Experimental pastures consisted of differing proportions of grass, legume and forage herb represented by perennial ryegrass (*Lolium perenne*), white clover (*Trifolium repens*) and ribwort plantain (*Plantago lanceolata*), respectively. N_2O isotopomers were measured using a cavity ring down spectroscopy (CRDS) instrument adapted with a small sample isotope module (SSIM) for the analysis of gas samples ≤20 mL. Site preference ($SP = \delta^{15}N^{\alpha} - \delta^{15}N^{\beta}$) and $\delta^{15}N^{bulk}$ ($(\delta^{15}N^{\alpha} + \delta^{15}N^{\beta}) / 2$) values were used to attribute N_2O production to nitrification, denitrification or a mixture of both nitrification and denitrification over a range of soil WFPS (%). Daily N_2O fluxes ranged from 8.26 to 86.86 g N_2O-N ha^{-1} d^{-1}. Overall, 34.2% of daily N_2O fluxes were attributed to nitrification, 29.0% to denitrification and 36.8% to a mixture of both. A significant diversity effect of white clover and

* Corresponding author at: UCD School of Agriculture and Food Science, University College Dublin, Belfield, Dublin 4, Ireland.
E-mail address: paul.murphy@ucd.ie (P.N.C. Murphy).

https://doi.org/10.1016/j.scitotenv.2021.146515

White clover (*Trifolium repens*)
Ribwort plantain (*Plantago lanceolata*)

ribwort plantain on predicted SP and $\delta^{15}N^{bulk}$ indicated that the inclusion of ribwort plantain may decrease N_2O emission through biological nitrification inhibition under drier soil conditions (31%–75% WFPS). Likewise, a sharp decline in predicted SP indicates that increased white clover content could increase N_2O emissions associated with denitrification under elevated soil moisture conditions (43%–77% WFPS). Biological nitrification inhibition from ribwort plantain inclusion in grassland swards and management of N fertiliser source and application timing to match soil moisture conditions could be useful N_2O mitigation strategies.

1. Introduction

Mitigating GHG emissions while sustaining food production for growing human populations is a major international challenge (Godfray et al., 2010; IPCC, 2019). Agricultural soils are a major source of N_2O (Bouwman et al., 2013; Fowler et al., 2013) and worryingly global tropospheric N_2O concentrations continue to rise (Thompson et al., 2019; Makowski, 2019). As human populations and activity increased alongside the use of synthetic N fertilisers since the mid-20th century so too did the harmful losses of reactive N to the environment (Galloway and Cowling, 2002; Steffen et al., 2007; Müller and Clough, 2014). This disturbance to the global N cycle has been reflected in N isotope-ratios of N_2O and NO_3^-, sampled from polar ice cores, indicating a distinction of the Anthropocene from the earlier Holocene (Waters et al., 2016; Prokopiou et al., 2017, 2018). An increase in N_2O site preference ($SP = \delta^{15}N^{\alpha}$–$\delta^{15}N^{\beta}$) since pre-industrial times, potentially signals a relative shift from denitrification to nitrification associated with increased N_2O emissions from agriculture (Prokopiou et al., 2017, 2018).

It is evident that multispecies grassland swards comprising of grasses, N fixing legumes and forage herbs can maintain dry matter (DM) production or even out-yield high N input grass monocultures at much lower annual N fertiliser rates (Grace et al., 2019; Kirwan et al., 2007; Nyfeler et al., 2009, 2011). This is often attributed to complementarity of functional group effects (e.g. contribution of biologically fixed N from legumes) (Loreau and Hector, 2001; Kahmen et al., 2006). Multispecies grasslands can also lower N losses (N_2O and NO_3^-), however, N fertiliser rates and legume content complicate these findings (Niklaus et al., 2006, 2016; Leimer et al., 2015, 2016; Malcolm et al., 2014; Scherer-Lorenzen et al., 2003). Biological nitrification inhibition could be another functional group effect associated with forage herbs such as ribwort plantain (*Plantago lanceolata*: PLAN) (Subbarao et al., 2007; de Klein et al., 2019). Compounds extracted from ribwort plantain have reduced N_2O emissions (Gardiner et al., 2018) while growing this species in swards of perennial ryegrass (*Lolium perenne*: PRG) and white clover (*Trifolium repens*: WC) has been shown to reduce NO_3^- leaching (Carlton et al., 2019). Using measurements of N_2O isotopomers could provide useful insights into the impact of such management practices on soil N cycling processes.

Soil derived N_2O can come from nitrification ($NH_4^+ \rightarrow NO_3^-$) and denitrification ($NO_3^- \rightarrow N_2$) pathways (Arnold, 1954; Gayon and Dupetit, 1882; Davidson and Verchot, 2000). Characteristic isotope effects that occur during these processes (Mariotti et al., 1981; Denk et al., 2017) make it possible to distinguish them by the site-specific isotope-ratios of $^{15}N/^{14}N$ in the alpha (α) and beta (β) position of N_2O, sometimes referred to as isotopomer measurements (Friedman and Bigeleisen, 1950; Toyoda and Yoshida, 1999). Isotopic N_2O measurements ($\delta^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$, $\delta^{15}N^{bulk}$, SP) by isotope ratio mass spectrometry (IRMS) or mid-infrared laser absorbance spectroscopy (Toyoda and Yoshida, 1999; Mohn et al., 2014) and are typically expressed as delta (δ) values in per mil (‰) (Coplen, 2011). These are calculated relative to the isotope-ratio of the international standard for N, atmospheric N_2 (3.677×10^{-3}) (Mariotti, 1983). $\delta^{15}N^{bulk}$ is equivalent to $(\delta^{15}N^{\alpha} + \delta^{15}N^{\beta})/2$ (Toyoda and Yoshida, 1999). Tracer experiments using ^{15}N enriched fertiliser, typically report isotope-ratio results in atom% units rather than per mil (‰) due to the much larger isotope-ratios observed compared to natural abundance studies. For example, Müller et al. (2014) and Lewicka-Szczebak et al. (2017) both reported bulk N_2O isotope-ratios in the region of 0 to 50 atom%, approximately equivalent to $\delta^{15}N^{bulk} = -1000‰$ to 271,000‰. N_2O from nitrification becomes highly depleted in ^{15}N (Yoshida, 1988). Whereas, denitrification preferentially selects isotopically light N_2O during further reduction to N_2, leaving the residual N_2O enriched in ^{15}N (Barford et al., 1999). N_2O produced from NH_4^+ will be enriched in ^{15}N at the α position and depleted at the β position due to the preferential cleavage of $^{14}N—^{16}O$ bonds over $^{15}N—^{16}O$ bonds from the intermediates formed during the nitrification reaction sequence, resulting in higher SP values (Popp et al., 2002; Toyoda et al., 2002). Denitrification is less discriminating against the heavier ^{15}N isotope than nitrification so shifts in SP may explain a shift in microbial process (i.e. nitrification or denitrification) due to a change in management practice (i.e. fertiliser application or irrigation) (Pérez et al., 2006; Sutka et al., 2006). These previous studies provide a general course of reasoning to distinguish N_2O from nitrification (higher SP values and lower $\delta^{15}N^{bulk}$ values) and N_2O from denitrification (lower SP values and higher $\delta^{15}N^{bulk}$ values).

Plotting SP vs $\delta^{15}N^{bulk}$ or $\delta^{18}O$ (aka 'isotopomer mapping' Lewicka-Szczebak et al., 2017) enables distinction of N_2O from nitrification (SP: 28 to 36‰ and $\delta^{15}N^{bulk}$: −60 to −30‰) and denitrification (SP: −10 to 0‰ and $\delta^{15}N^{bulk}$: −40 to 0‰) (Koba et al., 2009; Toyoda et al., 2011; Zou et al., 2014). Estimates of N_2O mixing from different processes and the further reduction of N_2O to N_2 have been made using 'isotopomer maps' (Well et al., 2012; Lewicka-Szczebak et al., 2017). Further reduction of N_2O to N_2 can increase SP values of residual N_2O and if overlooked could bias the interpretation of results towards nitrification sources (Well and Flessa, 2009). Several studies have found denitrification to be the dominant source of N_2O emissions from agricultural grassland, while noting shifts in SP related to fertiliser application and changes in soil conditions (Bol et al., 2003; Wolf et al., 2015; Buchen et al., 2018; Ibraim et al., 2019). However, nitrification and fungal denitrification, are difficult to distinguish from each other using isotopomer mapping alone as they have similar SP ranges (Wu et al., 2019). Congreaves et al. (2019) used isotopomer maps and soil specific measured endmember SP values for nitrification and denitrification to establish significant linear relationships to predict the fractions of N_2O from nitrification ($F_N = 3.19–0.041x\times$) and denitrification ($F_D = -2.19 + 0.041x$) based on soil WFPS. This showed, as with previous studies, that nitrification is a dominant source of N_2O when WFPS is <70% and denitrification becomes more dominant when WFPS is >70% but given the heterogeneous nature of soil both processes can occur simultaneously (Nõmmik, 1956; Linn and Doran, 1984; Stevens et al., 1997; Davidson, 1991; Abbasi and Adams, 2000; Bateman and Baggs, 2005).

The current study tested if an adaption to the approach of Congreaves et al. (2019) was applicable to distinguish if N_2O came from either nitrification or denitrification for isotopic N_2O measurements from a ^{15}N tracer field experiment. Bracken et al. (2020) quantified N_2O fluxes and cumulative N_2O emissions post a single application of a 2% ^{15}N labelled urea fertiliser (for the purpose of a ^{15}N tracing study) to plots of different sward composition managed under two contrasting soil moisture conditions. Bracken et al. (2020) inferred that denitrification was likely the most dominant source of N_2O under wet soil conditions and that sward composition influences soil N dynamics depending on the proportions of WC and PLAN. Given the similar range

of soil WFPS between the Bracken et al. (2020) and Congreaves et al. (2019) studies, this experiment aimed to assess if similar relationships between $F_{(N)}$ and $F_{(D)}$ with soil WFPS were observable under field conditions. N_2O isotopomers, sampled on the same days as the N_2O fluxes reported by Bracken et al. (2020), were measured using a novel CRDS technique (Bracken et al., 2021) and the results were used to produce an isotopomer map of SP and $\delta^{15}N^{bulk}$ (Decock and Six, 2013; Zou et al., 2014) to source partition nitrification from denitrification. Due to the 2% ^{15}N labelled urea fertiliser application, the soil emitted N_2O had a similar enrichment to ^{15}N tracer studies (e.g. Müller et al., 2014; Lewicka-Szczebak et al., 2017) rather than natural abundance studies meaning visual interpretation of N_2O production processes from previously reported ranges was not possible (Koba et al., 2009; Zou et al., 2014). The CRDS instrument used in the current study does not measure $\delta^{18}O$. Therefore, the limitations of the current study were: 1) distinguishing specific source pathways indicative of soil microbial communities based on previously reported SP and $\delta^{15}N^{bulk}$ natural abundance ranges (e.g. bacterial denitrification, nitrifier-denitrification, nitrification and fungal denitrification), and 2) assessing if further N_2O reduction to N_2 occurred.

The current study aimed to test the hypothesis proposed by Bracken et al. (2020) that denitrification was more dominant under elevated soil moisture conditions, whereas nitrification likely contributed more under ambient soil moisture conditions. Given that SP varies if N_2O is produced from nitrification or denitrification (Decock and Six, 2013), changes in SP caused by differences in proportions of PRG, WC and PLAN in the sward would be of interest as they may indicate an effect of sward composition on soil N cycling processes and resultant N_2O emissions. Therefore, this study also tests the hypothesis that SP of derived N_2O will change significantly based on the proportions of PRG, WC and PLAN within a sward.

2. Materials and methods

2.1. Experimental site

The experiment was located at University College Dublin (UCD) Lyons Farm (53° 18′ N, 6° 32′ W, ca. 80 m AOL) in Co. Kildare, Eastern Ireland. Climate conditions are cool temperate oceanic. Annual mean total rainfall and annual mean temperature for this site are 754.2 mm and 9.7 °C, respectively (Met Éireann, 2018). The soil type is a grey brown podzol with silty clay loam texture (Lalor, 2004). This would be classified as a luvisol under the World Reference Base (WRB) soil classification system (IUSS Working Group WRB, 2014). A detailed description of the sites physical and chemical soil properties can be found in Table 1 of Bracken et al. (2020).

Table 1
Statistical significance for the functional group identity and diversity effects and soil moisture interactions for SP and $\delta^{15}N^{bulk}$ (‰).

Effect type	Parameter	$SP_{(soil)}$	$\delta^{15}N^{bulk}$
Functional group identity effects	Grass intercept	NS	NS
	Legume	0.00684**	NS
	Herb	NS	NS
Functional group diversity effects	Grass × legume	NS	NS
	Grass × herb	NS	NS
	Legume × herb	0.03909*	NS
Functional group identity and soil moisture interaction effects	Grass × SM	NS	NS
	Legume × SM	0.00752**	0.00143**
	Herb × SM	NS	0.04608*
Functional group diversity and soil moisture interaction effects	Grass × legume × SM	0.02139*	0.00941**
	Grass × herb × SM	NS	NS
	Legume × herb × SM	0.03798*	0.03396*

SM = soil moisture. NS = not significant. See Section *Statistical analysis* for description of effect types.
*** <0.001.
** <0.01.
* <0.05.

The experimental plots used in this experiment were originally established in August 2013 as described by Grace et al. (2018). Prior to establishing the experimental pasture swards the site was under continuous tillage with a final crop of maize (*Zea mays*). During the Grace et al. (2018) study, the plots (1.95 × 10 m) used for the current experiment were managed at an annual N fertiliser rate of 90 kg N ha^{-1} yr^{-1} and all other macro and micronutrients were kept non-limiting. Herbage was cut to 4 cm and removed 8 times per year between April and October using a Haldrup forage harvester (Løgstør, Denmark) at 21 to 30 day intervals (Grace et al., 2018).

2.2. Experimental design

The Grace et al. (2018) study followed the diversity-interaction approach described by Kirwan et al. (2009) by using a constrained simplex experimental design. Eight plots with differing proportions of grasses, legumes and forage herbs, represented by PRG, WC and PLAN, were selected from the Grace et al. (2018) study. This design considers the sward/pasture as a mixture of component species (PRG, WC, PLAN) and assumes the measured responses depend on the relative proportions of the component species within the sward (Cornell, 2002). Given the diversity-interaction model (Simplex model) uses regression to determine coefficient estimates, replication of the sward mixtures is not necessary (Kirwan et al., 2009).

The eight sward mixtures are referred to by the ratios of their component species (PRG: WC: PLAN, Fig. S1 see Supplementary Material). *Lolium perenne* (PRG), *Trifolium repens* (WC) and *Plantago lanceolata* (PLAN) as single species representing the three components of the sward mixtures. Each mixture contained a minimum of 40% grass (PRG) as a practical agronomic constraint. Each of the eight plots was split into two distinct areas with a buffer zone of ≥1 m between each area within the plot. One area was maintained under ambient soil moisture conditions while the other area was watered to increase the soil water filled pore space (WFPS) to a target of >70%. Two stainless steel static chamber bases (40 cm × 40 cm) and 12 cm high were installed in each plot, one per distinct area, to a depth of 5 cm into the soil (de Klein and Harvey, 2012). Each chamber base was lined with a neoprene foam seal to create an air tight seal when closed with the corresponding 10 cm high stainless steel chamber lids. During gas sampling chambers were closed for 60 min and lids were weighed down with a 5 kg weight.

2.3. Soil moisture and water filled pore space (WFPS)

As described in Bracken et al. (2020) the mean soil bulk density and soil moisture content was determined and used to estimate the WFPS assuming a particle size density of 2.65 g cm^{-3} (Krol et al., 2015). At the beginning of the experiment, to increase the soil WFPS in one half of each plot to >70%, 7.5 L of water was applied by watering can in two applications (5 L followed by 2.5 L) which simulated a total of 30 mm of rainfall. Based on historical weather data the return period for this amount of rainfall at this site is approximately 1.09 years (Met Éireann, 2018). A subsequent water application of 3 L (equivalent to 12 mm rainfall) was applied to the area of elevated WFPS during the experiment when the WFPS of the two distinct areas came within 5%. Surface soil moisture (% volume, 0–6 cm depth) was measured on each sampling day using a ML2 Theta Probe (Delta-T Devices Ltd., HH2, UK) from four points around the outside of each static chamber. The average of these four measurements was used in Eq. (1) to track soil WFPS associated with each static chamber throughout the experimental monitoring period.

$$\text{WFPS}\ (\%) = \text{Soil Moisture}/(1-(\text{BD}/\text{PSD})) \quad (1)$$

whereby:

Soil moisture is % volume, average of four measurements using a ML2 Theta Probe;

BD is the mean soil bulk density of the plots (1.2 g cm^{-3});
PSD is an assumed soil particle density of 2.65 g cm^{-3} (Krol et al., 2015).

2.4. Fertiliser application

A 2% ^{15}N labelled urea fertiliser was prepared using 40.33285 g laboratory grade urea (Sigma Aldrich) and 0.82312 g of ^{15}N labelled urea (99.9% purity; Cambridge Isotope Laboratories, Inc.) dissolved in 2 L of 18.2 mQ water. A syringe was used to apply 66.67 mL of the ^{15}N labelled fertiliser to the soil surface in each of the chamber footings (0.16 m^2) and a further 37.5 mL to an adjacent area (0.09 m^2) for periodic soil sampling, equivalent to a rate of 40 kg N ha^{-1}. This was lightly watered in immediately after application using a watering can and rose head attachment to minimise N volatilisation to ammonia. No other macro or micro nutrients were applied to the plots prior to or during this experiment. For further details of the soil chemical properties refer to Table 1 of Bracken et al. (2020).

2.5. N_2O flux and isotopomer sampling

Gas samples were collected by syringe through the rubber septa of the static chambers once prior to fertiliser application and then regularly for 2 months post fertiliser application as described by Bracken et al. (2020). Gas sampling took place between 09.00 and 12.00 each sampling day to obtain the most representative average daily N_2O flux (Alves et al., 2012; Laville et al., 2011; Parkin, 2008; Smith and Dobbie, 2001). During the 60-min closure period chamber headspace samples (10 mL) were taken at times 0, 30 and 60 min after the static chambers were closed. These samples were injected into 7 mL pre-evacuated glass vials with double wadded PTFE/silicone septa (Labco Ltd., UK). Flux samples were analysed by gas chromatograph as described by Bracken et al. (2020).

Following the 60-min flux samples and prior to the removal of the static chamber lids, 20 mL samples for N_2O isotopic analysis were removed and injected into pre-evacuated 12 mL Exetainer vials capped with grey butyl rubber septa (Labco Ltd., UK) to achieve over pressure for storage. The syringe was flushed three times with ambient air prior to each sample removal. During sample removal, the syringe was plunged three times to evenly mix the gas inside chambers. The 60-min chamber closure period was considered suitable for sufficient N_2O build-up to enable distinction of soil emitted N_2O from ambient air N_2O if soil fluxes were reasonably high (Buchen et al., 2018; Petersen et al., 2020).

Isotope samples were measured by CRDS using a Picarro G5101-i combined with a SSIM unit for discrete gas samples (<20 mL) using a manual injection method and results were calibrated to the international isotope-ratio scale using a previously calibrated internal lab reference gas (Bracken et al., 2021). Eq. (2) was used to distinguish soil emitted N_2O $\delta^{15}N^{bulk}$, $\delta^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$ and SP, following the approach of Petersen et al. (2020), using a similar data screening rule to omit samples with N_2O concentrations <450 ppb. This "sample concentration rule" was necessary since N_2O isotopomers were measured using CRDS and Petersen et al. (2020) showed that laser based measurements become more variable towards ambient atmospheric N_2O concentrations (330 ppb).

$$\delta^{15}N^{x}_{(soil)} = \left(\delta^{15}N^{x}_{(sample)} \times N_2O_{(sample)} - \delta^{15}N^{x}_{(air)} \times N_2O_{(air)}\right) \div \left(N_2O_{(sample)} - N_2O_{(air)}\right) \quad (2)$$

$\delta^{15}N^{x}$ refers to either $\delta^{15}N^{bulk}$, $\delta^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$ or SP. 'Soil', 'air' and 'sample' refer to N_2O emitted from soil, N_2O in ambient air and mixture of these two (i.e. sample measurement), respectively. Average δ values of tropospheric N_2O in ambient air were used in Eq. (2) for $\delta^{15}N^{bulk}_{(air)}$ (6.8‰), $\delta^{15}N^{\alpha}_{(air)}$ (15.8‰), $\delta^{15}N^{\beta}_{(air)}$ (−2.3‰) and $SP_{(air)}$ (18.1‰) because *in situ* isotope-ratio values were not measured for ambient air during sampling in this experiment (Prokopiou et al., 2017, 2018). This is a limitation of the current study as Ostrom et al. (2021) recently showed δ values in ambient air can vary up to 30‰ which means substituting literature derived values could potentially lead to greater sampling error.

2.6. N_2O source partitioning using SP, $\delta^{15}N^{bulk}$ and soil WFPS

An isotopomer map was used to describe the linear relationship between SP and $\delta^{15}N^{bulk}$. The model of CRDS used in this study does not measure $\delta^{18}O$. Therefore, isotopomer maps using SP and $\delta^{18}O$ (Well et al., 2012; Lewicka-Szczebak et al., 2017) and the additional estimation of N_2O reduction to N_2 was beyond the scope of the current study. Adapting the approach applied by Congreaves et al. (2019), minimum and maximum $\delta^{15}N^{bulk}$ values were used in the linear equation describing this relationship ($y = -0.01145x - 126.5$; where y = SP and $x = \delta^{15}N^{bulk}$) to determine measured endmember SP values for nitrification ($SP_N = -86‰$) and denitrification ($SP_D = -144‰$). This was done given the observed N_2O enrichment was more similar to tracer studies, as such using literature derived endmember values from natural abundance studies was considered unsuitable. Eqs. (3) and (4) were then applied to calculate the fractions of nitrification (F_N) and denitrification (F_D) (Deppe et al., 2017; Lewicka-Szczebak et al., 2017; Congreaves et al., 2019). An assumption that the minimum and maximum measured enriched $\delta^{15}N^{bulk}$ values were suitable to distinguish nitrification and denitrification (Popp et al., 2002; Toyoda et al., 2002) was necessary to calculate $F_{(N)}$ and $F_{(D)}$ from Eqs. (3) and (4) below. The F_N and F_D values were determined from measured SP values (SP_x) and the endmember SP_N and SP_D values above using Eqs. (3) and (4).

$$F_N = SP_x - SP_D / SP_N - SP_D \quad (3)$$

$$F_D = 1 - F_N \quad (4)$$

F_N and F_D values >1 were considered to be either 100% nitrification or denitrification and the fractions were set to 1. Three groups were used to distinguish dominant (≥90%) N_2O production processes by the measured fractions of nitrification (F_N); Nitrification = $F_N \geq 0.9$, Denitrification = $F_N \leq 0.1$, Mixture of Nitrification and Denitrification = $0.1 \leq F_N \leq 0.9$. Linear regressions were used to describe the relationships of F_N and F_D with soil WFPS.

Given it was beyond the scope of the current study to determine further reduction of N_2O to N_2 due to complete denitrification, it was assumed to be negligible given the soil WFPS did not exceed 80% (Davidson, 1991; Cardenas et al., 2017). It was beyond the scope of the current study to determine reduction of N_2O to N_2 as done in previous studies (Lewicka-Szczebak et al., 2017; Deppe et al., 2017; Congreaves et al., 2019), as the CRDS model used in the current study does not measure $\delta^{18}O$. Likewise, samples were not collected to measure SP values of N_2O prior to any possible reduction to N_2 so this could not be determined. A calculation of net isotope effects ($\Delta\delta^{15}N$) or fractionation factors from the difference in ^{15}N enrichment of soil NH_4^+ or NO_3^- compared to ^{15}N enrichment of soil-emitted N_2O over the sampling period was also beyond the scope of the current study.

2.7. Meteorological data

Daily weather data, rainfall (mm) and mean air temperature (°C), were obtained from the Met Éireann meteorological station at Casement Aerodrome (53°30′ N, 6°44′ W), 5.8 km east of the experimental site and at a similar elevation (80 m AOL). Soil temperature (°C, 0–10 cm) was recorded on each sampling day from each plot using a TinyTag View 2 with a PB-5002-1M5 Thermistor Probe (Gemini Data Loggers).

2.8. Statistical analysis

SP and $\delta^{15}N^{bulk}$ results averaged over sampling dates were statistically analysed using a simplex model in R (R Core Team, 2017). The diversity-interaction modelling (Simplex model) approach of Kirwan et al. (2009) was adapted to determine identity effects and functional group diversity effects of the three plant functional groups (represented by PRG, WC and PLAN). The effect of the proportions of PRG, WC and PLAN on SP values under ambient and elevated soil moisture were assessed using the simplex model. When the response associated with a monoculture of one of the plant functional groups was significantly different to the response of a monoculture of another plant functional group this was considered an identity effect. Functional group diversity effects arise when the response of a mixture of plant functional groups is significantly different from the response that would be expected based on the proportional composition of functional groups in the mixture. Interactions between functional group identity effects and two soil moisture levels as well as three-way interactions between functional group diversity effects and soil moisture levels were also tested. The model outputs and simplex contour plots were produced using the "lm" function and "mixexp" package in R (Lawson and Willden, 2016). Tests of significance were performed at the $P < 0.05$ level. All other plots were produced using the "ggplot2" package in R (Wickham, 2009).

3. Results

A total of 272 N_2O isotopomer samples were measured. However, based on the "sample concentration rule" (see Materials and Methods), 38 sample results were retained for further analysis. The range of $\delta^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$, $\delta^{15}N^{bulk}$ and SP values presented in Table S1 (see Supplementary Material) show that isotopic N_2O measurements were similar to previously reported ranges in ^{15}N tracer studies and that Eq. (2) caused increased variation when sample concentrations are similar to ambient air (330 ppb), hence the need to apply the "sample concentration rule". The 38 retained samples accounted for 57.85% of total N_2O emissions. Daily N_2O fluxes for this subset of samples, which ranged from 8.26 to 86.86 g N_2O-N ha^{-1} d^{-1} overall, and peak N_2O fluxes, reflected fertiliser application and heavy rainfall events, and corresponding WFPS conditions, as detailed in Bracken et al. (2020). The highest daily N_2O flux occurred under elevated soil moisture conditions (Fig. 1, bottom). Daily N_2O fluxes ranged from 8.26 to 17.70 g N_2O-N ha^{-1} d^{-1} under ambient soil moisture conditions and from 8.91 to 86.86 g N_2O-N ha^{-1} d^{-1} under elevated soil moisture conditions. Ambient soil moisture conditions ranged from 51% to 67% WFPS and elevated soil moisture conditions ranged from 52% to 75% WFPS (Fig. 1, top). As reported in the Bracken et al. (2020) study, cumulative post fertiliser N_2O emissions ranged from 22.1 to 206.4 g N_2O-N ha^{-1} under ambient soil moisture and 62.5 to 434.3 g N_2O-N ha^{-1} under elevated soil moisture.

The temporal trend of $\delta^{15}N^{\alpha}$ shows that $\delta^{15}N^{\alpha}$ becomes particularly enriched shortly after fertiliser application but relatively much less enriched during and just after the heavy rainfall period (Fig. 2, left). The temporal trend of $\delta^{15}N^{\beta}$ shows that $\delta^{15}N^{\beta}$ becomes only slightly enriched relative to $\delta^{15}N^{\alpha}$ shortly after fertiliser application but becomes more enriched around the heavy rainfall period (Fig. 2, right). There was a highly significant ($P < 0.001$) negative linear relationship ($R^2 = 0.25$) between SP and $\delta^{15}N^{bulk}$ (Fig. 3, top). However, it is clear that the measured values ($SP_{(soil)}$: −218 to −35‰ and $\delta^{15}N^{bulk}_{(soil)}$: −3553 to 1562‰) are more similar to tracer studies and do not fall within the approximate ranges typically reported for natural abundance values of N_2O emitted from soils as a result of nitrification (SP: 28 to 36‰ and $\delta^{15}N^{bulk}$: −60 to −30‰) and denitrification (SP: −10 to 0‰ and $\delta^{15}N^{bulk}$: −40 to 0‰) (Fig. 3, bottom). The mean fraction of nitrification (F_N), estimated using the adapted isotopomer mapping approach, was 0.57 and the mean fraction of denitrification (F_D) was 0.43. N_2O emissions were mostly attributed to a mixture of both nitrification and denitrification (36.8%) overall. Nitrification (i.e. $F_N \geq 0.9$) was associated with 34.2% and denitrification (i.e. $F_D \geq 0.9$) was associated with 29.0% of N_2O emission overall.

There was no significant relationship detected between F_N or F_D and soil WFPS ($P = 0.16$). However, it is clear over the observed range of soil WFPS that the trend in F_N is to decrease while F_D increases with increasing WFPS (Fig. 4). The intersection of the F_N and F_D lines indicates that denitrification becomes the most dominant N_2O source at >66% WFPS. Of all the samples attributed to nitrification, 10 out of 13 were from static chambers in the "Wet" half of the plots but these samples were associated with a soil WFPS ≤66% except for one (74.8% WFPS). In general, low daily N_2O fluxes with correspondingly high SP values at lower soil WFPS indicated nitrification, while high daily N_2O fluxes with correspondingly low SP values at higher soil WFPS indicated denitrification (Fig. 5).

The statistical significances of the simplex model outputs for SP and $\delta^{15}N^{bulk}$ are presented in Table 1. There was a strongly significant legume (WC) functional group identity effect on SP ($P < 0.01$) and a significant interaction between legume (WC) × herb (PLAN) ($P < 0.05$). There was a strongly significant interaction between legume (WC) × soil moisture for SP ($P < 0.01$). There were significant three-way interactions between grass (PRG) × legume (WC) × soil moisture and between legume (WC) × herb (PLAN) × soil moisture for SP ($P < 0.05$). SP values decreased with increasing proportions of legume under elevated soil moisture conditions and SP values were lowest around the 50:50 proportion (mid-point) of the legume – herb and grass – legume axes under ambient soil moisture conditions (Fig. 6). Highest SP values were observed around the 25:75 proportion under elevated soil moisture conditions. These trends are also apparent in the contour plots presented in Fig. S2 (see Supplementary Material).

There were no significant functional group identity effects or interactions between functional groups (diversity effects) for $\delta^{15}N^{bulk}$. There was a strongly significant interaction between legume (WC) × soil moisture ($P < 0.01$) and a significant interaction between herb (PLAN) × soil moisture on $\delta^{15}N^{bulk}$ ($P < 0.05$). There was a strongly significant three-way interaction between grass (PRG) × legume (WC) × soil moisture ($P < 0.01$) and a significant three-way interaction between legume (WC) × herb (PLAN) × soil moisture on $\delta^{15}N^{bulk}$ ($P < 0.05$). $\delta^{15}N^{bulk}$ values were lowest near the 50:50 proportion of the legume – herb axis and the 75:25 proportion of the grass – legume axis under ambient soil moisture conditions (Fig. 7). These trends are also apparent in the contour plots presented in Fig. S3 (see Supplementary Material).

4. Discussion

In the current study, the subset of daily N_2O fluxes, representing the highest concentration samples from this experiment (i.e. >450 ppb N_2O), clearly represented the responses to fertiliser application and heavy rainfall previously reported by Bracken et al. (2020). Similar responses to perturbations like fertiliser application and rainfall have been observed in other grassland studies (Wolf et al., 2015; Ibraim et al., 2019; Buchen et al., 2018) which found denitrification to be the dominant source of N_2O production through the use of isotopic N_2O measurements. In this study, the measured $SP_{(soil)}$ and $\delta^{15}N^{bulk}_{(soil)}$ ranges are evidently more similar to those of ^{15}N tracer studies than natural abundance studies due to the ^{15}N labelled fertiliser source used in the experiment. It was assumed that the 2% ^{15}N labelled urea fertiliser applied in the present study would be quickly and almost fully hydrolysed to NH_4^+ post application, thus, enriching the soil NH_4^+ pool (Abbasi and Adams, 2000). It is understood that nitrification is more discriminatory of the heavy isotope (^{15}N) than denitrification, thus producing more depleted N_2O $\delta^{15}N^{bulk}$ values (Popp et al., 2002; Toyoda et al., 2002). This would explain the congregation of depleted points to the left of the isotopomer map (Fig. 3) which in general also have higher SP values. The samples classified as nitrification in this study were mostly depleted

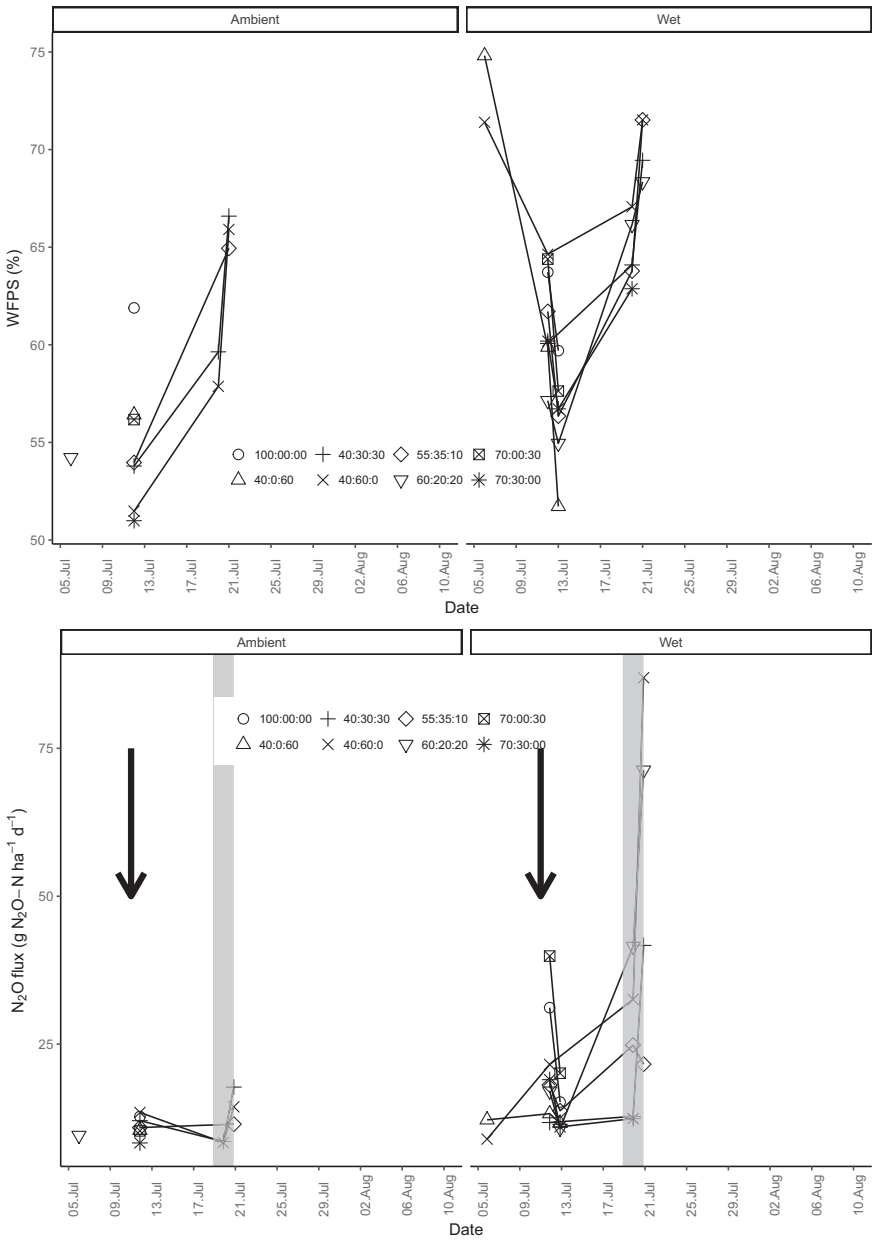


Fig. 1. Temporal plot of soil WFPS and daily N_2O fluxes subset data based on Eq. (2) concentration rule (Legend = Mixture Ratio; Black arrows = fertiliser application; shaded area = heavy rainfall). Full dataset presented in Bracken et al. (2020).

with very low $\delta^{15}N^{bulk}$ and occurred just after the application of the labelled urea fertiliser and prior to the heavy rainfall event outlined in Fig. 1.

More samples were classified as denitrification post this heavy rainfall event which was approximately 1 week after fertiliser application. If nitrification was more dominant before the rainfall event this would have allowed sufficient time for much of the labelled NH_4^+ to be converted to NO_3^-. The temporal trends in $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$ (Fig. 2) further highlight that nitrification was more prevalent before the heavy rainfall event and that denitrification occurred more after this event. If the subsequent N_2O emissions were then derived mostly from denitrification this would explain the congregation of more enriched points to the right of the isotopomer map (Fig. 3) which in general also have lower SP values. Combining isotope effects reported for different reactions in the N cycle from an extensive range of literature sources, Denk et al. (2017) highlighted that SP values are clearly higher for nitrification sources of N_2O production compared to denitrification N_2O sources (with the exception of fungal denitrification). SP is defined as $\delta^{15}N^{\alpha}$ - $\delta^{15}N^{\beta}$, therefore, when nitrification is more dominant, $\delta^{15}N^{\alpha}$ would be relatively high and $\delta^{15}N^{\beta}$ low, with the opposite expected for denitrification. In a similar way to Congreaves et al. (2019), this study generally found that higher daily N_2O fluxes and low corresponding SP values were associated with denitrification, while low daily N_2O fluxes and high corresponding SP values were associated with nitrification (Fig. 5).

Based on the current results N_2O was determined to be mostly derived from a mixture of nitrification and denitrification, with nitrification being slightly more dominant than denitrification overall. This was contrary to the hypothesis of Bracken et al. (2020). However, the variation of measured $\delta^{15}N^{x}_{(sample)}$ values and calculated $\delta^{15}N^{x}_{(soil)}$ values presented in Table S1 suggests large sampling artefacts may have occurred, particularly for samples close in N_2O concentration to ambient air. This could be due to a number of reasons including; 1) the ^{15}N enriched fertiliser source increasing the range of measured isotoperatios, 2) potential systematic errors associated with the CRDS sample analysis technique used, previously described by Bracken et al. (2021), and 3) generally large variation associated with laser spectroscopy

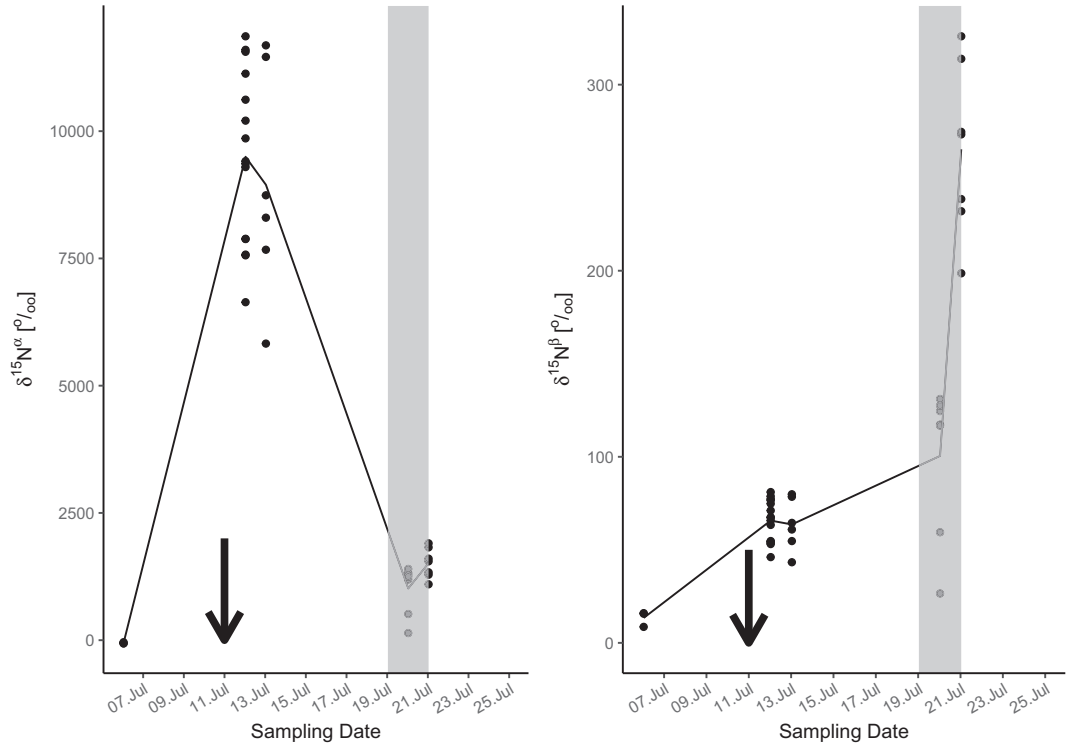


Fig. 2. Temporal plot of $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$ changes over the sampling period. Solid line = mean $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$ on each sampling date. Black arrows = ^{15}N labelled urea fertiliser application; shaded area = heavy rainfall. (See main text for further explanation of large δ value scales).

measurement of N_2O isotope-ratios when sample concentrations are near that of ambient air (Petersen et al., 2020). Likewise, Ostrom et al. (2021) recently found that in situ ambient air isotopic N_2O measurements can vary by 30‰ indicating that substitution of literature derived values of ambient air rather than measured values into Eq. (2) is another possible source of error when calculating $\delta^{15}N^{x}_{(soil)}$. Inferences related to fractions of nitrification and denitrification and the trends in N_2O emissions observed by Bracken et al. (2020) were therefore limited in this study by the reduced sample size post data screening using the "sample concentration rule". Future field studies applying this approach should

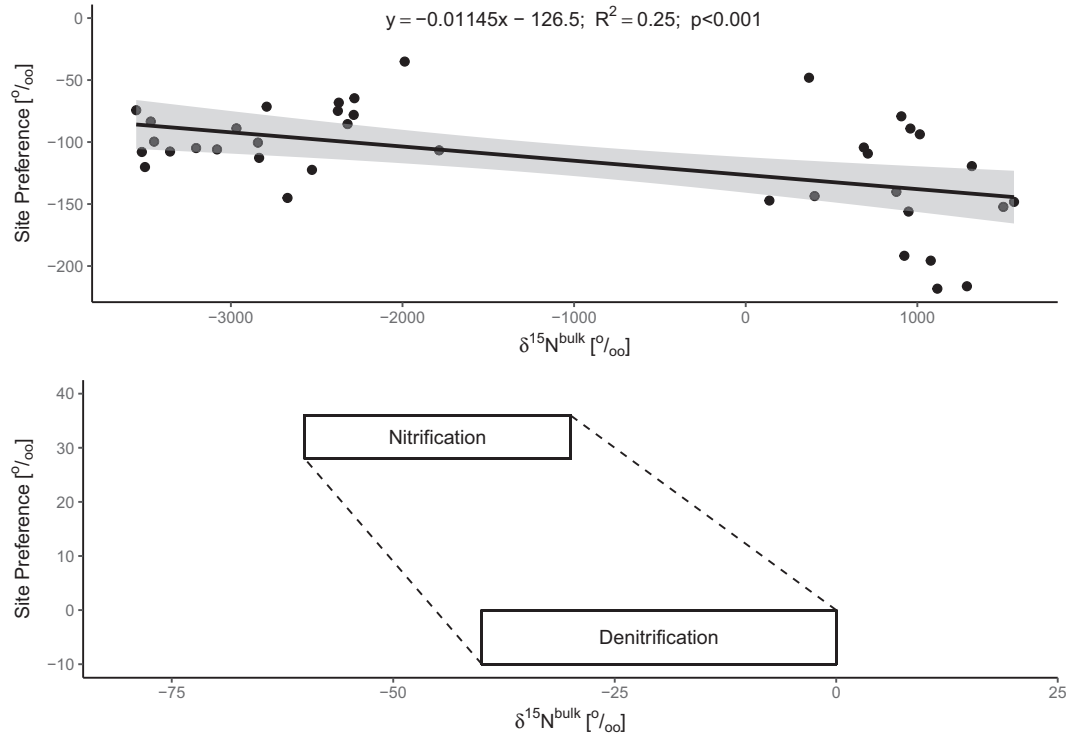


Fig. 3. Isotopomer map of SP and $\delta^{15}N^{bulk}$ values used to determine nitrification (SP_N) and denitrification (SP_D) endmember values from measured data (top). (See main text for further explanation of large δ value and SP scales). Typical literature reported endmember ranges from nitrification and denitrification processes (bottom); adapted from Zou et al. (2014).

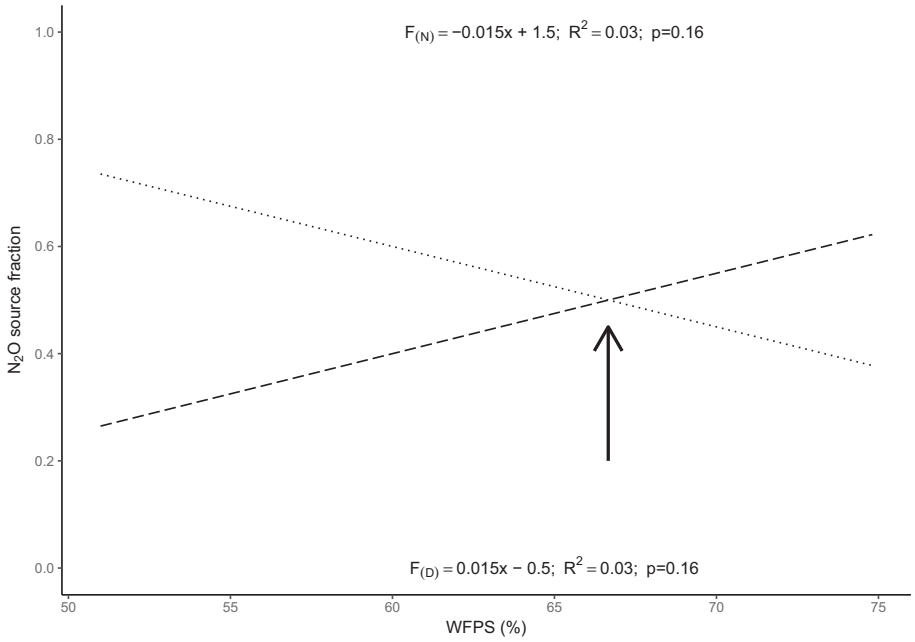


Fig. 4. N_2O source fractions over the observed range of soil WFPS. Dotted line = predicted fraction of nitrification (F_N) and dashed line = predicted fraction of denitrification (F_D). Arrow = intersection of F_N and F_D.

aim to measure in situ N_2O concentrations and isotope-ratios of ambient air to use in Eq. (2).

Of the remaining samples 37% were from ambient (drier) plots while 63% were from wet plots. However, there was considerable overlap in estimated soil WFPS ranges for those remaining samples (ambient plots: 51% to 67% and wet plots: 52% to 75%). Given previous studies found denitrification typically dominates when soil WFPS is >70% (Linn and Doran, 1984; Bateman and Baggs, 2005), the uncontrolled drop in WFPS of the "Wet" plots may explain why denitrification was not found to be the dominant source of N_2O in this study. Simultaneous nitrification and denitrification due to spatial heterogeneity of aerobic and anaerobic sites in the field soil may have occurred, thus mixing N_2O from both processes and making the isotopic signatures of these pathways more difficult to distinguish (Abbasi and Adams, 2000). Hence, why results of this study would suggest mixing of N_2O from both nitrification and denitrification was more prevalent (36.8%).

The predicted trend of F_D with WFPS (Fig. 4) suggests that denitrification would have been more dominant on occasions when soil WFPS was >66%, although these relationships were weak and not significant in this study. This again may have been due to the heterogeneous nature of field soil conditions and the lack of regulation over the soil WFPS in this experiment compared to the controlled conditions in soil incubation studies (Well et al., 2006; Bergstermann et al., 2011; Congreaves et al., 2019). It is also worth noting that the relationship between F_N or F_D and WFPS without correcting the calculated values of $F_N > 1$ and $F_D > 1$ back to 1 was almost significant ($P = 0.06$). Congreaves et al.

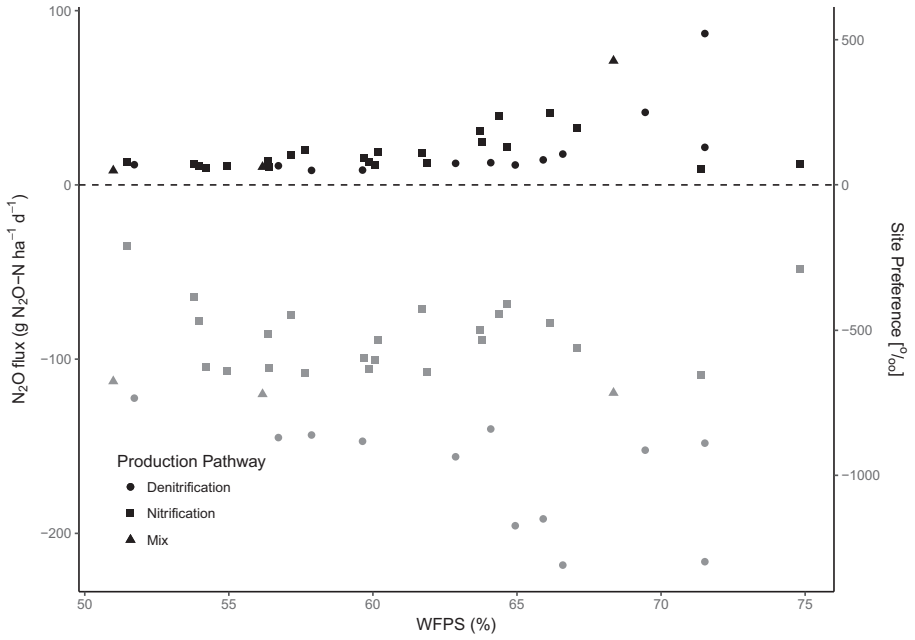


Fig. 5. Daily N_2O fluxes (black, left axis) and corresponding site preference (grey, right axis) influenced by soil WFPS. (see main text for further explanation of large SP scale).

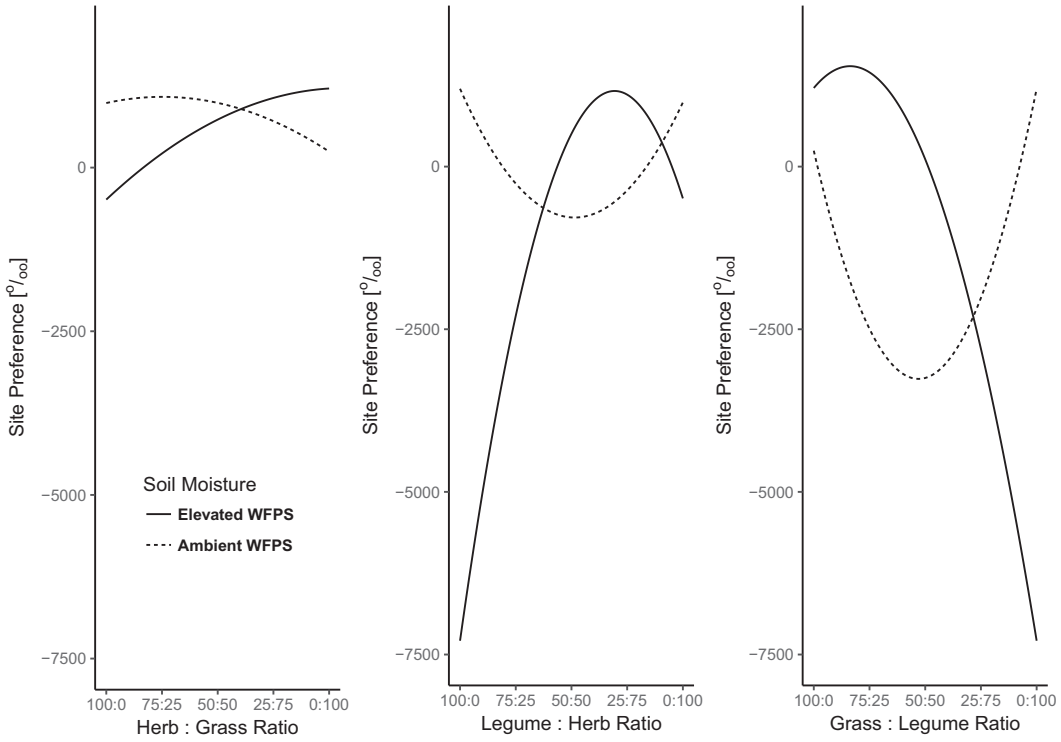


Fig. 6. Simplex model effects plots of predicted average site preference (‰) with increasing proportion of individual component species within the sward mixtures under elevated soil WFPS (solid line) and ambient soil WFPS (dotted line). (see main text for further explanation of large SP scales).

(2019) found significant linear relationships between F_N or F_D and WFPS for different soil types using a controlled soil incubation study with high temporal resolution N_2O isotopomer measurements. Higher temporal N_2O isotopomer measurements was recommended previously by Decock and Six (2013) who noted no discernible relationship between SP and WFPS from earlier sources of literature. In the current study, WFPS was estimated over a soil depth of 0–6 cm and it is possible that the evolved N_2O was influenced by soil moisture at greater depths.

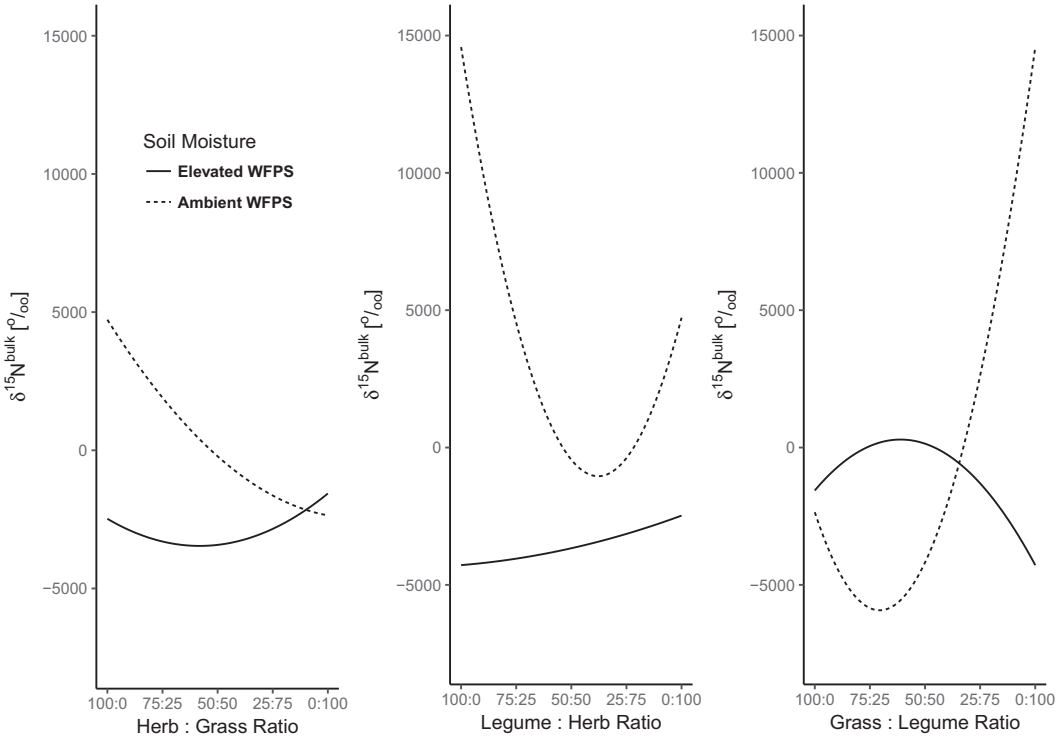


Fig. 7. Simplex model effects plots of predicted average $\delta^{15}N^{bulk}$ (‰) with increasing proportion of individual component species within the sward mixtures under elevated soil WFPS (solid line) and ambient soil WFPS (dotted line). (see main text for further explanation of large δ value scales).

Future field studies applying this approach should aim to utilise stricter controls of soil WFPS (e.g. maintain elevated soil moisture conditions >70% WFPS with continued irrigation) or perhaps measure soil moisture over a greater soil depth to determine if this improves the model fit between $F_{(N)}$ and $F_{(D)}$ and soil WFPS. Maintaining an elevated soil WFPS would be expected to restrict oxygen availability and encourage more activity from soil microorganisms that produce N_2O under anaerobic conditions (Bateman and Baggs, 2005).

This study may have also biased results towards nitrification by not being able to determine if N_2O emissions were influenced by further reduction to N_2 during complete denitrification. Previous studies using natural abundance isotopic N_2O measurements found that SP values increase after further reduction to N_2 (Well et al., 2012; Lewicka-Szczebak et al., 2017). These researchers used SP and $\delta^{18}O$ to estimate N_2O reduction to N_2 but this could not be considered in the current study given the CRDS instrument used does not measure $\delta^{18}O$. Likewise, it was considered unfeasible to apply literature reported fractionation factors (Denk et al., 2017) as there would be too much uncertainty of their applicability to the current study given the application of the ^{15}N labelled urea fertiliser. Although fungal denitrification was assumed to be negligible in the current study, if it did occur it may have biased calculations of F_N and F_D in favour of nitrification. To avoid biasing results towards nitrification, going forward, future studies should consider analysing additional N_2O samples to estimate further reduction to N_2, particularly when soil WFPS exceeds 80%, along with soil samples to characterise microbial communities and assess if fungal denitrification was influential (Well et al., 2012; Lewicka-Szczebak et al., 2017; Congreaves et al., 2019).

The diversity effect on SP, noted by the significant interaction between legume × herb and the significant three-way interaction between legume × herb × soil moisture under ambient soil moisture conditions is particularly interesting. As was highlighted by Bracken et al. (2020), significantly higher cumulative N_2O emissions were associated with higher legume proportions under both soil moisture conditions. The predicted lowest values of SP at the 50:50 proportion of the legume – herb axis (Fig. 6) indicates a diversity effect of WC and PLAN in lowering the proportion of N_2O derived from nitrification (Denk et al., 2017). Cumulative emissions decreased with higher herb and lower legume proportions, as found by Bracken et al. (2020). The inclusion of legumes and herbs such as white clover and ribwort plantain can not only reduce the requirements for high fertiliser N inputs (Nyfeler et al., 2009, 2011), but possibly also reduce N_2O emissions due to a biological nitrification inhibition effect caused by the presence ribwort plantain (de Klein et al., 2019). The fact that this diversity effect was significant for ambient soil moisture conditions would be consistent with the prevalence of nitrification under these drier soil moisture conditions and inhibition of nitrification in the presence of PLAN. Conversely, the lack of such a significant diversity effect under wet soil conditions might be explained by the prevalence of denitrification.

Similarly, the higher predicted $\delta^{15}N^{bulk}$ associated with higher herb proportion under ambient soil moisture conditions on both the herb – grass and legume – herb axes (Fig. 7) also suggests that ribwort plantain could have a biological inhibition effect on nitrification, as N_2O derived from nitrification is usually more depleted in ^{15}N (Popp et al., 2002; Toyoda et al., 2002). Gardiner et al. (2018) have shown how compounds, such as aucubin, produced by ribwort plantain can inhibit nitrification and reduce N_2O emissions. Carlton et al. (2019) also reported significantly less ammonia oxidising bacteria, that can produce N_2O during nitrification, related to ribwort plantain grown in swards with PRG and WC. Further studies are necessary to improve our understanding of the long-term effects of ribwort plantain on N_2O production processes at a field scale, but the results of this study and Bracken et al. (2020) would be consistent with ribwort plantain having a role in biological nitrification inhibition and suppression of N_2O emissions.

Under wet soil moisture conditions the predicted SP values steeply decline with increased proportions of legumes (Fig. 6) which would indicate that denitrification is increasingly important as the source of N_2O as sward legume content increases, under wet conditions. This supports the findings of Bracken et al. (2020) who found the greatest N_2O emissions under wet soil conditions with increasing proportions of legumes (approximately twice as great as those under ambient soil conditions). Hatch et al. (1990, 1991) showed net N mineralisation was greater under grass-clover swards after soil rewetting. This would suggest that there may already be higher levels of mineral N in soil solution associated with greater biological N fixation with increasing sward legume content, and that N applied as fertiliser and/or biologically fixed N that has been mineralized is then more vulnerable to denitrification under such wet soil conditions. The implication of these results suggest that particular care is needed in managing N in legume-containing swards to avoid excess mineral N in soil solution, particularly under wet soil conditions when it is vulnerable to conversion to N_2O via denitrification. Avoiding application of N fertiliser when soils are wet or when they are likely to become wet, might be a possible management strategy to avoid such emissions. Improved monitoring and forecasting of soil moisture conditions might aid soil managers in making better decisions in this regard. There is a much flatter decline in $\delta^{15}N^{bulk}$ under wet soil conditions with increasing legume proportions. This would be expected if denitrification is more likely under these conditions since there would be less discrimination of the heavier ^{15}N isotope (Popp et al., 2002; Toyoda et al., 2002). Advances in soil sensor and associated technologies and precision modelling and mapping approaches might facilitate the development of decision support systems for such a precision agriculture approach to mitigate N_2O and other N emissions (Thomas et al., 2016).

The present study aimed to assess the potential of using an adapted version of the isotopomer mapping approach described by Congreaves et al. (2019) for a field study in which a ^{15}N labelled fertiliser was applied. Even though this approach is generally applied in natural abundance studies it proved suitable in the current study as measured endmember values based on the mixing line presented in Fig. 3 (top) enabled the determination $F_{(N)}$ and $F_{(D)}$. The current study highlights that this adapted isotopomer mapping approach is another complementary source partitioning method that can be applied to enriched N_2O data typically measured for the purpose of a ^{15}N tracer experiment to provide more insights into the details of the soil N cycle (Müller et al., 2007, 2014). Combining such a field study with controlled incubations of the same soil could provide more detailed information relating to precise transition stages from nitrification to denitrification related to changes in soil WFPS (Congreaves et al., 2019). Given the numerous isotope effects of different soil N transformations (Denk et al., 2017) that could impact N_2O isotopomer measurements it is recommended to carefully consider these during the experimental design of such studies. Combining field studies with controlled incubation experiments and careful modelling of measured isotope effects could help increase our understanding of these processes at a field scale, as has been previously suggested by Decock and Six (2013). This could improve our ability to determine the effectiveness of management strategies, such as adopting multispecies swards that include ribwort plantain, to biologically inhibit nitrification and mitigate N_2O emissions.

5. Conclusion

The current study attributed N_2O emissions from this grassland soil mostly to a mixture of nitrification and denitrification using an N_2O isotopomer mapping approach. The range of estimated WFPS under both ambient and elevated soil moisture conditions widely overlapped and rarely exceeded 70%. This may explain why denitrification was not detected as the dominant N_2O source as formerly predicted. Simplex modelled SP and $\delta^{15}N^{bulk}$ outputs indicated that the inclusion of ribwort plantain may biologically inhibit nitrification under drier soil moisture conditions (31% to 75% WFPS) but not under wetter soil conditions (43% to 77% WFPS). Predicted SP and $\delta^{15}N^{bulk}$ under elevated soil moisture conditions (43% to 74% WFPS) suggest that increased denitrification

may occur with higher proportions of legumes when soils are wet. Such information is particularly useful to suggest possible management options that may help mitigate N_2O emissions. Appropriate management of N fertiliser source and application timing to soil moisture conditions could be a useful management strategy to lower N_2O emissions. The combination of field scale measurements of N_2O emissions and isotopomers with controlled higher frequency measurements from ^{15}N tracer or natural abundance soil incubation studies would likely provide more detailed information required to improve the accuracy of field and farm scale models of N cycling and the impacts of different management strategies on N use and losses from such systems.

Funding

The Teagasc Walsh Fellowship Scheme, Ireland (Ref: 2016075) and the UCD Seed Funding Scheme, Ireland (Ref: 42767) financially supported this work. The experimental plots used in this study were part of the SmartGrass project, funded by the Department of Agriculture, Food and the Marine (RSF 11/S/147: Smartgrass).

CRediT authorship contribution statement

Conor J. Bracken: Methodology, Formal analysis, Investigation, Conceptualization, Writing – original draft, Visualization, Project administration, Data curation. **Gary J. Lanigan:** Conceptualization, Methodology, Resources, Validation, Writing – review & editing, Supervision, Funding acquisition. **Karl G. Richards:** Conceptualization, Methodology, Resources, Validation, Writing – review & editing, Supervision, Funding acquisition. **Christoph Müller:** Conceptualization, Methodology, Resources, Validation, Writing – review & editing, Supervision, Funding acquisition. **Saoirse R. Tracy:** Conceptualization, Methodology, Resources, Validation, Writing – review & editing, Supervision, Funding acquisition. **James Grant:** Methodology, Formal analysis, Validation, Visualization. **Dominika J. Krol:** Validation, Writing – review & editing. **Helen Sheridan:** Resources, Writing – review & editing, Conceptualization. **Mary Bridget Lynch:** Resources, Writing – review & editing, Conceptualization. **Cornelia Grace:** Resources, Conceptualization. **Rochelle Fritch:** Resources, Conceptualization. **Paul N.C. Murphy:** Conceptualization, Methodology, Resources, Validation, Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to gratefully acknowledge the technical staff at UCD Lyons Farm; UCD School of Agriculture and Food Science and Teagasc Environmental Research Center for their assistance with this work. We would also like to thank the two anonymous reviewers for their constructive comments during the peer-review process that helped improve the quality of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.146515.

References

Abbasi, M.K., Adams, W.A., 2000. Estimation of simultaneous nitrification and denitrification in grassland soil associated with urea-N using ^{15}N and nitrification inhibitor. Biol. Fertil. Soils 31, 38–44.

Alves, B.J.R., Smith, K.A., Flores, R.A., Cardoso, A.S., Oliveira, W.R.D., Jantalia, C.P., Urquiaga, S., Boddey, R.M., 2012. Selection of the most suitable sampling time for static chambers for the estimation of daily mean N_2O flux from soils. Soil Biol. Biochem. 46, 129–135.

Arnold, P.W., 1954. Losses of nitrous oxide from soil. J. Soil Sci. 5, 116–128.

Barford, C.C., Montoya, J.P., Altabet, M.A., Mitchell, R., 1999. Steady-state nitrogen isotope effects of N_2 and N_2O production in Paracoccus denitrificans. Appl. Environ. Microbiol. 65, 989–994.

Bateman, E.J., Baggs, E.M., 2005. Contributions of nitrification and denitrification to N_2O emissions from soils at different water-filled pore space. Biol. Fertil. Soils 41, 379–388.

Bergstermann, A., Cárdenas, L., Bol, R., Gilliam, L., Goulding, K., Meijide, A., Scholefield, D., Vallejo, A., Well, R., 2011. Effect of antecedent soil moisture conditions on emissions and isotopologue distribution of N_2O during denitrification. Soil Biol. Biochem. 43, 240–250.

Bol, R., Toyoda, S., Yamulki, S., Hawkins, J.M.B., Cardenas, L., Yoshida, N., 2003. Dual isotope and isotopomer ratios of N_2O emitted from a temperate grassland soil after fertiliser application. Rapid Commun. Mass Spectrom. 17, 2550–2556.

Bouwman, A.F., Beusen, A.H.W., Griffioen, J., Van Groenigen, J.W., Hefting, M.M., Oenema, O., van Puijenbroek, P.J.T.M., Seitzinger, S., Slomp, C.P., Stehfest, E., 2013. Global trends and uncertainties in terrestrial denitrification and N_2O emissions. Philosophical Transactions of the Royal Society London B368, 20130112.

Bracken, C.J., Lanigan, G.J., Richards, K.G., Müller, C., Tracy, S.R., Grant, J., Krol, D.J., Sheridan, H., Lynch, M.B., Grace, C., Fritch, R., Murphy, P.N.C., 2020. Sward composition and soil moisture conditions affect nitrous oxide emissions and soil nitrogen dynamics following urea-nitrogen application. Sci. Total Environ. 722 (137780), 1–12.

Bracken, C.J., Lanigan, G.J., Richards, K.G., Müller, C., Tracy, S.R., Well, R., Carolan, R., Murphy, P.N.C., 2021. Development and verification of a novel isotopic N_2O measurement technique for discrete static chamber samples using Cavity Ring Down Spectroscopy. Rapid Commun. Mass Spectrom. 35, e9049. https://doi.org/10.1002/rcm.9049.

Buchen, C., Lewicka-Szczebak, D., Flessa, H., Well, R., 2018. Estimating N_2O processes during grassland renewal and grassland conversion to maize cropping using N_2O isotopocules. Rapid Commun. Mass Spectrom. 32, 1053–1067.

Cardenas, L.M., Bol, R., Lewicka-Szczebak, D., Gregory, A.S., Mathews, G.P., Whalley, W.R., Misselbrook, T.H., Scholefield, D., Well, R., 2017. Effect of soil saturation on denitrification in a grassland soil. Biogeosciences 14, 4691–4710.

Carlton, A.J., Cameron, K.C., Di, H.J., Edwards, G.R., Clough, T.J., 2019. Nitrate leaching losses are lower from ryegrass/white clover forages containing plantain than from ryegrass/white clover forages under different irrigation. N. Z. J. Agric. Res. 62, 150–172.

Congreaves, K.A., Phan, T., Farrell, R.E., 2019. A new look at an old concept: using $^{15}N_2O$ isotopomers to understand the relationship between soil moisture and N_2O production pathways. Soil 5, 265–274.

Coplen, T.B., 2011. Guidelines and recommended terms for expression of stable isotope-ratio and gas-ratio measurement results. Rapid Commun. Mass Spectrom. 25, 2538–2560.

Core Team, R., 2017. R: a language and environment for statistical computing. R Foundation for Statistical Computing. Austria. URL, Vienna https://www.R-project.org/.

Cornell, J.A., 2002. Experiments with Mixtures: Designs, Models, and the Analysis of Mixture Data. 3rd ed. Wiley, Chichester.

Davidson, E.A., 1991. Fluxes of nitrous oxide and nitric oxide from terrestrial ecosystems. In: Rogers, J.E., Whitman, W.B. (Eds.), Microbial Production and Consumption of Greenhouse Gases: Methane, Nitrogen Oxides, and Halomethanes. American Society for Microbiology, Washington DC.

Davidson, E.A., Verchot, L.V., 2000. Testing the hole-in-the-pipe model of nitric and nitrous oxide emissions from soils using the TRAGNET database. Glob. Biogeochem. Cycles 14, 1035–1043.

Decock, C., Six, J., 2013. How reliable is the intramolecular distribution of ^{15}N in N_2O to source partition N_2O emitted from soil? Soil Biol. Biochem. 65, 114–127.

Denk, T.R.A., Mohn, J., Decock, C., Lewicka-Szczebak, D., Harris, E., Butterbach-Bahl, K., Kiese, R., Wolf, B., 2017. The nitrogen cycle: a review of isotope effects and isotope modelling approaches. Soil Biol. Biochem. 105, 121–137.

Deppe, M., Well, R., Giesemann, A., Spott, O., Flessa, H., 2017. Soil N_2O fluxes and related processes in laboratory incubations simulating ammonium fertilizer depots. Soil Biol. Biochem. 104, 68–80.

Éireann, Met, 2018. 30 year averages. Casement Available at. https://www.met.ie/climate-ireland/1981-2010/casement.html [accessed: 15/11/2018].

Fowler, D., Coyle, M., Skiba, U., Sutton, M.A., Cape, J.N., Reis, S., Sheppard, L.J., Jenkins, A., Grizzetti, B., Galloway, J.N., Vitousek, P., Leach, A., Bouwman, A.F., Butterbach-Bahl, K., Dentener, F., Stevenson, D., Amann, M., Voss, M., 2013. The global nitrogen cycle in the twenty-first century. Philos. Trans. R. Soc. B 368, 1–13.

Friedman, L., Bigeleisen, J., 1950. Oxygen and nitrogen isotope effects in the decomposition of ammonium nitrate. J. Chem. Phys. 18, 1325–1331.

Galloway, J.N., Cowling, E.B., 2002. Reactive nitrogen and the world: 200 years of change. Ambio 31, 64–71.

Gardiner, C.A., Clough, T.J., Cameron, K.C., Di, H.J., Edwards, G.R., De Klein, C.A., 2018. Potential inhibition of urine patch nitrous oxide emissions by Plantago lanceolata and its metabolite aucubin. N. Z. J. Agric. Res. 61, 495–503.

Gayon, U., Dupetit, G., 1882. Sur la transformation des nitrates en nitrites. Comptes Rendus hebdomadaires des Séance de l'Academie des Sciences (Paris) 95, 1365–1367.

Godfray, H.C.J., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., Pretty, J., Robinson, S., Thomas, S.M., Toulmin, C., 2010. Food security: the challenge of feeding 9 billion people. Science 327, 812–818.

Grace, C., Boland, T.M., Sheridan, H., Brennan, E., Fritch, R., Kirwan, L., Lynch, M.B., 2018. The effect of varying levels of nitrogen input on the annual and seasonal dry matter yield of multispecies mixtures compared to a perennial ryegrass monoculture. In:

Grace, C. (Ed.), (2018) The potential of multispecies swards compared to perennial ryegrass only swards for dry matter yield, chemical composition and animal performance. PhD Thesis. University College Dublin.
Grace, C., Boland, T.M., Sheridan, H., Brennan, E., Fritch, R., Lynch, M.B., 2019. The effect of grazing versus cutting on dry matter production of multispecies and perennial ryegrass-only swards. Grass Forage Sci. 00, 1–13.
Hatch, D.J., Jarvis, S.C., Philipps, L., 1990. Field measurement of nitrogen mineralization using soil core incubation and acetylene inhibition of nitrification. Plant Soil 124, 97–107.
Hatch, D.J., Jarvis, S.C., Reynolds, S.E., 1991. An assessment of the contribution of net mineralization to N cycling in grass swards using a field incubation method. Plant Soil 138, 23–32.
Ibraim, E., Wolf, B., Harris, E., Gasche, R., Wei, J., Yu, L., Kiese, R., Eggleston, S., Butterbach-Bahl, K., Zeeman, M., Tuzson, B., Emmenegger, L., Six, J., Henne, S., Mohn, J., 2019. Attribution of N_2O sources in a grassland soil with laser spectroscopy based isotopocule analysis. Biogeosciences 16, 3247–3266.
IPCC, 2019. Climate Change and Land: an IPCC Special Report on Climate Change, Desertification, Land Degradation, Sustainable Land Management, Food Security, and Greenhouse Gas Fluxes in Terrestrial Ecosystems. IPCC Secretariat, Geneva, Switzerland.
IUSS Working Group, 2014. World reference base for soil resources 2014. International soil classification system for naming soils and creating legends for soil maps. World Soil Resources Report No. 106. FAO, Rome.
Kahmen, A., Renker, C., Unsicker, S.B., Buchmann, N., 2006. Niche complementarity for nitrogen: an explanation for the biodiversity and ecosystem functioning relationship? Ecology 87, 1244–1255.
Kirwan, L., Lüscher, A., Sebastià, M.T., Finn, J.A., Collins, R.P., Porqueddu, C., Helgadottir, A., Baadshaug, O.H., Brophy, C., Coran, C., Dalmannsdóttir, S., Delgado, I., Elgersma, A., Fothergill, M., Frankow-Lindberg, B.E., Golinski, P., Grieu, P., Gustavsson, A.M., Höglind, M., Huguenin-Elie, O., Iliadis, C., Jørgensen, M., Kadziuliene, Z., Karyotis, T., Lunnan, T., Malengier, M., Maltoni, S., Meyer, V., Nyfeler, D., Nykanen-Kurki, P., Parente, J., Smit, H.J., Thumm, U., Connolly, J., 2007. Evenness drives consistent diversity effects in intensive grassland systems across 28 European sites. J. Ecol. 95, 530–539.
Kirwan, L., Connolly, J., Finn, J.A., Brophy, C., Lüscher, A., Nyfeler, D., Sebastià, M.T., 2009. Diversity–interaction modelling: estimating contributions of species identities and interactions to ecosystem function. Ecology 90, 2032–2038.
de Klein, C.A.M., Harvey, M., 2012. Nitrous Oxide Chamber Methodology Guidelines. Wellington, Ministry of Primary Industries.
de Klein, C.A.M., van der Weerden, T.J., Luo, J., Cameron, K.C., Di, H.J., 2019. A review of plant options for mitigating nitrous oxide emissions from pasture-based systems. N. Z. J. Agric. Res. 63, 29–43.
Koba, K., Osaka, K., Tobari, Y., Toyoda, S., Ohte, N., Katsuyama, M., Suzuki, N., Itoh, M., Yamagishi, H., Kawasaki, M., Kim, S.J., Yoshida, N., Nakajima, T., 2009. Biogeochemistry of nitrous oxide in groundwater in a forested ecosystem elucidated by nitrous oxide isotopomer measurements. Geochim. Cosmochim. Acta 73, 3115–3133.
Krol, D.J., Forrestal, P.J., Lanigan, G.J., Richards, K.G., 2015. In situ N_2O emissions are not mitigated by hippuric and benzoic acids under denitrifying conditions. Sci. Total Environ. 511, 362–368.
Lalor, S.T.J., 2004. Soils of UCD research farm, Lyons Estate. Celbridge, Co. Kildare. Department of Crop Science, H. & Forestry, University College Dublin (MSc Thesis).
Laville, P., Lehuger, S., Loubet, B., Chaumartin, F., Cellier, P., 2011. Effect of management, climate and soil conditions on N_2O and NO emissions from an arable crop rotation using high temporal resolution measurements. Agric. For. Meteorol. 151, 228–240.
Lawson, J., Willden, C., 2016. Mixture experiments in R using mixexp. Journal of Statistical Software, Code Snippets 72 (2), 1–20.
Leimer, S., Oelmann, Y., Wirth, C., Wilcke, W., 2015. Time matters for plant diversity effects on nitrate leaching from temperate grassland. Agric. Ecosyst. Environ. 211, 155–163.
Leimer, S., Oelmann, Y., Eisenhauer, N., Milcu, A., Roscher, C., Scheu, S., Weigelt, A., Wirth, C., Wilcke, W., 2016. Mechanisms behind plant diversity effects on inorganic and organic N leaching from temperate grassland. Biogeochemistry 131, 339–353.
Lewicka-Szczebak, D., Augusin, J., Giesemann, A., Well, R., 2017. Quantifying N_2O reduction to N_2 based on N_2O isotopocules – validation with independent methods (helium incubation and ^{15}N gas flux method). Biogeosciences 14, 711–732.
Linn, D.M., Doran, J.W., 1984. Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. Soil Sci. Soc. Am. J. 48, 1267–1272.
Loreau, M., Hector, A., 2001. Partitioning selection and complementarity in biodiversity experiments. Nature 412, 72–76.
Makowski, D., 2019. N_2O increasing faster than expected. Nat. Clim. Chang. 9, 909–910. https://doi.org/10.1038/s41558-019-0642-2.
Malcolm, B.J., Cameron, K.C., Di, H.J., Moir, J.L., 2014. The effect of four different pasture species compositions on nitrate leaching losses under high N loading. Soil Use Manag. 30, 58–68.
Mariotti, A., 1983. Atmospheric nitrogen is a reliable standard for natural ^{15}N abundance measurements. Nature 303 (5919), 685–687.
Mariotti, A., Germon, J.C., Hubert, P., Kaiser, P., Letolle, R., Tardieux, A., Tardieux, P., 1981. Experimental determination of nitrogen kinetic isotope fractionation: some principles; illustration for the denitrification and nitrification processes. Plant Soil 62, 413–430.
Mohn, J., Wolf, B., Toyoda, S., Lin, C.-T., Liang, M.-C., Brüggemann, N., Wissel, H., Steiker, A.E., Dyckmans, J., Szwec, L., Ostrom, N.E., Casciotti, K.L., Forbes, M., Giesemann, A., Well, R., Doucett, R.R., Yarnes, C.T., Ridley, A.R., Kaiser, J., Yoshida, N., 2014. Interlaboratory assessment of nitrous oxide isotopomer analysis by isotope ratio mass spectrometry and laser spectroscopy: current status and perspectives. Rapid Commun. Mass Spectrom. 28, 1995–2007.
Müller, C., Clough, T.J., 2014. Advances in understanding nitrogen flows and transformations: gaps and research pathways. J. Agric. Sci. (Camb.) 152, S34–S44.
Müller, C., Rütting, T., Kattge, J., Laughlin, R.J., Stevens, R.J., 2007. Estimation of parameters in complex ^{15}N tracing models by Monte Carlo sampling. Soil Biol. Biochem. 39, 715–726.
Müller, C., Laughlin, R.J., Spott, O., Rütting, T., 2014. Quantification of N_2O emission pathways via a 15N tracing model. Soil Biol. Biochem. 72, 44–54.
Niklaus, P.A., Wardle, D.A., Tate, K.R., 2006. Effects of plant species diversity and composition on nitrogen cycling and the trace gas balance of soils. Plant Soil 282, 83–98.
Niklaus, P.A., Le Roux, X., Poly, F., Buchmann, N., Scherer-Lorenzen, M., Weigelt, A., Barnard, R.L., 2016. Plant species diversity affects soil–atmosphere fluxes of methane and nitrous oxide. Oecologia 181, 919–930.
Nõmmik, H., 1956. Investigations on denitrification in soil. Acta Agric. Scand. 6, 195–228.
Nyfeler, D., Huguenin-Elie, O., Suter, M., Frossard, E., Connolly, J., Luscher, A., 2009. Strong mixture effects among four species in fertilized agricultural grassland led to persistent and consistent transgressive overyielding. J. Appl. Ecol. 46, 683–691.
Nyfeler, D., Huguenin-Elie, O., Suter, M., Frossard, E., Luscher, A., 2011. Grass-legume mixtures can yield more nitrogen than legume pure stands due to mutual stimulation of nitrogen uptake from symbiotic and non-symbiotic sources. Agric. Ecosyst. Environ. 140, 155–163.
Ostrom, P.H., DeCamp, S., Gandhi, H., Haslun, J., Ostrom, N.E., 2021. The influence of tillage and fertilizer on the flux and source of nitrous oxide with reference to atmospheric variation using laser spectroscopy. Biogeochemistry https://doi.org/10.1007/s10533-020-00742-y.
Parkin, T.B., 2008. Effect of sampling frequency on estimates of cumulative nitrous oxide emissions. J. Environ. Qual. 37, 1390–1395.
Pérez, T., Garcia-Montiel, D.C., Trumbore, S.E., Tyler, S.C., de Camargo, P., Moreira, M., Piccolo, M., Cerri, C.C., 2006. Nitrous oxide nitrification and denitrification ^{15}N enrichment factors from Amazon forest soils. Ecol. Appl. 16, 2153–2167.
Petersen, S.O., Well, R., Taghizadeh-Toosi, A., Clough, T., 2020. Seasonally distinct sources of N_2O in acid organic soil drained for agriculture as revealed by N_2O isotopomer analysis. Biogeochemistry 147, 15–33.
Popp, B.N., Westley, M.B., Toyoda, S., Miwa, T., Dore, J.E., Yoshida, N., Rust, T.M., Sansone, F.J., Russ, M.E., Ostrom, N.E., Ostrom, P.H., 2002. Nitrogen and oxygen isotopomeric constraints on the origins and sea-to-air flux of N_2O in the oligotrophic subtropical North Pacific gyre. Glob. Biogeochem. Cycles 16 (12–1) (12-10).
Prokopiou, M., Martinerie, P., Sapart, C.J., Witrant, E., Monteil, G., Ishijima, K., Bernard, S., Kaiser, J., Levin, I., Blunier, T., Etheridge, D., Dlugokencky, E., van de Wal, R.S.W., Röckmann, T., 2017. Constraining N_2O emissions since 1940 using firn air isotope measurements in both hemispheres. Atmos. Chem. Phys. 17, 4539–4564.
Prokopiou, M., Sapart, C.J., Rosen, J., Sperlich, P., Blunier, T., Brook, E., van de Wal, R.S.W., Röckmann, T., 2018. Changes in the isotopic signature of atmospheric nitrous oxide and its global average source during the last three millennia. J. Geophys. Res.-Atmos. 123, 10757–10773.
Scherer-Lorenzen, M., Palmborg, C., Prinz, A., Schulze, E.-D., 2003. The role of plant diversity and composition for nitrate leaching in grasslands. Ecology 84, 1539–1552.
Smith, K.A., Dobbie, K., 2001. The impact of sampling frequency and sampling times on chamber-based measurements of N_2O emissions from fertilized soils. Glob. Chang. Biol. 7, 933–945.
Steffen, W., Crutzen, P.J., McNeill, J.R., 2007. The Anthropocene: are humans now overwhelming the great forces of nature? Ambio 36, 614–621.
Stevens, R.J., Laughlin, R.J., Burns, L.C., Arah, J.R.M., Hood, R.C., 1997. Measuring the contributions of nitrification and denitrification to the flux of nitrous oxide from soil. Soil Biol. Biochem. 29, 139–151.
Subbarao, G.V., Rondon, M., Ito, O., Ishikawa, T., Rao, I.M., Nakahara, K., Lascano, C., Berry, W.L., 2007. Biological nitrification inhibition (BNI) - is it a widespread phenomenon? Plant Soil 294, 5–18.
Sutka, R.L., Ostrom, N.E., Ostrom, P.H., Breznak, J.A., Gandhi, H., Pitt, A.J., Li, F., 2006. Distinguishing nitrous oxide production from nitrification and denitrification on the basis of isotopomer abundances. Appl. Environ. Microbiol. 72, 638–644.
Thomas, I.A., Jordan, P., Mellander, P.-E., Fenton, O., Shine, O., hUallacháin, D.Ó., Creamer, R., McDonald, N.T., Dunlop, P., Murphy, P.N.C., 2016. Improving the identification of hydrologically sensitive areas using LiDAR DEMs for the delineation and mitigation of critical source areas of diffuse pollution. Sci. Total Environ. 556, 276–290.
Thompson, R.L., Lassaletta, L., Patra, P.K., Wilson, C., Wells, K.C., Gressent, A., Koffi, E.N., Chipperfield, M.P., Winiwarter, W., Davidson, E.A., Tian, H., Canadell, J.G., 2019. Acceleration of global N_2O emissions seen from two decades of atmospheric inversion. Nat. Clim. Chang. 9, 993–998. https://doi.org/10.1038/s41558-019-0613-7.
Toyoda, S., Yoshida, N., 1999. Determination of nitrogen isotopomers of nitrous oxide on a modified isotope ratio mass spectrometer. Anal. Chem. 71, 4711–4718.
Toyoda, S., Yoshida, N., Miwa, T., Matsui, Y., Yamagishi, H., Tsunogai, U., Nojiri, Y., Tsurushima, N., 2002. 'Production mechanism and global budget of N_2O inferred from its isotopomers in the western North Pacific. Geophys. Res. Lett. 29, 2001GL014311.
Toyoda, S., Yano, M., S-i, Nishimura, Akiyama, H., Hayakawa, A., Koba, K., Sudo, K., Yagi, K., Makabe, A., Tobari, Y., Ogawa, N.O., Ohkouchi, N., Yamada, K., Yoshida, N., 2011. Characterization and production and consumption processes of N_2O emitted from temperate agricultural soils determined via isotopomer ratio analysis. Glob. Biogeochem. Cycles 25, GB2008. https://doi.org/10.1029/2009GB003769.
Waters, C., Zalasiewicz, J., Summerhayes, C., Barnosky, A.D., Poirier, C., Galuszka, A., Cearreta, A., Edgeworth, M., Ellis, E.C., Ellis, M., Jeandel, C., Leinfelder, R., McNeill, J.R., Richter, D. deB, Steffen, W., Syvitski, J., Vidas, D., Wagreich, M., Williams, M., Zhisheng, A., Grinevald, J., Odada, E., Oreskes, N., Wolfe, A.P., 2016. 'The Anthropocene is functionally and stratigraphically distinct from the Holocene. Science 351 (11d2622), 1–10.

Well, R., Flessa, H., 2009. Isotopologue enrichment factors of N_2O reduction in soils. Rapid Commun. Mass Spectrom. 23, 2996–3002.
Well, R., Kurganova, I., Lopes de Gerenyu, V., Flessa, H., 2006. Isotopomer signatures of soil-emitted N_2O under different moisture conditions - a microcosm study with arable loess soil. Soil Biol. Biochem. 38, 2923–2933.
Well, R., Eschenbach, W., Flessa, H., Von der Heide, C., Weymann, D., 2012. Are dual isotope and isotopomer ratios of N_2O useful indicators for N_2O turnover during denitrification in nitrate-contaminated aquifers? Geochim. Cosmochim. Acta 90, 265–282.
Wickham, H., 2009. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York.
Wolf, B., Merbold, L., Decock, C., Tuzson, B., Harris, E., Six, J., Emmenegger, L., Mohn, J., 2015. First on-line isotopic characterization of N_2O above intensively managed grassland. Biogeosciences 12, 2517–2531.
Wu, D., Well, R., Cárdenas, L.M., Fuß, R., Lewicka-Szczebak, D., Köster, J.R., Brüggemann, N., Bol, R., 2019. Quantifying N_2O reduction to N_2 during denitrification in soils via isotopic mapping approach: model evaluation and uncertainty analysis. Environ. Res. 179, 1–8.
Yoshida, N., 1988. ^{15}N-depleted N_2O as a product of nitrification. Nature 335, 528–529.
Zou, Y., Hirono, Y., Yanai, Y., Hattori, S., Toyoda, S., Yoshida, N., 2014. Isotopomer analysis of nitrous oxide accumulated in soil cultivated with tea (Camellia sinensis) in Shizuoka, central Japan. Soil Biol. Biochem. 77, 276–291.