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1 Title: Sward composition and soil moisture conditions affect nitrous oxide emissions and

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## 2 soil nitrogen dynamics following urea-nitrogen application

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#### 30 Abstract

Increased emissions of N<sub>2</sub>O, a potent greenhouse gas (GHG), from agricultural soils is a major 31 32 concern for the sustainability of grassland agriculture. Emissions of N2O are closely associated 33 with the rates and forms of N fertilisers applied as well as prevailing weather and soil conditions. Evidence suggests that multispecies swards require less fertiliser N input, and may 34 cycle N differently, thus reducing N loss to the environment. This study used a restricted 35 36 simplex-centroid experimental design to investigate N<sub>2</sub>O emissions and soil N cycling following application of urea-N (40 kg N ha<sup>-1</sup>) to eight experimental swards (7.8 m<sup>2</sup>) with 37 38 differing proportions of three plant functional groups (grass, legume, herb) represented by perennial ryegrass (PRG, Lolium perenne), white clover (WC, Trifolium repens) and ribwort 39 plantain (PLAN, Plantago lanceolata), respectively. Swards were maintained under two 40 41 contrasting soil moisture conditions to examine the balance between nitrification and denitrification. Two N<sub>2</sub>O peaks coincided with fertiliser application and heavy rainfall events; 42 13.4 and 17.7 g N<sub>2</sub>O-N ha<sup>-1</sup> day<sup>-1</sup> (ambient soil moisture) and 39.8 and 86.9 g N<sub>2</sub>O-N ha<sup>-1</sup> day<sup>-1</sup> 43  $^{1}$  (wet soil moisture). Overall, cumulative N<sub>2</sub>O emissions post-fertiliser application were higher 44 under wet soil conditions. Increasing legume (WC) proportions from 0% to 60% in 45 multispecies swards resulted in model predicted N<sub>2</sub>O emissions increasing from 22.3 to 96.2 g 46 N<sub>2</sub>O-N ha<sup>-1</sup> (ambient soil conditions) and from 59.0 to 219.3 g N<sub>2</sub>O-N ha<sup>-1</sup> (wet soil conditions), 47 48 after a uniform N application rate. Soil N dynamics support denitrification as the dominant 49 source of N<sub>2</sub>O especially under wet soil conditions. Significant interactions of PRG or WC 50 with PLAN on soil mineral N concentrations indicated that multispecies swards containing PLAN potentially inhibit nitrification and could be a useful mitigation strategy for N loss to 51 52 the environment from grassland agriculture.

- 53 Keywords: Nitrous oxide, soil nitrogen cycling, multispecies swards, perennial ryegrass
- 54 (Lolium perenne), white clover (Trifolium repens), ribwort plantain (Plantago lanceolata).

# 55 Highlights

56	•	Measurement of N <sub>2</sub> O emissions and N cycling from varying sward compositions.
57	•	Post N application (40 kg N ha <sup>-1</sup> ) $N_2O$ loss increased with white clover proportion.
58	•	$N_2O$ emissions from PRG were 2.5 fold higher in wet soil (WFPS >60%) compared to
59		ambient.
60	•	Soil N dynamics suggest denitrification as dominant $N_2O$ source when WFPS >60%.
61	•	Plantago lanceolata (forage herb) potentially regulates N cycling pathways.

#### 62 1. Introduction

63 Improving the sustainability of food production systems, while also reducing associated GHG 64 emissions, is a major global challenge (IPCC 2019). Nitrous oxide (N<sub>2</sub>O), is a potent GHG, with the tropospheric concentration continuing to increase (Thompson et al. 2019; Makowski, 65 2019). Anthropogenic soil N<sub>2</sub>O emissions are governed by the rate and form of N applied as 66 67 well as other biotic and abiotic factors, such as microbial community composition and activity, 68 soil texture, and climatic conditions (Braker and Conrad, 2011; Butterbach-Bahl et al. 2013). 69 Soil N loss is therefore a significant economic and environmental barrier to achieving 70 sustainable food production.

Nitrogen can be lost from agricultural soils in a number of ways that include nitrate (NO<sub>3</sub><sup>-</sup>)
leaching, and gaseous N forms such as nitric oxide (NO), ammonia (NH<sub>3</sub>), dinitrogen (N<sub>2</sub>) and
N<sub>2</sub>O. Despite over a century of research into the N cycle, there are still numerous questions
regarding N transformations and losses from terrestrial ecosystems (Müller and Clough, 2014).
Thus, it is imperative to continue research into soil N cycling; developing food production
systems that are N-use efficient while mitigating the threats posed by N loss to the environment.

77

### 78 1.1 N<sub>2</sub>O losses from agricultural grassland soils

Several N transformation pathways can lead to N<sub>2</sub>O production from soil (Butterbach-Bahl et al. 2013; Müller et al. 2014; Zhang et al. 2015). Nitrification is the oxidative conversion of NH<sub>4</sub><sup>+</sup> to NO<sub>3</sub><sup>-</sup> during which N<sub>2</sub>O can be lost to the atmosphere (Davidson and Verchot, 2000). Denitrification reduces NO<sub>3</sub><sup>-</sup> to N<sub>2</sub>O and finally to N<sub>2</sub> (Arnold, 1954; Gayon and Dupetit, 1882). Microbial activity (fungal and bacterial) regulates N<sub>2</sub>O production from nitrification and denitrification in soil (Baggs, 2011). With conditions conducive to nitrification, Conrad et al. (1983), found that soil N<sub>2</sub>O emissions associated with NH<sub>4</sub><sup>+</sup>-N fertiliser application 86 increased when compared to those associated with NO<sub>3</sub><sup>-</sup>N. Egginton and Smith (1986) showed that when conditions favoured denitrification, soil N<sub>2</sub>O emissions associated with NO<sub>3</sub><sup>-</sup>N 87 fertiliser application increased compared to those associated with NH<sub>4</sub><sup>+</sup>-N. This contrast 88 89 emphasizes the importance of selecting the appropriate N fertiliser type and rate, to suit the land management practices, to align with antecedent soil and weather conditions, in order to 90 91 reduce N loss as N<sub>2</sub>O. Increased availability of NO<sub>3</sub>-N, together with wetter soil conditions, has been shown to increase soil denitrification rates producing higher N<sub>2</sub>O emissions (Arnold, 92 93 1954; De Klein and van Logtestijn, 1994; Dobbie and Smith, 2003a, 2003b; Harty et al., 2017). 94 Dobbie and Smith (2003b) and Krol et al. (2016) observed that rainfall around the time of N application and its effect on water-filled pore space (WFPS) was a key driver of N<sub>2</sub>O emissions. 95 96

97 1.2 The influence of soil WFPS on  $N_2O$  production

Soil moisture is a major contributory factor to N<sub>2</sub>O emissions via its influence on N 98 transformation pathways. Below 60 - 70% WFPS, Nõmmik (1956) found that the microbial 99 100 activity resulting in denitrification was negligible. Davidson (1991) stated that nitrification is the dominant source of  $N_2O$  when WFPS < 70%. However, even in a predominantly aerobic 101 soil (conducive to nitrification), Burford and Stefanson, (1973) found anaerobic microsites 102 103 within the soil that gave rise to N<sub>2</sub>O produced by denitrification. The dynamic and heterogeneous nature of soil moisture means that conditions promoting nitrification and 104 105 denitrification can often occur simultaneously (Abbasi and Adams, 2000). Furthermore, soils 106 with an increased soil organic matter (SOM) content often show N<sub>2</sub>O production associated 107 with the turnover or organic N (Zhang et al., 2015). The interaction between N inputs, microbial activity and soil WFPS is complex. Improved knowledge of these interacting factors under 108 109 different agricultural systems is essential for the development of N<sub>2</sub>O mitigation options and improving N fertiliser use efficiency. 110

111

#### 112 *1.3 Dry matter production and N recovery in multispecies swards*

113 Multispecies swards composed of different plant functional groups (e.g. grasses, N fixing 114 legumes and herbs) have been investigated as alternatives to PRG monocultures due to their potential to meet primary productivity needs while requiring less fertiliser N inputs (Husse et 115 116 al., 2017; Nyfeler et al., 2009; Nyfeler et al., 2011; Suter et al 2015; Lüscher et al., 2014). Niche differentiation and complementarity resulting from differential resource use by the 117 118 individual plants within mixtures, benefiting the mixture as a whole (Loreau et al., 2001), are 119 often cited as the mechanisms by which multispecies swards produce greater dry matter (DM) yields compared to monocultures. For example; deeper rooting species grown in mixtures can 120 121 improve nutrient uptake from greater soil depths (Hoekstra et al., 2015; Jumpponen et al., 2002; 122 Massey et al., 2013). Cong et al., (2017, 2018) and Elgersma et al. (2014) found that herbs, such as *Plantago lanceolata*, have positive effects on DM yields when included in multispecies 123 124 swards with Cong et al., (2017) also reporting a significant increase in root biomass of swards 125 containing *Plantago lanceolata*.

It has been shown that the inclusion of legumes in sward mixtures for their contribution of 126 biologically fixed N, is a suitable means to replace fertiliser N requirements and maintain or 127 128 often increase DM yields and N recovery compared to PRG monocultures (Grace et al. 2019; Kirwan et al. 2007; Nyfeler et al. 2009; Nyfeler et al. 2011). Multispecies swards are also 129 130 considered more resilient than monocultures to environmental stresses such as drought which 131 may be vital for maintaining DM production and adapting to more frequent adverse weather conditions resulting from anthropogenic climate change (Finn et al., 2018; Hoekstra et al., 132 133 2015; Isbell et al., 2017).

134

#### 135 *1.4 N<sub>2</sub>O emissions and N cycling associated with multispecies swards*

136 How multispecies swards influence N<sub>2</sub>O emissions is still not understood. Niklaus et al. (2006) proposed that plant community composition impacts N cycling, soil properties related to gas 137 138 diffusivity and interactions of plants with soil microbial communities which influence soil N<sub>2</sub>O 139 emissions. They found some reduction in N<sub>2</sub>O associated with species diversity but observed increased N<sub>2</sub>O emissions in the presence of legumes. Allan et al. (2013) found no significant 140 141 effects on N<sub>2</sub>O emissions from multispecies swards but did find a significant legume effect on soil NO<sub>3</sub><sup>-</sup>. Abalos et al. (2014) only examined grass species diversity (not plant functional 142 143 group diversity) but found that certain grass mixtures led to a N<sub>2</sub>O reduction through greater 144 productivity and complementarity in root morphology. Niklaus et al. (2016) found that species richness reduced N<sub>2</sub>O emissions over time except from legume containing swards when 145 146 fertiliser was added. Many authors have found that multispecies swards can reduce NO<sub>3</sub><sup>-</sup> 147 leaching and propose high winter activity and differences in root system architecture to explain this reduction (Leimer et al., 2015, 2016; Malcolm et al., 2014; Scherer-Lorenzen et al., 2003). 148 149 Some studies have found a reduction in N<sub>2</sub>O emissions associated with the application of 150 compounds extracted from Plantago lanceolata leaves (Dietz et al., 2013; Gardiner et al., 2018). Recently, Carlton et al. (2019) reported that swards of perennial ryegrass, white clover 151 and ribwort plantain (Plantago lanceolata) had significantly lower nitrate leaching than 152 153 compositions of just perennial ryegrass and white clover, proposing that root exudates from ribwort plantain had an inhibitory effect on nitrification. These authors also found a lower 154 155 abundance of ammonia oxidising bacteria (AOB), highlighting the importance of the 156 interactions between multispecies swards and soil microbial communities in regulating soil N cycling. There is a growing interest in the use of plants as mitigation options for N<sub>2</sub>O emissions 157 from agricultural grasslands (De Klein et al., 2019). More research is needed to determine what 158 impact growing plants such as *Plantago lanceolata* in multispecies swards has on soil N 159 cycling and N<sub>2</sub>O emission over time. 160

We hypothesised that plant functional group (grass, legume, herb; represented by PRG, WC and PLAN) identity effects and plant functional group diversity effects (interaction between functional groups) may significantly affect N<sub>2</sub>O emissions depending on their proportions and soil moisture conditions. To test these hypotheses, we carried out an experiment that focused on plant functional group identity and diversity effects on N<sub>2</sub>O emissions, post N fertiliser application, from an agricultural grassland soil managed under two contrasting soil moisture conditions.

#### 168 2. Materials and Methods

#### 169 2.1 Experimental site

This experiment was carried out at University College Dublin (UCD) Lyons Farm (3°18' N, 170 6° 32' W, ca. 80 m AOL) in Co. Kildare, Eastern Ireland. The general climate is cool temperate 171 oceanic. The mean monthly total rainfall accumulation (1981 to 2010) for July and August is 172 54.2 – 72.3 mm, respectively, with an annual mean total rainfall of 754.2 mm (Met Éireann, 173 2018). The mean temperature (1981 to 2010) for July and August is between 15.7 and 15.4 °C, 174 respectively, with an annual mean temperature of 9.7 °C (Met Éireann, 2018). The soil type 175 176 has been previously classified as a grey brown podzolic soil with a silty clay loam texture (Lalor, 2004) (a Luvisol under the World Reference Base (WRB) soil classification system 177 (IUSS Working Group WRB, 2014)). Further details of the site's soil characteristics are 178 179 presented in Table 1.

The experimental swards used in this experiment were established in August 2013 as part of a 180 multi-species grassland sward experiment (Grace et al., 2018). Prior to this, the site had been 181 managed under continuous tillage, most recently in maize (Zea mays). Plots (1.95 x 10 m), 182 comprising of various seed mixes, were established in August 2013 (Grace et al., 2018). From 183 2013 to 2016 the subset of plots used in this experiment received an annual fertiliser N rate of 184 90 kg N ha<sup>-1</sup> yr<sup>-1</sup> and herbage was cut and removed 8 times per year (Grace et al., 2018). For 185 this experiment, subplots of 1.95 x 4 m were used. Each plot was harvested to a height of 4 cm 186 187 between April - October 2017 using a Haldrup forage harvester (Løgstør, Denmark) at 21 - 30 day intervals. 188

189

#### 190 2.2 Experimental design

191 Following the diversity-interaction modelling approach described by Kirwan et al. (2009), a

192 constrained simplex experiment was set up by Grace et al. (2018). A subset of eight plots from 193 the Grace et al. (2018) study were used for this experiment. The simplex experimental design 194 treats a sward as a mixture of component species (PRG, WC, PLAN) and assumes that the 195 measured responses depend on the relative proportions of the component species within the mixture (Cornell, 2002). The estimate of the response variables of a specific composition 196 197 derives from the compositions included in the design (Lawson and Wilden, 2016). Eight plots of pasture mixtures consisting of different proportions of three plant functional groups (grasses, 198 199 legumes and forage herbs, represented by PRG, WC and PLAN) were selected from the larger 200 experiment (Figure 1). As the diversity-interaction model (Simplex model) is based on a regression approach, it does not require replication of sward mixtures (Kirwan et al. 2009). 201

The eight plots are referred to by the ratios of the different plant functional groups included within the original seed rates (Grace et al. 2018) e.g. grass monoculture = 100:0:0. A single species represented each functional group; the grass species was perennial ryegrass (PRG, *Lolium perenne*), the legume species was white clover (WC, *Trifolium repens*) and the forage herb species was ribwort plantain (PLAN, *Plantago lanceolata*). A practical agronomic constraint was imposed on the simplex design such that there must be a minimum of 40% grass (PRG) in each mixture.

Two stainless steel collars for static chambers to measure N<sub>2</sub>O emissions were installed in each plot on 28 June 2017. This was approximately one week prior to the first sampling day. Chamber bases were only removed to facilitate grass harvesting and were returned to the same position immediately following this. The collars were inserted into the soil to a depth of  $\geq$  5 cm (De Klein and Harvey, 2012). The collars were square (40 cm × 40 cm) and 12 cm high, and had a rim lined with a neoprene foam seal to prevent gas diffusion when the chambers were closed (Minet et al., 2016). The corresponding stainless steel static chamber lid height was 10 cm. Lids were weighed down during sampling with a 5 kg weight to provide an air-tight seal.

217

### 218 2.3 Soil bulk density and water filled pore space (WFPS)

Six soil samples were taken from each plot using stainless steel bulk density rings on 13 June 2017. Gravimetric soil moisture and soil bulk density was measured by difference after drying 2017 for 24 hours at 105°C. The gravimetric soil moisture content and mean soil bulk density (1.16 2017 g cm<sup>-3</sup>) were used to calculate the mean water filled pore space (WFPS) of the plots, based on 2017 an assumed particle size density of 2.65 g cm<sup>-3</sup> (Krol et al., 2015).

Half of each plot was kept at ambient soil moisture while the other was watered to achieve a 224 225 higher WFPS. To do this, 7.5 L of water was applied in two applications using a watering can fitted with a rose head (5 L on 30 June 2017 and 2.5 L on 05 July 2017) which simulated 30 226 mm of rainfall in total. The estimated return period for 30 mm of rainfall in one day at this site 227 228 is 1.09 years based on the available historical weather data (Met Éireann, 2018). The 30 year 229 averages from the nearby weather station show that the greatest total daily rainfall recorded for July was 33.7 mm with a mean monthly total of 54.2 mm (Met Éireann, 2018). The target 230 231 WFPS for the wet soil moisture conditions was 70 - 80 %. To maintain the desired separation of WFPS between the ambient and wet soil moisture conditions, the wet areas received a 232 second water application of 3L (equivalent of 12 mm rainfall) on 17 July 2017. The area 233 234 incorporating the other static chamber in each plot was maintained under ambient soil moisture conditions. A buffer area ( $\geq 1$  m) was used to separate the ambient and wet soil moisture areas. 235

236

### 237 2.4 Fertiliser application

238 While the larger plot area received no fertiliser application throughout 2017, fertiliser N was applied by syringe in the form of a urea solution, at a typical rate of 40 kg N ha<sup>-1</sup> for the time 239 of year (Wall and Plunkett, 2016), to the base of each static chamber  $(0.16 \text{ m}^2)$  and to an area 240 adjacent to the chambers to be used for periodic soil sampling  $(0.09 \text{ m}^2)$ . The fertiliser urea 241 solution was prepared by dissolving a total of 41.16 g of lab grade urea in 2 L of 18 mQ water. 242 243 At the base of each chamber 66.67 ml of the fertiliser was applied and 37.5 ml was applied to each of the adjacent areas to be used for periodic soil sampling. No other macro or micro 244 nutrients were applied immediately prior to or during the experimental period. Table 1 presents 245 the most recently measured soil chemical properties of these plots. They had a mean soil pH of 246 247 7.2 and a Morgan's-extractable phosphorous (P) and potassium (K) content of 29.2 and 175.0 mg L<sup>-1</sup>, indicating that P and K were non-limiting based on the Irish Soil Index System (Wall 248 and Plunkett, 2016). Plots were harvested to 4 cm on 06 July 2017 prior to fertiliser application 249 250 on 11 July 2017 (Fig 2).

251

### 252 2.5 Sampling N<sub>2</sub>O emissions and calculating daily flux

253 Background N<sub>2</sub>O fluxes were measured on one occasion five days prior to fertiliser application 254 and then regularly for a two-month period post fertiliser application. Gas samples were taken 255 by syringe, through a rubber septum port on the lids of the static chambers, four times per week 256 for the first two weeks, twice per week for the next two weeks and then once per week for the 257 following month (Harty et al., 2016). In general, daily N<sub>2</sub>O fluxes are controlled by soil 258 temperature (Livesley et al., 2008). Therefore, it is necessary to choose the most appropriate 259 sampling time to represent the average daily flux (Laville et al., 2011). Gas samples were taken 260 in the mornings between 09.00 and 12.00 to obtain the most representative estimate of average daily N<sub>2</sub>O flux (Alves et al., 2012; Parkin 2008; Smith and Dobbie 2001). Headspace samples 261

(10 ml) were taken during a 60-minute closure period at times 0, 30 and 60 minutes after the
static chambers were closed. 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 within chambers. Ten ml gas samples were injected into 7 ml pre-evacuated glass vials
with double-wadded PTFE/silicone septa (Labco, UK) to achieve overpressure for storage.

The N<sub>2</sub>O concentration was measured by gas chromatography (GC) using a Bruker Scion 456 267 GC with a <sup>63</sup>Ni electron capture detector (Bruker, Germany) in combination with a Combi-268 269 PAL xt® auto-sampler (CTC Analytics AG, Switzerland). Five calibration standards were run 270 at the beginning of each sample batch with verification standards run after every 10 samples. Occasionally, the first air sample (T(0 min)) concentrations were higher than ambient. De Klein 271 272 and Harvey (2012) discussed a number of issues that can impact T(0 min) and defined outliers. 273 The T(0 min) outliers were substituted with the average of the T(0) concentrations for that sampling date to avoid introducing sources of additional variation, as outlined by De Klein and 274 275 Harvey (2012). Daily N<sub>2</sub>O fluxes were calculated based on the change in N<sub>2</sub>O concentration 276 over the three sampling time points using the following equation (De Klein and Harvey 2012):

277 Eq. 1: 
$$F(daily) = (\Delta C/\Delta t) x ((M x P) / (R x T)) x (V/A)$$

278 Whereby:

279 F(daily) is the daily N<sub>2</sub>O flux (g N<sub>2</sub>O-N ha<sup>-1</sup> day<sup>-1</sup>);

280  $\Delta C/\Delta t$  is the slope of the line between the N<sub>2</sub>O concentrations (ppm) at the three sampling time 281 points;

282 M is the molar mass of  $N_2O$ -N (28 g mol<sup>-1</sup>);

283 P is the atmospheric pressure (Pa) measured at Casement Aerodrome (53°30'N, -6°44'W)

meteorological station (approx. 5.8 km east of the experimental site and similar elevation) atthe time and date of sampling;

286 T is the air temperature (K) measured at the plot at the time of sampling;

- 287 R is the ideal gas constant (8.314 J  $K^{-1}$  mol<sup>-1</sup>);
- 288 V is the headspace volume of the closed chamber (approx.  $0.026 \text{ m}^3$ );

and A is the area covered by the base of the gas chamber (approx.  $0.1695 \text{ m}^2$ ).

Cumulative N<sub>2</sub>O emissions for the two-month period post fertiliser application (g N<sub>2</sub>O-N ha<sup>-1</sup>) were determined by integrating the daily N<sub>2</sub>O fluxes from Eq. 1 using the trapezoidal integration method (de Klein and Harvey, 2012; Harty et al., 2016) to interpolate between sampling dates.

294

#### 295 2.6 Dry matter yields

296 Herbage was harvested on three occasions from within each chamber base, using a small 297 handheld pruning shears, to a height of 4 cm above the soil surface (Burchill et al., 2014); at 15 days, 26 days, and 31 days post fertiliser application. Fresh herbage was separated into each 298 299 plant functional group and weighed, followed by oven drying at 65°C for 48 hours (Burchill et 300 al., 2014) and reweighing, to determine the herbage dry weight of each plant functional group 301 within the sward mixtures. For each sward mixture, the individual plant functional group dry weights were summed to determine the total dry weight yielded for each sward mixture. Yields 302 were expressed in units of kilograms of dry matter per hectare (kg DM ha<sup>-1</sup>). Herbage yields 303 for each sampling date were summed to get the total post fertiliser yield. The average 304 305 percentage inclusion of PRG, WC and PLAN in the herbage collected during this experiment (4 harvest dates, Figure 2) for each sward mixture was determined to compare the present 306

307 botanical compositions of the swards for the short duration of this experiment within the static chambers (0.16 m<sup>2</sup>) with the ratios of the original seeding rates for the field plots (Table S1 308 Supplementary Information). A more representative and longer-term quantification of the 309 persistence of each species in the experimental swards from the entire plot area  $(19.5 \text{ m}^2)$  is 310 presented by Grace et al. (2018). 311

312

#### 2.7 Soil sampling and KCl-extractable TON and $NH_4^+$ 313

314 Two molar potassium chloride (KCl) was used to extract mineral N from soil samples that were 315 taken periodically. These samples were taken from the fertilised area adjacent to the static chambers to avoid any physical disturbance of the area within the chamber. This extracted soil 316 317 N represents the mineral N that might potentially be found in soil solution, and thus be available for plant uptake, or be vulnerable to loss via volatilisation or leaching (Maynard and Kalra, 318 1993; Müller et al., 1998). The soil samples were analysed to determine the levels of soluble 319 soil mineral N as total oxidisable N (TON; the sum of  $NO_3^-$  and  $NO_2^-$ ) and  $NH_4^+$ . Soil samples 320 321 were taken to a depth of 10 cm using a 2 cm diameter soil corer. On each sampling date, 4 322 evenly spaced cores were taken and placed in a labelled zip-lock bag and brought to the lab 323 immediately for further processing. The first set of soil samples were taken five days prior to 324 fertiliser application to determine the background levels of KCl-extractable TON and NH<sub>4</sub><sup>+</sup>. 325 Soil samples were taken on four subsequent occasions (6, 15, 29 and 66 days after fertiliser 326 application). Upon completion of the N<sub>2</sub>O sampling period (77 days after fertiliser application), 30 cm deep intact soil cores were taken from within each chamber using a 5 cm diameter corer 327 328 (Eijkelkamp Soil & Water, Netherlands). Each core was split into three depths; 0 - 10 cm, 10-20 cm and 20 - 30 cm to assess the concentrations of KCl-extractable mineral N over depth 329 in the soil. 330

331 Soil samples were processed within 24 hours by sieving the fresh soil through 2 mm soil sieves. Soil sieves were cleaned with deionized water and dried between each sample. Fresh sieved 332 soil (20 g) was weighed into centrifuge containers and 50 ml of 2 M KCl solution was added 333 334 (1:2.5 ratio). The remaining soil from each sample was weighed and dried at 105°C for 24 hours and reweighed to measure the gravimetric soil moisture content. The centrifuge 335 336 containers were shaken for 1 hour and soil solutions were then filtered into 50 ml plastic containers through Whatman no. 2 filter paper. Samples were immediately placed into a freezer 337 for storage. The frozen KCl extracts were defrosted overnight prior to chemical analysis. 338

The methods used by Saghir et al., (1993); Stevens and Laughlin, (1995); Watson and Mills, (1998); Watson et al., (2000) were adapted to measure TON and  $NH_4^+$  colorimetrically using a Shimadzu UV-1280 spectrophotometer with the wavelength set at 520 nm and 625 nm, respectively.

The limits of detection (LOD) for the analyses of TON and  $NH_4^+$  concentrations, were 0.248 ppm and 0.001 ppm, respectively, based on the mean concentration + 3 x standard deviation 0 ppm calibration standard. All of the samples analysed for TON were above the LOD. However, for the final set of soil samples, to analyse  $NH_4^+$  over depth, many of the values were below the LOD, particularly at the two lowest depths; 10 - 20 cm and 20 - 30 cm. Prior to statistical analyses the values <LOD were corrected to zero.

349

#### 350 2.8 Meteorological and soil data

Average daily air temperature (°C) and rainfall (mm) for the study period were acquired from the Met Éireann meteorological station at Casement Aerodrome (53°30′N, -6°44′W), approximately 5.8 km east of UCD Lyons Farm and with similar elevation (80 m) above sea level (Met Éireann, 2017). Surface soil moisture (% volume, 0 – 6 cm depth) and temperature 355 (°C, 0 – 10 cm depth) were recorded on each sampling date using a ML2 Theta Probe (Delta356 T Devices Ltd., HH2, UK) and a TinyTag View 2 with a PB-5002-1M5 Thermistor Probe
357 (Gemini Data Loggers, UK), respectively.

358

#### 359 2.9 Statistical analysis

360 Results were statistically analysed using a simplex model in R (R Core Team, 2017). Identity effects and diversity effects of the three plant functional groups (represented by PRG, WC and 361 PLAN) were modelled as described by Connolly et al., (2009) and Kirwan et al., (2009). 362 Functional group identity effects occur when the response associated with a monoculture of 363 one of the plant functional groups is significantly different to the response of a monoculture of 364 another plant functional. Functional group diversity effects occur when the response of the 365 mixture of plant functional groups is significantly different from the response that would be 366 367 expected based on the proportional composition of functional groups in the mixture. 368 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 369 370 also tested. The model outputs and simplex contour plots were produced using the "lm" 371 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 372 373 R (Wickham, 2009).

The effect of events such as fertiliser application and heavy rainfall which occurred during the experimental period on temporal variations of TON and  $NH_4^+$  was analysed by fitting soil sampling dates as a time parameter to the original simplex model. A general correlation model (with any correlation possible between sampling times) was used for testing TON responses and a compound symmetry model with constant correlation between sampling times was used for testing  $NH_4^+$  responses. Full models were initially fitted to the data followed by reduced models, removing insignificant terms and observing hierarchy. Final model selection was based on parsimony, Akaike Information Criteria (AIC) and likelihood ratio tests between the model options for incorporating the time factor. A similar approach was taken to incorporate soil depths (0 – 10 cm, 10 – 20 cm, 20 – 30 cm) into the simplex model to statistically analyse the TON and  $NH_4^+$  concentrations measured from the final set of destructive soil cores used to determine the effect of depth on TON and  $NH_4^+$ .

#### 386 **3. Results**

### 387 3.1. Temporal trends in rainfall, temperature, N<sub>2</sub>O fluxes and mineral N

There was a clear initial separation in WFPS of the ambient (approx. 60 %) and wet (> 70%) 388 chamber areas across all plots achieved by the additional water added to the wet chamber areas 389 at the beginning of the experiment (Fig 2). Due to several days of persistent heavy rainfall (15.1 390 mm) in mid-July the mean WFPS for the ambient and wet chambers were within 5% of each 391 392 other (Fig 2). There was a significant grass (PRG) functional group identity effect on soil bulk density (Table 2). The PRG only plot had a lower soil bulk density of 1.17 g cm<sup>-3</sup>. The 393 maximum model estimated bulk density was 1.26 g cm<sup>-3</sup> at a predicted legume (WC) to grass 394 (PRG) ratio of 56:44. The lowest estimated bulk density was 1.14 g cm<sup>-3</sup> at a predicted grass 395 (PRG) to herb (PLAN) ratio of 64:36. 396

397 Higher N<sub>2</sub>O fluxes corresponded with fertiliser application (11 July 2019), heavy rainfall and reductions in soil and air temperatures (Figs 2 and 3). Daily N<sub>2</sub>O fluxes initially peaked one 398 day after fertiliser application. The highest initial peaks were 13.4 g N<sub>2</sub>O-N ha<sup>-1</sup> day<sup>-1</sup> from the 399 40:60:0 sward mixture (ambient soil moisture) and 39.8 g N<sub>2</sub>O-N ha<sup>-1</sup> day<sup>-1</sup> from the 70:0:30 400 sward mixture (wet soil moisture). The highest daily N<sub>2</sub>O fluxes from both ambient and wet 401 soil conditions occurred on 21 July 2017, coinciding with a period of high rainfall and WFPS; 402 17.7 g N<sub>2</sub>O-N ha<sup>-1</sup> day<sup>-1</sup> from the 40:30:30 sward mixture (ambient soil moisture) and 86.9 g 403 N<sub>2</sub>O-N ha<sup>-1</sup> day<sup>-1</sup> from the 40:60:0 sward mixture (wet soil moisture) (the mixture with the 404 405 highest proportion of WC) (Fig 3).

For the majority of the sward mixes the temporal trend for soil TON concentrations was to initially decline from approximately  $5.0 - 10.0 \text{ mg kg}^{-1}$  and then level out between 2.0 - 6.0mg kg<sup>-1</sup> after 20 days (Fig 3). Unlike TON concentrations, NH<sub>4</sub><sup>+</sup> concentrations, did not appear to have an obvious temporal trend (declining / increasing) over time and ranged from approximately 0 - 4.0 mg/kg for most of the sward mixes (Fig 3). 411

#### 412 3.2 Cumulative post-fertiliser N<sub>2</sub>O emission

The highest cumulative N<sub>2</sub>O emission for the two-month period post-fertiliser application under ambient soil conditions was 206.4 g N<sub>2</sub>O-N ha<sup>-1</sup> from the 40:30:30 mixture. The highest cumulative N<sub>2</sub>O emission for the two-month period post-fertiliser application under wet soil conditions was 434.3 g N<sub>2</sub>O-N ha<sup>-1</sup> from the 40:60:0 mixture. Cumulative post-fertiliser N<sub>2</sub>O emissions ranged from 22.1 - 206.4 g N<sub>2</sub>O-N ha<sup>-1</sup> for the ambient soil and from 62.5 - 434.3 g N<sub>2</sub>O-N ha<sup>-1</sup> for the wet soil.

There was a strongly significant grass (PRG) and legume (WC) functional group identity effect (P<0.01) on cumulative N<sub>2</sub>O emissions (Table 2), with emissions increasing with increasing WC proportion and decreasing with increasing PRG proportion for both wet and ambient soil moisture conditions (Figs. 4 and 5). There was a significant grass (PRG) x soil moisture interaction (P<0.05, Table 2), with N<sub>2</sub>O emissions being much higher under wet soil conditions than ambient (Fig 4).

425

### 426 *3.3 Dry matter yields*

Cumulative DM yield post-fertiliser application ranged from 760 - 3060 kg DM ha<sup>-1</sup>. The 427 428 70:30:0 sward mixture (ambient soil moisture) produced the highest cumulative DM yield, while the 70:0:30 sward mixture (wet soil moisture) produced the lowest cumulative DM yield. 429 There was a significant legume (WC) and herb (PLAN) functional group identity effect on DM 430 431 yields with model predictions of DM yields increasing with increasing proportions of WC and PLAN (P<0.05; Table 2). The proportions of PRG, WC and PLAN (kg DM ha<sup>-1</sup>) within each 432 of the mixtures at the time of the experiment is expressed as a percentage of the total DM (kg 433 DM ha<sup>-1</sup>) and provided in Table S1 (Supplementary Information). 434

#### 436 *3.4 Soil mineral nitrogen dynamics*

There was a highly significant grass (PRG) functional group identity effect and time effect on
TON concentrations in soil KCl extracts (P<0.001). There was also a strongly significant herb</li>
(PLAN) x grass (PRG) functional group diversity effect (P<0.01) as shown by the curved</li>

- response with lower TON concentrations at the 50:50 mixed proportions of herb (PLAN) and
- 441 grass (PRG) than at the 100% point of either herb (PLAN) or grass (PRG) (Fig S1, left).

There was a significant legume (WC) functional group identity effect (P<0.05) on  $NH_4^+$ 442 concentrations in soil KCl extracts, with NH4<sup>+</sup> concentrations tending to increase markedly 443 444 with increasing WC content under wet soil conditions but having the opposite trend under ambient soil moisture conditions (Fig S2, right). There was no significant effect of time on 445 446 NH4<sup>+</sup>. There was a significant legume (WC) x herb (PLAN) functional group diversity effect with a curved response showing concentration predictions higher near the 50:50 mixed 447 proportions of legume (WC) and herb (PLAN) than at the 100% point of either legume (WC) 448 449 or herb (PLAN (P<0.05; Fig S2).

450 Results of the last soil sampling 77 days post-fertilisation, which included three sampling depths, showed that there was a significant effect of depth (P<0.05) on TON concentrations, 451 with concentrations tending to decrease with depth. Mean concentrations across all sward 452 mixes were 3.99 (0 – 10 cm), 3.32 (10 – 20 cm) and 3.15 (20 – 30 cm) mg kg<sup>-1</sup>. There were 453 also significant grass (PRG) (P<0.001) and legume (WC) functional group identity effects 454 (P<0.05; Fig S3, right), with TON tending to increase in concentration with increasing PRG 455 proportion and decrease with increasing WC proportion. There was a significant herb (PLAN) 456 x grass (PRG) functional group diversity effect with a curved TON response showing 457 concentrations lowest around the 50:50 herb (PLAN) to grass (PRG) ratio (P<0.05; Fig S3, 458 459 left).

Using the corrected values for  $NH_4^+$  over depth; there was a strongly significant effect of depth 460 on NH4<sup>+</sup> concentrations, with concentrations tending to decrease with depth. Mean 461 concentrations across all sward mixes were 0.87 (0 - 10 cm), 0.05 (10 - 20 cm) and 0.05 (20 462 -30 cm) mg kg<sup>-1</sup>. There was a significant herb (PLAN) x soil moisture interaction (P<0.05; 463 Fig S4, middle). There was a significant three-way interaction of the herb (PLAN) x grass 464 (PRG) functional groups with soil moisture (P<0.05; Fig S4, left and middle). Around the 50:50 465 herb (PLAN) to grass (PRG) ratio NH4<sup>+</sup> concentrations were higher under wet soil moisture 466 conditions and lower under ambient for all three soil depths. 467

#### 468 4. Discussion

#### 469 4.1. Temporal $N_2O$ emissions

470 It is clear from this study that N<sub>2</sub>O emissions from multispecies swards were strongly impacted by fertiliser N management practices and soil moisture conditions. Higher N<sub>2</sub>O emissions 471 472 occurred directly post-fertilisation and under wetter soil conditions. N<sub>2</sub>O emissions peaked when WFPS was above 60%, suggesting denitrification as a dominant source of N<sub>2</sub>O emission 473 474 over nitrification. In temperate grasslands, peak N<sub>2</sub>O emissions have been related to fertiliser 475 N application timing while also inferring that rainfall contributed to greater emissions and 476 seasonal variability of N<sub>2</sub>O (Jackson et al., 2015; Jones et al., 2007; Liu et al., 2015). Average daily N<sub>2</sub>O fluxes, based on data reported by those studies as well as studies in Ireland by Harty 477 et al. (2016) and Krol et al. (2016), range from 0 g N<sub>2</sub>O-N ha<sup>-1</sup> d<sup>-1</sup> for unfertilized control plots 478 to approximately 30 g N<sub>2</sub>O-N ha<sup>-1</sup> d<sup>-1</sup> for N fertilized plots, with some large daily peaks 479 reported > 1000 g N<sub>2</sub>O-N ha<sup>-1</sup> d<sup>-1</sup>. The observed daily N<sub>2</sub>O fluxes presented in Fig 3 fall within 480 481 these previously reported ranges.

Temporal N<sub>2</sub>O emissions appeared to be directly related to soil TON concentrations which 482 decreased as N<sub>2</sub>O emissions peaked, especially under wet soil conditions (Fig 3). Hatch et al. 483 (1990; 1991) found that peak daily rates of net mineralization could range from 0.7 - 4.1 kg N 484  $ha^{-1} d^{-1}$  and that peak rates were related to re-wetting of soil after dryer weather. They found 485 that total net mineralization was highest under grass/clover swards. In the current study such 486 487 increased rates of daily mineralization, particularly in clover containing swards, may have increased the amounts of mineral N available to be lost as N<sub>2</sub>O during the second large peak in 488 489 Fig. 3. Krol et al. (2016) statistically related N<sub>2</sub>O emissions with soil moisture at the time of N 490 application and cumulative rainfall post application. Decreasing concentrations of soil TON, when WFPS is high, supports denitrification of NO<sub>3</sub><sup>-</sup> in soil solution to N<sub>2</sub> and N<sub>2</sub>O as the main 491 pathway for N<sub>2</sub>O production. The loss of N<sub>2</sub> was not quantified but may have accounted for a 492

substantial amount of the N loss particularly from plots under wet soil conditions (Selbie et al.,2015).

Despite the application of N as urea the  $NH_4^+$  concentrations were much more constant over time. This might suggest that urea was rapidly converted to  $NH_4^+$  which was then consumed through plant uptake or rapidly nitrified to  $NO_3^-$ . Other reasons may be mineralization of organic N replacing  $NH_4^+$ -N taken up from soil solution by plants or converted to  $NO_3^-$  by nitrification (Müller and Clough 2014; Müller et al., 2004, 2011). Adsorption of  $NH_4^+$  to organic matter and soil particles may also have occurred (Harty et al., 2017).

501 Plots under wet soil conditions mostly remained above 60% WFPS as planned but occasionally were below 60%. Therefore, it was considered that the greater N<sub>2</sub>O emissions from plots under 502 503 wet soil conditions were due to a contribution from both nitrification and denitrification sources 504 (Abassi and Adams, 2000; Davidson, 1991; Nõmmik, 1956). It is also notable that the larger N<sub>2</sub>O peaks under ambient soil conditions (Fig 3) occurred shortly after the heavy rainfall period 505 when the WFPS for these plots was >60%. Under wet soil conditions, soils had >60% WFPS 506 for a considerably longer period of time (~39 days; 66 % of time) compared to ambient soil 507 conditions (~22 days; 37 % of time) and mean cumulative N<sub>2</sub>O emissions were considerably 508 higher (214.06 g N<sub>2</sub>O-N ha<sup>-1</sup> and 108.65 g N<sub>2</sub>O-N ha<sup>-1</sup>, respectively). 509

510

### 511 4.2 Cumulative N<sub>2</sub>O emissions and DM yields

The model predictions for an increase in WC proportion from 0% - 60% (i.e. within the constraints of the seeding rates of Grace et al. 2018) showed an increase in cumulative N<sub>2</sub>O emissions from 22.3 to 96.2 g N<sub>2</sub>O-N ha<sup>-1</sup> under ambient soil conditions and from 59.0 to 219.3 g N<sub>2</sub>O-N ha<sup>-1</sup> under wet soil conditions, respectively (grass to legume ratio). The PRG only plots may have been N limited whereas the plots containing higher WC proportions may not have been as N-limited due to biological N fixation. This may also explain the significantincrease in DM yield with increasing proportions of legume and herb.

519 The significant legume (WC) functional group identity effect on cumulative N<sub>2</sub>O emissions 520 indicates that applying the same N application rate, to multispecies swards with high proportions of legume, compared to PRG monocultures, is an inappropriate N management 521 522 practice that can result in greater N<sub>2</sub>O emissions, particularly during periods of high WFPS. Multispecies swards with higher proportions of WC and PLAN were found to have higher DM 523 524 yields than the PRG only sward. Larger proportions of legumes within the sward mixtures likely provided sufficient biologically fixed N to support DM production and the additional N 525 applied as fertiliser was then underutilised by the plants, making it prone to loss to the 526 527 environment. Mixtures containing legumes have shown potential for reducing synthetic 528 fertiliser N requirements while maintaining or increasing DM production compared to high fertiliser N input grass monocultures (Grace et al., 2019; Kirwan et al., 2007; Nyfeler et al., 529 530 2009), but benefits associated with mixtures greatly diminish if managed at high N fertiliser 531 rates (Nyfeler et al., 2011).

Cumulative N<sub>2</sub>O emissions were also higher under wet soil conditions (Figs. 4 and 5). There 532 was a significant grass (PRG) x soil moisture interaction, with 2.5 fold higher N<sub>2</sub>O emissions 533 534 from the grass monocultures under wet soil conditions than ambient. Drier soil conditions were expected to favour N<sub>2</sub>O production from nitrification over denitrification. Interestingly, there 535 536 were no significant interactions of soil moisture and the other mixture components; legumes 537 (WC) and herbs (PLAN), nor were there any significant 3-way interactions of the functional groups with soil moisture. Perhaps, this is due to differences in the root systems of the mixtures 538 and their effects on soil structure and porosity compared to the grass monocultures (Gould et 539 540 al., 2016; Niklaus et al., 2006). The present study noted that predicted soil bulk density increased with an increasing proportion of legume (WC). A higher soil bulk density in swards 541

542 with greater WC content supports the argument for greater contribution to the overall N<sub>2</sub>O emissions from denitrification due to the soil being more compact. Harrison-Kirk et al. (2015) 543 found that N<sub>2</sub>O and N<sub>2</sub> production ratios indicated that denitrification was more dominant 544 545 under conditions of compaction and reduced porosity, after successive saturation and drying cycles. In the present study, the lowest model-predicted bulk density was 1.14 g cm<sup>-3</sup> resulting 546 547 from a simulated 64:36 ratio of grass (PRG) to herb (PLAN). Plots were established in 2013 providing four years of plant growth and root establishment. Consequently, PLAN roots over 548 549 time may have transformed the porous architecture of the soil, thus reducing the soil bulk 550 density and altering soil gas diffusivity properties as outlined by Friedl et al. (2018). The effects of different plants on long-term soil structure and subsequent N<sub>2</sub>O emissions and N cycling is 551 552 an area of growing research interest that requires further investigation (De Klein et al., 2019).

553

### 554 **4.3 Soil TON and NH**<sub>4</sub><sup>+</sup> concentrations over time and depth

The changes in TON concentrations over time may be attributed to disturbances such as the 555 fertiliser application and heavy rainfall. Assuming that TON is largely NO<sub>3</sub>, which is very 556 mobile in soil, the decrease in TON concentration over time, despite fertiliser N application, 557 might suggest that  $NO_3^-$  was being removed from the soil solution through plant uptake, 558 559 through a combination of plant uptake, immobilisation, conversion to N<sub>2</sub> or N<sub>2</sub>O, and leaching (Müller and Clough 2014; Müller et al., 2004, 2011). The fact TON concentrations decreased 560 significantly over time, while NH<sub>4</sub><sup>+</sup> concentrations did not, indicates that denitrification was 561 562 likely the most dominant N<sub>2</sub>O production pathway during this experiment. Given the relatively restricted drainage of the plots used, additional residual soil N from swards with higher WC 563 proportions, that may have been leached from more freely draining soils (Leimer et al., 2015, 564 2016; Scherer-Lorenzen et al., 2003), would have been available for conversion to N<sub>2</sub>O by 565

566 denitrification particularly under the wet soil moisture conditions. This would be consistent 567 with the higher  $N_2O$  emissions observed under wet soil moisture conditions.

568 The significant diversity effect of herb (PLAN) x grass (PRG) on TON concentrations analysed over time and the significant diversity effect of legume (WC) x herb (PLAN) on  $NH_4^+$  analysed 569 over time suggest that perhaps  $NH_4^+$  was slower to convert to  $NO_3^-$  under multispecies swards 570 571 with PLAN. Carlton et al. (2019) found significantly lower NO<sub>3</sub><sup>-</sup> losses from mixtures containing Plantago lanceolata (PLAN) compared to those of just PRG and WC, associated 572 with nitrification inhibition and a reduction in ammonia oxidizing bacteria. The long-term 573 574 establishment of the plots used here may have allowed biological nitrification inhibitors from 575 root exudates or leaf litter of PLAN (Dietz et al., 2013; Gardiner et al., 2018) to build up prior 576 to this experiment or may have led to the differential soil microbial population development.

The current study indicates that PLAN growing in multispecies swards could potentially be an 577 alternative biological nitrification inhibition option (as opposed to synthetic inhibitors, Di et 578 al., 2014; Harty et al., 2016; Zaman et al., 2008) to be used as a mitigation strategy for both 579 N<sub>2</sub>O emissions and nitrate leaching, while improving N use efficiency and the sustainability of 580 grassland based agricultural systems (Carlton et al., 2019; De Klein et al., 2019). However, this 581 study made it clear that future research should take a balanced N fertiliser management 582 583 approach, when comparing N<sub>2</sub>O emissions from multispecies swards, accounting for biological 584 N fixation from legumes. There is a need to consider the effects of all components of 585 multispecies swards as part of long term systems experiments to ensure that differences in N 586 fertiliser management (including rate and timing) and species composition are quantified. This would enable appropriate management advice options to suit prevailing weather and soil 587 conditions. 588

### 589 **5.** Conclusion

Increasing legume (WC) proportions from 0% to 60% in multispecies swards resulted in model 590 predicted N<sub>2</sub>O emissions increasing from 22.3 g N<sub>2</sub>O-N ha<sup>-1</sup> to 96.2 g N<sub>2</sub>O-N ha<sup>-1</sup> (ambient soil 591 conditions) and from 59.0 g N<sub>2</sub>O-N ha<sup>-1</sup> to 219.3 g N<sub>2</sub>O-N ha<sup>-1</sup> (wet soil conditions), after a 592 uniform N application rate. Appropriate timing and application of lower quantities of fertiliser 593 N to multispecies swards containing WC compared to PRG monocultures is important to 594 595 mitigating N<sub>2</sub>O emissions, particularly in wet soil conditions. Consideration of biologically fixed N from WC and mineralization of organic N under multispecies swards is necessary to 596 597 develop appropriate N fertiliser management strategies for multispecies swards.

Soil moisture had a significant interaction with PRG resulting in over 2.5 times higher 598 cumulative N<sub>2</sub>O emissions under wet conditions compared to ambient from the PRG 599 600 monoculture. Soil mineral N dynamics suggested denitrification was the dominant production pathway of N<sub>2</sub>O, particularly under wet soil conditions, but that nitrification may also have 601 contributed, particularly when WFPS dropped below 60%. Future <sup>15</sup>N tracing studies could 602 provide clearer insights on the effect of multispecies swards and soil moisture conditions on 603 different N transformation pathways resulting in N<sub>2</sub>O production. Multispecies swards could 604 help reduce reliance on feriliser N inputs, while maintaining DM production needs. Swards 605 606 containing Plantago lanceolata (PLAN) showed potential for regulating soil N cycling (biological nitrification inhibition) which could be a useful strategy for mitigating N losses to 607 608 the environment, either as N<sub>2</sub>O or leached NO<sub>3</sub><sup>-</sup> and improving the sustainability of grassland agriculture. 609

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#### Tables 896

#### **Table 1:** Site description and summary of soil properties. 897

Soil Phys	sical Properties					
Soil Type	e So	oil Texture		Sand (%)	Silt (%)	Clay
Luvisol	Cl	ay Loam		24.83 (± 0.65)	43.57 (± 0.76)	31.63
Soil Che	mical Properties					
рН	SOM (LOI%)	TN (%)	TC (%)	P (mg L <sup>-1</sup> soil)	K (mg L <sup>-1</sup> soil)	Mg (mg L <sup>-1</sup> s
7.24 (± 0.10)	5.71 (± 0.26)	0.306 (± 0.01)	3.083 (± 0.26)	29.20 (± 4.66)	175.00 (± 16.46)	87.33 (± 6.81)
				Index 4	Index 4	Index 3

(SOM: Soil Organic Matter, LOI: Loss on Ignition, TN: Total Nitrogen, TC: Total Carbon, P: Phosphorus, 898

Calcium, S: Sulphur). Index refers to 1 - 4 scale (3 is adequate, 4 is high); Irish Soil Index (Wall and Plunket 899

**Table 2:** Statistical significance for the functional group identity and diversity effects and soil moisture inter 901 cumulative N<sub>2</sub>O loss (g N<sub>2</sub>O-N ha<sup>-1</sup>) and cumulative DM yield (kg DM ha<sup>-1</sup>).

Effect Type	Parameter	Bulk Density	N <sub>2</sub> O
Eventional Crown Identity Effects	Grass Intercept	2.39 <sup>e-12</sup> ***	1.26 <sup>e-06</sup> ***
Functional Group Identity Effects	Legume	NS	0.00356 **
	Herb	NS	NS
	Grass x Legume	NS	NS
Functional Group Diversity Effects	Grass x Herb	NS	NS
	Legume x Herb	NS	NS
Eunstianal Crown Identity and Sail	Grass x SM	NS	0.04082 *
Moisture Interaction Effects	Legume x SM	NS	NS 0.04082 * NS
Moisture Interaction Effects	Herb x SM	NS	NS
Eurotional Group Diversity and Sail	Grass x Legume x SM	NS	NS
Moisture Interaction Effects	Grass x Herb x SM	NS	NS
Moisture Interaction Effects	Legume x Herb x SM	NS	NS

SM = soil moisture. NS = not significant. \*\*\* < 0.001, \*\* < 0.01, \* < 0.05. See Section 2.9 Statistical analysis

# 903 Figures



Figure 1: The simplex experimental design demonstrating the eight proportions of each of the
functional groups (grass: legume: herb) with constraint imposed (minimum of 40 % grass
inclusion in each mixture). Adapted from Grace et al., (2018).





909 Figure 2: Estimated soil WFPS (%), rainfall (mm), air and soil temperature data as recorded on sampling d
910 application. Upper plot: grey arrows = water applications to wet soil. Lower plot: small black arrows = herba





**Figure 3:** Daily N<sub>2</sub>O emissions (g N<sub>2</sub>O-N ha<sup>-1</sup> day<sup>-1</sup>); KCl-extractable soil TON-N and  $NH_4^+$ -N concentr mixtures; black arrow = fertiliser application date, shaded area = heavy rainfall period. (Mixture Ratio = prop



Figure 4: Effects plot of predicted cumulative N<sub>2</sub>O emission (g N<sub>2</sub>O-N ha<sup>-1</sup>) with increasing
proportions of individual plant functional groups under wet (solid line) and ambient (dotted
line) soil moisture conditions. Horizontal lines: PRG monoculture (100:0:0) response.
(Cumulative N<sub>2</sub>O emissions were for the two-month post fertiliser sampling period).





920 Figure 5: Contour plots of the two-month post fertiliser cumulative N<sub>2</sub>O emissions (g N<sub>2</sub>O-N

921	$ha^{-1}$	post-fertiliser	application	under an	nbient and	l wet soi	l moisture	conditions.
		1	11					



Figure S1: Effects plots of predicted TON (mg kg<sup>-1</sup> soil) for each soil sampling date with
increasing proportions of individual plant functional groups under wet and ambient soil
moisture conditions. Horizontal lines: PRG monoculture (100:0:0) response.



927

**Figure S2:** Effects plots of predicted  $NH_4^+$  (mg kg<sup>-1</sup> soil) for each soil sampling date with increasing proportions of individual plant functional groups under wet and ambient soil moisture conditions. Horizontal lines: PRG monoculture (100:0:0) response.



Figure S3: Effects plots of predicted TON (mg kg<sup>-1</sup> soil) for each soil sampling depth with
increasing proportions of individual plant functional groups under wet and ambient soil
moisture conditions. Horizontal lines: PRG monoculture (100:0:0) response.





**Figure S4:** Effects plots of predicted  $NH_4^+$  (mg kg<sup>-1</sup> soil) for each soil sampling depth with increasing proportions of individual plant functional groups under wet and ambient soil moisture conditions. Horizontal lines: PRG monoculture (100:0:0) response.

Orig	<b>Original Species Ratios</b>			2017 Species Ratio		
PRG	WC	PLAN	PRG	WC	PLAN	
100	0	0	100	0	0	
40	0	60	35	34	31	
55	35	10	41	59	0	
70	30	0	54	46	0	
40	30	30	39	61	0	
70	0	30	67	0	33	
60	20	20	34	66	0	
40	60	0	27	73	0	

**Table S1:** Original species ratios based on seeding rates (Grace et al. 2018) and 2017 speciesratios as a percentage of total DM.