



## Source partitioning using N<sub>2</sub>O isotopomers and soil WFPS to establish dominant N<sub>2</sub>O production pathways from different pasture sward compositions



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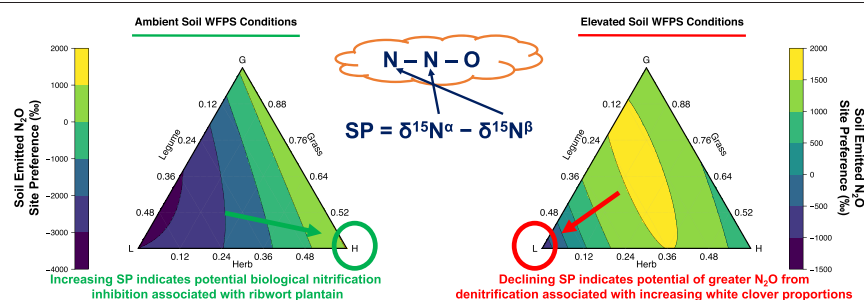
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### HIGHLIGHTS

- N<sub>2</sub>O samples from static chambers (<20 ml) analysed using novel CRDS technique.
- Site preference (‰) and soil WFPS (%) used to determine N<sub>2</sub>O production pathways.
- Daily N<sub>2</sub>O fluxes attributed to 34.2% nitrification and 29.0% denitrification.
- δ<sup>15</sup>N<sup>bulk</sup> and SP (drier soil) indicate ribwort plantain may inhibit nitrification.
- SP drop with increasing WC (wet soil) implies stimulation of denitrification.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Nitrous oxide (N<sub>2</sub>O) 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<sub>2</sub>O 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<sub>2</sub>O fluxes and associated isotopomers from eight experimental plots (7.8 m<sup>2</sup>) post a urea-N fertiliser application (40 kg N ha<sup>-1</sup>). 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<sub>2</sub>O 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 δ<sup>15</sup>N<sup>bulk</sup> ( $(\delta^{15}N^{\alpha} + \delta^{15}N^{\beta}) / 2$ ) values were used to attribute N<sub>2</sub>O production to nitrification, denitrification or a mixture of both nitrification and denitrification over a range of soil WFPS (%). Daily N<sub>2</sub>O fluxes ranged from 8.26 to 86.86 g N<sub>2</sub>O-N ha<sup>-1</sup> d<sup>-1</sup>. Overall, 34.2% of daily N<sub>2</sub>O 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

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

ribwort plantain on predicted SP and  $\delta^{15}\text{N}^{\text{bulk}}$  indicated that the inclusion of ribwort plantain may decrease  $\text{N}_2\text{O}$  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  $\text{N}_2\text{O}$  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  $\text{N}_2\text{O}$  mitigation strategies.

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## 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  $\text{N}_2\text{O}$  (Bouwman et al., 2013; Fowler et al., 2013) and worryingly global tropospheric  $\text{N}_2\text{O}$  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  $\text{N}_2\text{O}$  and  $\text{NO}_3^-$ , 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  $\text{N}_2\text{O}$  site preference ( $\text{SP} = \delta^{15}\text{N}^\alpha - \delta^{15}\text{N}^\beta$ ) since pre-industrial times, potentially signals a relative shift from denitrification to nitrification associated with increased  $\text{N}_2\text{O}$  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 ( $\text{N}_2\text{O}$  and  $\text{NO}_3^-$ ), 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  $\text{N}_2\text{O}$  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  $\text{NO}_3^-$  leaching (Carlton et al., 2019). Using measurements of  $\text{N}_2\text{O}$  isotopomers could provide useful insights into the impact of such management practices on soil N cycling processes.

Soil derived  $\text{N}_2\text{O}$  can come from nitrification ( $\text{NH}_4^+ \rightarrow \text{NO}_3^-$ ) and denitrification ( $\text{NO}_3^- \rightarrow \text{N}_2$ ) 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  $^{15}\text{N}/^{14}\text{N}$  in the alpha ( $\alpha$ ) and beta ( $\beta$ ) position of  $\text{N}_2\text{O}$ , sometimes referred to as isotopomer measurements (Friedman and Bigeleisen, 1950; Toyoda and Yoshida, 1999). Isotopic  $\text{N}_2\text{O}$  measurements ( $\delta^{15}\text{N}^\alpha$ ,  $\delta^{15}\text{N}^\beta$ ,  $\delta^{15}\text{N}^{\text{bulk}}$ , 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 ( $\delta$ ) values in per mil (‰) (Coplen, 2011). These are calculated relative to the isotope-ratio of the international standard for N, atmospheric  $\text{N}_2$  ( $3.677 \times 10^{-3}$ ) (Mariotti, 1983).  $\delta^{15}\text{N}^{\text{bulk}}$  is equivalent to  $(\delta^{15}\text{N}^\alpha + \delta^{15}\text{N}^\beta)/2$  (Toyoda and Yoshida, 1999). Tracer experiments using  $^{15}\text{N}$  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  $\text{N}_2\text{O}$  isotope-ratios in the region of 0 to 50 atom%, approximately equivalent to  $\delta^{15}\text{N}^{\text{bulk}} = -1000\text{‰}$  to 271,000‰.  $\text{N}_2\text{O}$  from nitrification becomes highly depleted in  $^{15}\text{N}$  (Yoshida, 1988). Whereas, denitrification preferentially selects isotopically light  $\text{N}_2\text{O}$  during further reduction to  $\text{N}_2$ , leaving the residual  $\text{N}_2\text{O}$  enriched in  $^{15}\text{N}$  (Barford et al., 1999).  $\text{N}_2\text{O}$  produced from  $\text{NH}_4^+$  will be enriched in  $^{15}\text{N}$  at the  $\alpha$  position and depleted at the  $\beta$  position due to the preferential cleavage of  $^{14}\text{N}-^{16}\text{O}$  bonds over  $^{15}\text{N}-^{16}\text{O}$  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  $^{15}\text{N}$  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  $\text{N}_2\text{O}$  from nitrification (higher SP values and lower  $\delta^{15}\text{N}^{\text{bulk}}$  values) and  $\text{N}_2\text{O}$  from denitrification (lower SP values and higher  $\delta^{15}\text{N}^{\text{bulk}}$  values).

Plotting SP vs  $\delta^{15}\text{N}^{\text{bulk}}$  or  $\delta^{18}\text{O}$  (aka 'isotopomer mapping' Lewicka-Szczebak et al., 2017) enables distinction of  $\text{N}_2\text{O}$  from nitrification (SP: 28 to 36‰ and  $\delta^{15}\text{N}^{\text{bulk}}$ : -60 to -30‰) and denitrification (SP: -10 to 0‰ and  $\delta^{15}\text{N}^{\text{bulk}}$ : -40 to 0‰) (Koba et al., 2009; Toyoda et al., 2011; Zou et al., 2014). Estimates of  $\text{N}_2\text{O}$  mixing from different processes and the further reduction of  $\text{N}_2\text{O}$  to  $\text{N}_2$  have been made using 'isotopomer maps' (Well et al., 2012; Lewicka-Szczebak et al., 2017). Further reduction of  $\text{N}_2\text{O}$  to  $\text{N}_2$  can increase SP values of residual  $\text{N}_2\text{O}$  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  $\text{N}_2\text{O}$  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  $\text{N}_2\text{O}$  from nitrification ( $F_N = 3.19 - 0.041x$ ) 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  $\text{N}_2\text{O}$  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 (Nömmik, 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  $\text{N}_2\text{O}$  came from either nitrification or denitrification for isotopic  $\text{N}_2\text{O}$  measurements from a  $^{15}\text{N}$  tracer field experiment. Bracken et al. (2020) quantified  $\text{N}_2\text{O}$  fluxes and cumulative  $\text{N}_2\text{O}$  emissions post a single application of a 2%  $^{15}\text{N}$  labelled urea fertiliser (for the purpose of a  $^{15}\text{N}$  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  $\text{N}_2\text{O}$  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-Szczepak 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 <sub>(soil)</sub>	$\delta^{15}N^{bulk}$
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<sup>-1</sup> yr<sup>-1</sup> 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<sup>-3</sup> (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.

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

whereby:

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

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

#### 2.4. Fertiliser application

A 2%  $^{15}\text{N}$  labelled urea fertiliser was prepared using 40.33285 g laboratory grade urea (Sigma Aldrich) and 0.82312 g of  $^{15}\text{N}$  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  $^{15}\text{N}$  labelled fertiliser to the soil surface in each of the chamber footings ( $0.16 \text{ m}^2$ ) and a further 37.5 mL to an adjacent area ( $0.09 \text{ m}^2$ ) for periodic soil sampling, equivalent to a rate of  $40 \text{ kg N ha}^{-1}$ . 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. $\text{N}_2\text{O}$ 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  $\text{N}_2\text{O}$  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  $\text{N}_2\text{O}$  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 plungered three times to evenly mix the gas inside chambers. The 60-min chamber closure period was considered suitable for sufficient  $\text{N}_2\text{O}$  build-up to enable distinction of soil emitted  $\text{N}_2\text{O}$  from ambient air  $\text{N}_2\text{O}$  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  $\text{N}_2\text{O}$   $\delta^{15}\text{N}^{\text{bulk}}$ ,  $\delta^{15}\text{N}^{\alpha}$ ,  $\delta^{15}\text{N}^{\beta}$  and SP, following the approach of Petersen et al. (2020), using a similar data screening rule to omit samples with  $\text{N}_2\text{O}$  concentrations <450 ppb. This ‘sample concentration rule’ was necessary since  $\text{N}_2\text{O}$  isotopomers were measured using CRDS and Petersen et al. (2020) showed that laser based measurements become more variable towards ambient atmospheric  $\text{N}_2\text{O}$  concentrations (330 ppb).

$$\delta^{15}\text{N}^{\text{X}}_{(\text{soil})} = \left( \delta^{15}\text{N}^{\text{X}}_{(\text{sample})} \times \text{N}_2\text{O}_{(\text{sample})} - \delta^{15}\text{N}^{\text{X}}_{(\text{air})} \times \text{N}_2\text{O}_{(\text{air})} \right) \div (\text{N}_2\text{O}_{(\text{sample})} - \text{N}_2\text{O}_{(\text{air})}) \quad (2)$$

$\delta^{15}\text{N}^{\text{X}}$  refers to either  $\delta^{15}\text{N}^{\text{bulk}}$ ,  $\delta^{15}\text{N}^{\alpha}$ ,  $\delta^{15}\text{N}^{\beta}$  or SP. ‘Soil’, ‘air’ and ‘sample’ refer to  $\text{N}_2\text{O}$  emitted from soil,  $\text{N}_2\text{O}$  in ambient air and mixture of these two (i.e. sample measurement), respectively. Average  $\delta$  values of tropospheric  $\text{N}_2\text{O}$  in ambient air were used in Eq. (2) for  $\delta^{15}\text{N}^{\text{bulk}}_{(\text{air})}$  (6.8‰),  $\delta^{15}\text{N}^{\alpha}_{(\text{air})}$  (15.8‰),  $\delta^{15}\text{N}^{\beta}_{(\text{air})}$  (−2.3‰) and  $\text{SP}_{(\text{air})}$  (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  $\delta$  values in ambient air can vary up to 30‰ which means substituting literature derived values could potentially lead to greater sampling error.

#### 2.6. $\text{N}_2\text{O}$ source partitioning using SP, $\delta^{15}\text{N}^{\text{bulk}}$ and soil WFPS

An isotopomer map was used to describe the linear relationship between SP and  $\delta^{15}\text{N}^{\text{bulk}}$ . The model of CRDS used in this study does not measure  $\delta^{18}\text{O}$ . Therefore, isotopomer maps using SP and  $\delta^{18}\text{O}$  (Well et al., 2012; Lewicka-Szczebak et al., 2017) and the additional estimation of  $\text{N}_2\text{O}$  reduction to  $\text{N}_2$  was beyond the scope of the current study. Adapting the approach applied by Congreaves et al. (2019), minimum and maximum  $\delta^{15}\text{N}^{\text{bulk}}$  values were used in the linear equation describing this relationship ( $y = -0.01145x - 126.5$ ; where  $y = \text{SP}$  and  $x = \delta^{15}\text{N}^{\text{bulk}}$ ) to determine measured endmember SP values for nitrification ( $\text{SP}_\text{N} = -86\text{‰}$ ) and denitrification ( $\text{SP}_\text{D} = -144\text{‰}$ ). This was done given the observed  $\text{N}_2\text{O}$  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 ( $F_\text{N}$ ) and denitrification ( $F_\text{D}$ ) (Deppe et al., 2017; Lewicka-Szczebak et al., 2017; Congreaves et al., 2019). An assumption that the minimum and maximum measured enriched  $\delta^{15}\text{N}^{\text{bulk}}$  values were suitable to distinguish nitrification and denitrification (Popp et al., 2002; Toyoda et al., 2002) was necessary to calculate  $F_\text{N}$  and  $F_\text{D}$  from Eqs. (3) and (4) below. The  $F_\text{N}$  and  $F_\text{D}$  values were determined from measured SP values ( $\text{SP}_\text{X}$ ) and the endmember  $\text{SP}_\text{N}$  and  $\text{SP}_\text{D}$  values above using Eqs. (3) and (4).

$$F_\text{N} = \text{SP}_\text{X} - \text{SP}_\text{D} / \text{SP}_\text{N} - \text{SP}_\text{D} \quad (3)$$

$$F_\text{D} = 1 - F_\text{N} \quad (4)$$

$F_\text{N}$  and  $F_\text{D}$  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 ( $\geq 90\%$ )  $\text{N}_2\text{O}$  production processes by the measured fractions of nitrification ( $F_\text{N}$ ); Nitrification =  $F_\text{N} \geq 0.9$ , Denitrification =  $F_\text{N} \leq 0.1$ , Mixture of Nitrification and Denitrification =  $0.1 \leq F_\text{N} \leq 0.9$ . Linear regressions were used to describe the relationships of  $F_\text{N}$  and  $F_\text{D}$  with soil WFPS.

Given it was beyond the scope of the current study to determine further reduction of  $\text{N}_2\text{O}$  to  $\text{N}_2$  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  $\text{N}_2\text{O}$  to  $\text{N}_2$  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  $\delta^{18}\text{O}$ . Likewise, samples were not collected to measure SP values of  $\text{N}_2\text{O}$  prior to any possible reduction to  $\text{N}_2$  so this could not be determined. A calculation of net isotope effects ( $\Delta\delta^{15}\text{N}$ ) or fractionation factors from the difference in  $^{15}\text{N}$  enrichment of soil  $\text{NH}_4^+$  or  $\text{NO}_3^-$  compared to  $^{15}\text{N}$  enrichment of soil-emitted  $\text{N}_2\text{O}$  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 ( $^\circ\text{C}$ ), were obtained from the Met Éireann meteorological station at Casement Aerodrome ( $53^\circ 30' \text{ N}$ ,  $6^\circ 44' \text{ W}$ ), 5.8 km east of the experimental site and at a similar elevation (80 m AOL). Soil temperature ( $^\circ\text{C}$ , 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  $\delta^{15}\text{N}^{\text{bulk}}$  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  $\text{N}_2\text{O}$  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  $\delta^{15}\text{N}^{\alpha}$ ,  $\delta^{15}\text{N}^{\beta}$ ,  $\delta^{15}\text{N}^{\text{bulk}}$  and SP values presented in Table S1 (see Supplementary Material) show that isotopic  $\text{N}_2\text{O}$  measurements were similar to previously reported ranges in  $^{15}\text{N}$  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  $\text{N}_2\text{O}$  emissions. Daily  $\text{N}_2\text{O}$  fluxes for this subset of samples, which ranged from 8.26 to 86.86  $\text{g N}_2\text{O-N ha}^{-1} \text{d}^{-1}$  overall, and peak  $\text{N}_2\text{O}$  fluxes, reflected fertiliser application and heavy rainfall events, and corresponding WFPS conditions, as detailed in Bracken et al. (2020). The highest daily  $\text{N}_2\text{O}$  flux occurred under elevated soil moisture conditions (Fig. 1, bottom). Daily  $\text{N}_2\text{O}$  fluxes ranged from 8.26 to 17.70  $\text{g N}_2\text{O-N ha}^{-1} \text{d}^{-1}$  under ambient soil moisture conditions and from 8.91 to 86.86  $\text{g N}_2\text{O-N ha}^{-1} \text{d}^{-1}$  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  $\text{N}_2\text{O}$  emissions ranged from 22.1 to 206.4  $\text{g N}_2\text{O-N ha}^{-1}$  under ambient soil moisture and 62.5 to 434.3  $\text{g N}_2\text{O-N ha}^{-1}$  under elevated soil moisture.

The temporal trend of  $\delta^{15}\text{N}^{\alpha}$  shows that  $\delta^{15}\text{N}^{\alpha}$  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  $\delta^{15}\text{N}^{\beta}$  shows that  $\delta^{15}\text{N}^{\beta}$  becomes only slightly enriched relative to  $\delta^{15}\text{N}^{\alpha}$  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  $\delta^{15}\text{N}^{\text{bulk}}$  (Fig. 3, top). However, it is clear that the measured values (SP<sub>(soil)</sub>: -218 to -35‰ and  $\delta^{15}\text{N}^{\text{bulk}}_{(\text{soil})}$ : -3553 to 1562‰) are more similar to tracer studies and do not fall within the approximate ranges typically reported for natural abundance values of  $\text{N}_2\text{O}$  emitted from soils as a result of nitrification (SP: 28 to 36‰ and  $\delta^{15}\text{N}^{\text{bulk}}$ : -60 to -30‰) and denitrification (SP: -10 to 0‰ and  $\delta^{15}\text{N}^{\text{bulk}}$ : -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.  $\text{N}_2\text{O}$  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  $\text{N}_2\text{O}$  emission overall.

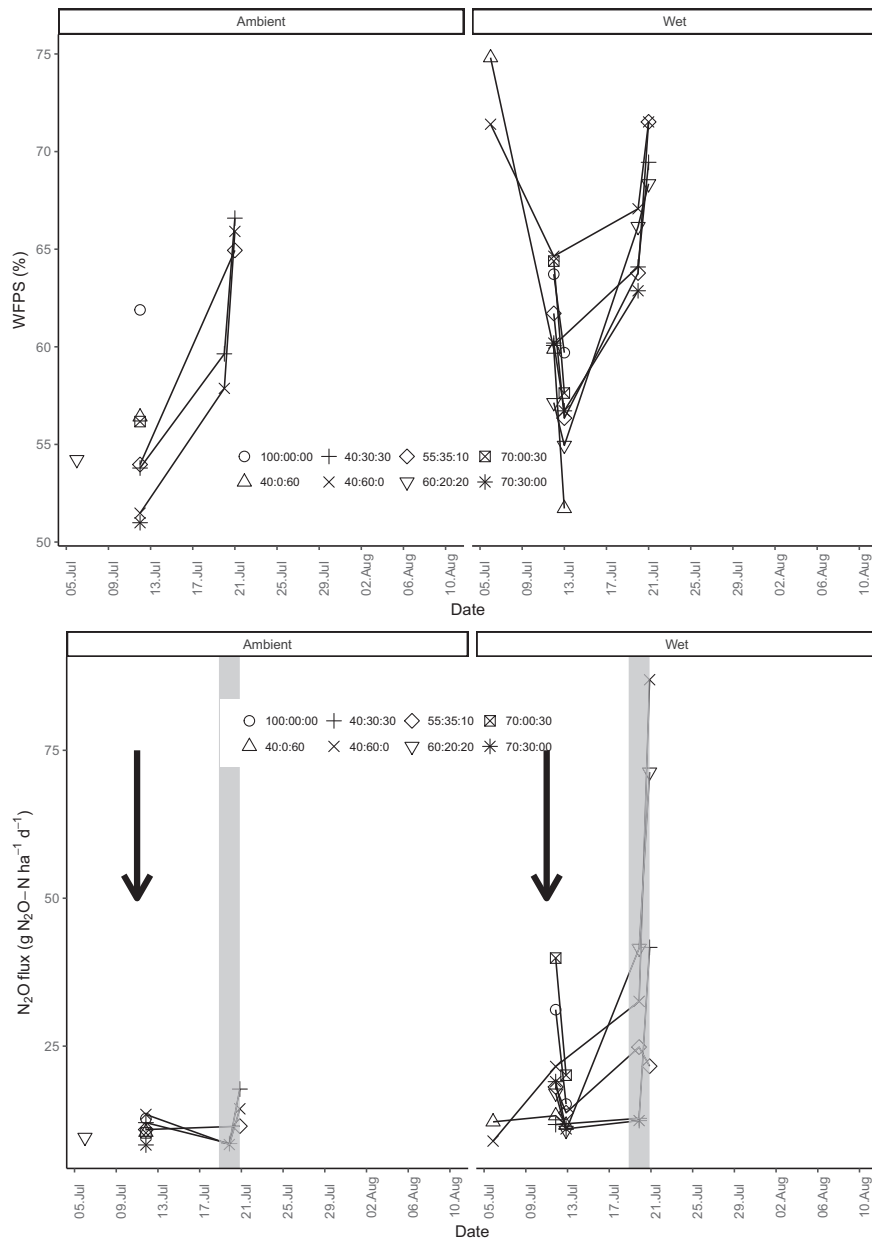
There was no significant relationship detected between  $F_N$  or  $F_D$  and soil WFPS ( $P = 0.16$ ). However, it is clear over the observed range of soil WFPS that the trend in  $F_N$  is to decrease while  $F_D$  increases with increasing WFPS (Fig. 4). The intersection of the  $F_N$  and  $F_D$  lines indicates that denitrification becomes the most dominant  $\text{N}_2\text{O}$  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  $\leq 66\%$  except for one (74.8% WFPS). In general, low daily  $\text{N}_2\text{O}$  fluxes with correspondingly high SP values at lower soil WFPS indicated nitrification, while high daily  $\text{N}_2\text{O}$  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  $\delta^{15}\text{N}^{\text{bulk}}$  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)  $\times$  herb (PLAN) ( $P < 0.05$ ). There was a strongly significant interaction between legume (WC)  $\times$  soil moisture for SP ( $P < 0.01$ ). There were significant three-way interactions between grass (PRG)  $\times$  legume (WC)  $\times$  soil moisture and between legume (WC)  $\times$  herb (PLAN)  $\times$  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  $\delta^{15}\text{N}^{\text{bulk}}$ . There was a strongly significant interaction between legume (WC)  $\times$  soil moisture ( $P < 0.01$ ) and a significant interaction between herb (PLAN)  $\times$  soil moisture on  $\delta^{15}\text{N}^{\text{bulk}}$  ( $P < 0.05$ ). There was a strongly significant three-way interaction between grass (PRG)  $\times$  legume (WC)  $\times$  soil moisture ( $P < 0.01$ ) and a significant three-way interaction between legume (WC)  $\times$  herb (PLAN)  $\times$  soil moisture on  $\delta^{15}\text{N}^{\text{bulk}}$  ( $P < 0.05$ ).  $\delta^{15}\text{N}^{\text{bulk}}$  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  $\text{N}_2\text{O}$  fluxes, representing the highest concentration samples from this experiment (i.e. >450 ppb  $\text{N}_2\text{O}$ ), 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  $\text{N}_2\text{O}$  production through the use of isotopic  $\text{N}_2\text{O}$  measurements. In this study, the measured SP<sub>(soil)</sub> and  $\delta^{15}\text{N}^{\text{bulk}}_{(\text{soil})}$  ranges are evidently more similar to those of  $^{15}\text{N}$  tracer studies than natural abundance studies due to the  $^{15}\text{N}$  labelled fertiliser source used in the experiment. It was assumed that the 2%  $^{15}\text{N}$  labelled urea fertiliser applied in the present study would be quickly and almost fully hydrolysed to  $\text{NH}_4^+$  post application, thus, enriching the soil  $\text{NH}_4^+$  pool (Abbasi and Adams, 2000). It is understood that nitrification is more discriminatory of the heavy isotope ( $^{15}\text{N}$ ) than denitrification, thus producing more depleted  $\text{N}_2\text{O}$   $\delta^{15}\text{N}^{\text{bulk}}$  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



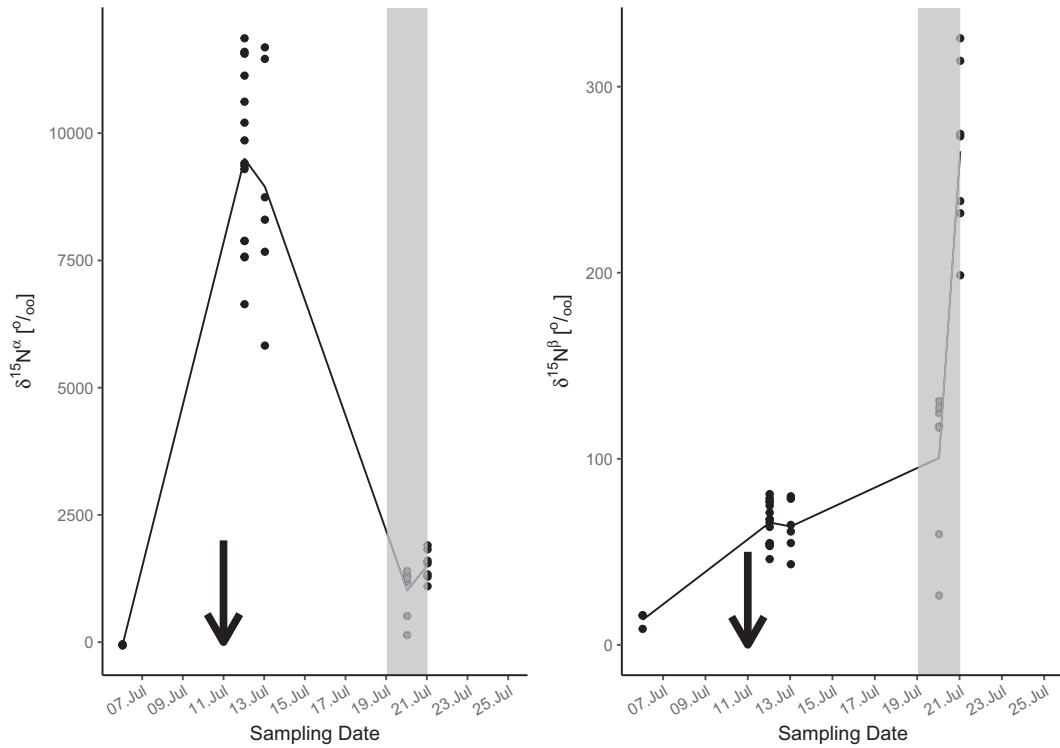
**Fig. 1.** Temporal plot of soil WFPS and daily N<sub>2</sub>O fluxes subset data based on Eq. (2) concentration rule (Legend = Mixture Ratio; Black arrows = fertiliser application; shaded area = heavy rainfall). Full dataset presented in Bracken et al. (2020).

with very low  $\delta^{15}\text{N}^{\text{bulk}}$  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  $\text{NH}_4^+$  to be converted to  $\text{NO}_3^-$ . The temporal trends in  $\delta^{15}\text{N}^{\alpha}$  and  $\delta^{15}\text{N}^{\beta}$  (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<sub>2</sub>O 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<sub>2</sub>O production compared to denitrification N<sub>2</sub>O sources (with the exception of fungal denitrification). SP is defined as  $\delta^{15}\text{N}^{\alpha}$  -

$\delta^{15}\text{N}^{\beta}$ , therefore, when nitrification is more dominant,  $\delta^{15}\text{N}^{\alpha}$  would be relatively high and  $\delta^{15}\text{N}^{\beta}$  low, with the opposite expected for denitrification. In a similar way to Congreaves et al. (2019), this study generally found that higher daily N<sub>2</sub>O fluxes and low corresponding SP values were associated with denitrification, while low daily N<sub>2</sub>O fluxes and high corresponding SP values were associated with nitrification (Fig. 5).

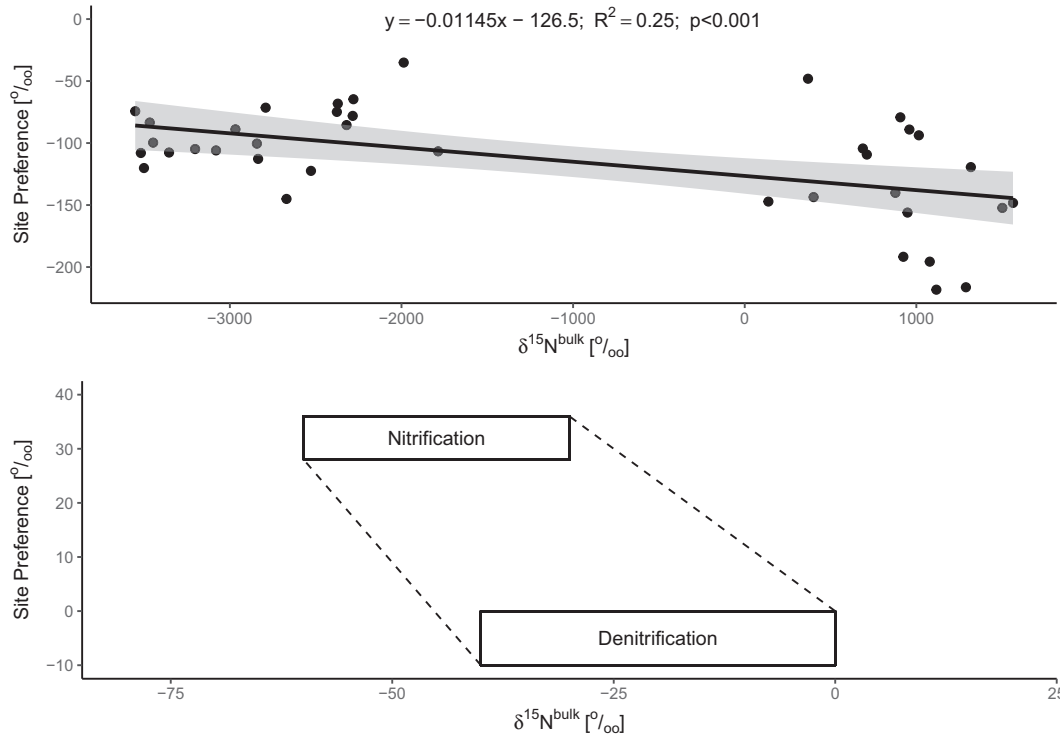
Based on the current results N<sub>2</sub>O was determined to be mostly derived from a mixture of nitrification and denitrification, with nitrification being slightly more dominant than denitrification overall. This was contrary to the hypothesis of Bracken et al. (2020). However, the variation of measured  $\delta^{15}\text{N}_{(\text{sample})}^{\text{x}}$  values and calculated  $\delta^{15}\text{N}_{(\text{soil})}^{\text{x}}$  values presented in Table S1 suggests large sampling artefacts may have occurred, particularly for samples close in N<sub>2</sub>O concentration to ambient air. This could be due to a number of reasons including; 1) the <sup>15</sup>N enriched fertiliser source increasing the range of measured isotope ratios, 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



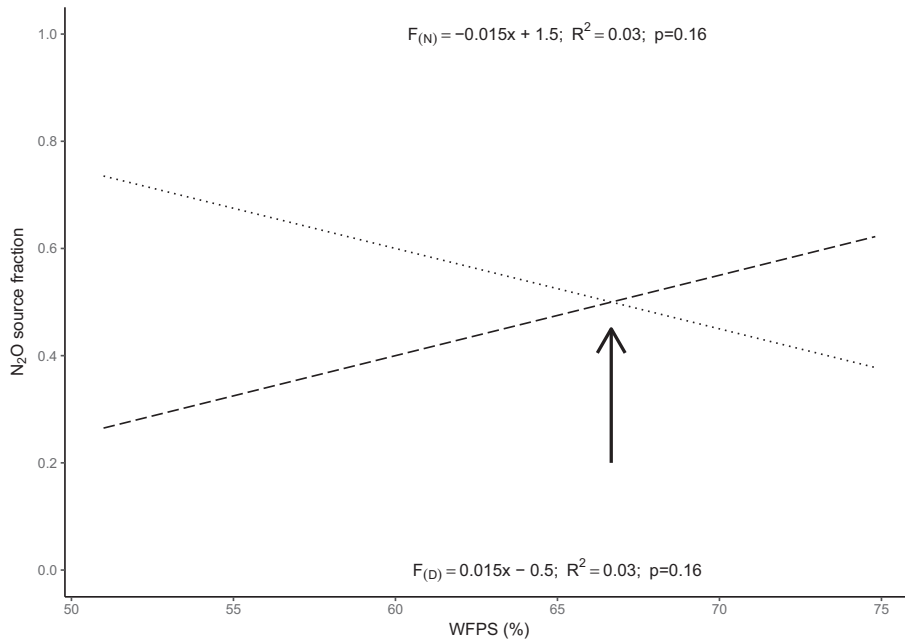
**Fig. 2.** Temporal plot of  $\delta^{15}\text{N}^\alpha$  and  $\delta^{15}\text{N}^\beta$  changes over the sampling period. Solid line = mean  $\delta^{15}\text{N}^\alpha$  and  $\delta^{15}\text{N}^\beta$  on each sampling date. Black arrows =  $^{15}\text{N}$  labelled urea fertiliser application;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

measurement of  $\text{N}_2\text{O}$  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  $\text{N}_2\text{O}$  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  $\delta^{15}\text{N}^x_{(\text{soil})}$ . Inferences related to fractions of nitrification and denitrification and the trends in  $\text{N}_2\text{O}$  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  $\delta^{15}\text{N}^{\text{bulk}}$  values used to determine nitrification ( $\text{SP}_\text{N}$ ) and denitrification ( $\text{SP}_\text{D}$ ) endmember values from measured data (top). (See main text for further explanation of large  $\delta$  value and SP scales). Typical literature reported endmember ranges from nitrification and denitrification processes (bottom); adapted from Zou et al. (2014).



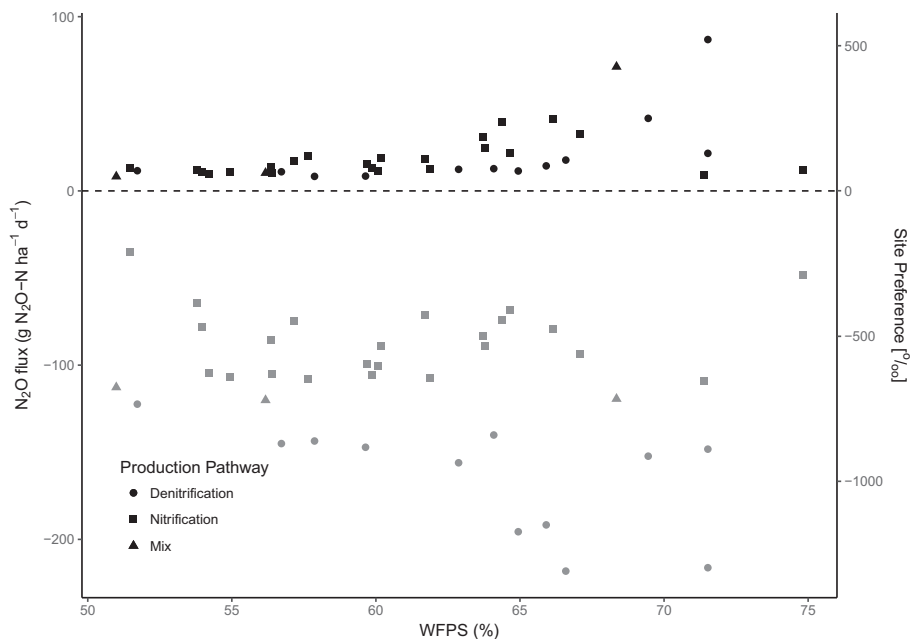
**Fig. 4.** N<sub>2</sub>O source fractions over the observed range of soil WFPS. Dotted line = predicted fraction of nitrification (F<sub>N</sub>) and dashed line = predicted fraction of denitrification (F<sub>D</sub>). Arrow = intersection of F<sub>N</sub> and F<sub>D</sub>.

aim to measure in situ N<sub>2</sub>O concentrations and isotope-ratios of ambient air to use in Eq. (2).

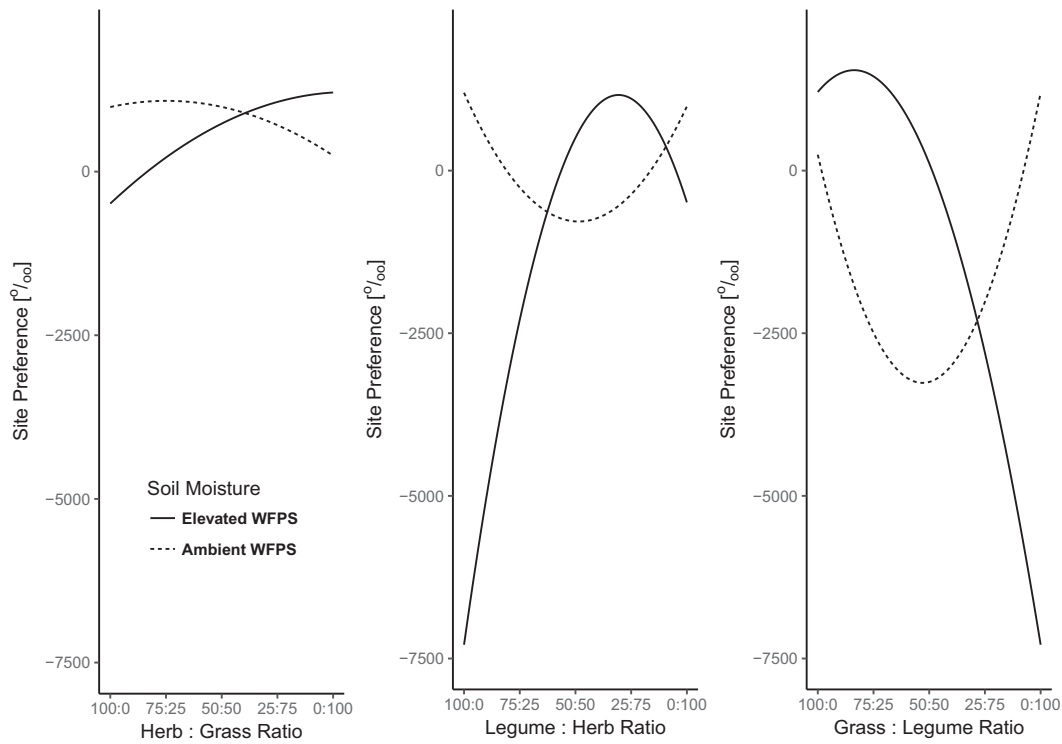
Of the remaining samples 37% were from ambient (drier) plots while 63% were from wet plots. However, there was considerable overlap in estimated soil WFPS ranges for those remaining samples (ambient plots: 51% to 67% and wet plots: 52% to 75%). Given previous studies found denitrification typically dominates 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<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O from both nitrification and denitrification was more prevalent (36.8%).

The predicted trend of F<sub>D</sub> 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<sub>N</sub> or F<sub>D</sub> and WFPS without correcting the calculated values of F<sub>N</sub> > 1 and F<sub>D</sub> > 1 back to 1 was almost significant (P = 0.06). Congreaves et al.



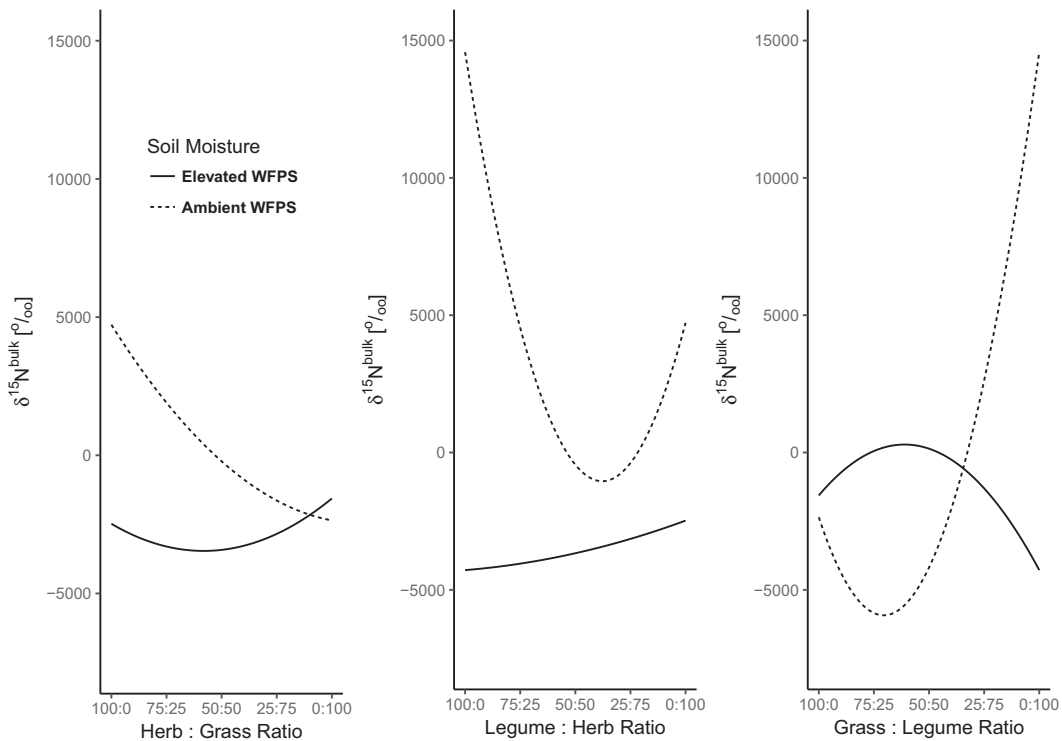
**Fig. 5.** Daily N<sub>2</sub>O fluxes (black, left axis) and corresponding site preference (grey, right axis) influenced by soil WFPS. (see main text for further explanation of large SP scale).



**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  $\delta$  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-Szczepak 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-Szczepak et al., 2017; Congreaves et al., 2019).

The diversity effect on SP, noted by the significant interaction between legume  $\times$  herb and the significant three-way interaction between legume  $\times$  herb  $\times$  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 acucubin, 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<sub>2</sub>O emissions. Appropriate management of N fertiliser source and application timing to soil moisture conditions could be a useful management strategy to lower N<sub>2</sub>O emissions. The combination of field scale measurements of N<sub>2</sub>O emissions and isotopomers with controlled higher frequency measurements from <sup>15</sup>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.

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## 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.

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## Appendix A. Supplementary data

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

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