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Geoderma

Application of a triple 15N tracing technique to elucidate N transformations in a UK grassland soil
--Manuscript Draft--

Manuscript Number:	GEODER_2020_2065R2
Article Type:	Research Paper
Keywords:	nitrous oxide; denitrification; nitrification; heterotrophic nitrification
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Abstract:	To identify the production and consumption pathways and temporal dynamics of N_2O emitted from soil, this study uses ^{15}N-labelled substrate-N to quantify the underlying gross N transformation rates using the $Ntrace$ analysis tool and link them to N-emissions. In three experiments twelve soil cores each were incubated in a lab incubation system to measure gaseous emissions, while parallel incubations under the same conditions were set up for destructive soil sampling at 7 time points. Using the triple labelling technique (applying NH_4NO_3 with either the NH_4^+-N or the NO_3^--N, or both being ^{15}N labelled), this study investigated the effects of 55, 70 and 85% water filled pore space (deemed to promote nitrification, both nitrification and denitrification, and denitrification, respectively) in a clay soil on gaseous N emissions and investigates the source and processes leading to N_2O emissions. To assess the utilisation of applied NO_3^- vs. nitrified NO_3^- from applied NH_4^+, the ^{15}N tracing tool $Ntrace$ was used to quantify the rates of immobilisation of NO_3^- and NH_4^+, oxidation of NH_4^+, mineralisation of organic N and subsequent nitrification by the analysis of the ^{15}N in the soil. Gross transformation rates were calculated, indicating the relative importance of added NO_3^- and NO_3^- derived from nitrified added NH_4^+. Results show an important contribution of heterotrophic nitrification (organic N oxidation to NO_3^-) which was highest at the 55% water filled pore space (WFPS), decreasing in its contribution to N-transformation processes with increasing WFPS, while nitrification (NH_4^+ oxidation to NO_3^-) was contributing the most at 70% WFPS. The contribution of denitrification increased with increasing WFPS, but only became dominant at 85% WFPS. While denitrification still showed to be most important at high and nitrification at lower WFPS, the actual % WFPS values were not as expected and highlight the fact that WFPS is a contributor, but not the sole/most important parameter determining the type of N-transformation processes taking place.

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Dear Editor:

I am submitting on behalf of my co-authors the revised manuscript and relevant response documents for your consideration to publish.

Details are below,

With best regards,

Laura

Type of Contribution: regular paper.

Number of pages: 38

Number of words: 8357 (including all text, no tables).

Number of tables: 2

Number of figures: 8

Full title: **Application of a triple ^{15}N tracing technique to elucidate N transformations in a UK grassland soil**

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1 **Application of a triple ^{15}N tracing technique to elucidate N**

2 **transformations in a UK grassland soil**

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27

28   **Abstract**

29   To identify the production and consumption pathways and

30   temporal dynamics of N_2O emitted from soil, this study uses [15]N-

31   labelled substrate-N to quantify the underlying gross N

32   transformation rates using the *Ntrace* analysis tool and link them

33   to N-emissions. In three experiments twelve soil cores each were

34   incubated in a lab incubation system to measure gaseous

35   emissions, while parallel incubations under the same conditions

36   were set up for destructive soil sampling at 7 time points. Using

37   the triple labelling technique (applying NH_4NO_3 with either the

38   NH_4^+-N or the NO_3^--N, or both being ^{15}N labelled), this study

39   investigated the effects of 55, 70 and 85% water filled pore

40   space (deemed to promote nitrification, both nitrification and

41   denitrification, and denitrification, respectively) in a clay soil on

2

42 gaseous N emissions and investigates the source and processes

43 leading to N_2O emissions.

44 To assess the utilisation of applied NO_3^- vs. nitrified NO_3^- from

45 applied NH_4^+, the ^{15}N tracing tool *Ntrace* was used to quantify

46 the rates of immobilisation of NO_3^- and NH_4^+, oxidation of NH_4^+,

47 mineralisation of organic N and subsequent nitrification by the

48 analysis of the ^{15}N in the soil. Gross transformation rates were

49 calculated, indicating the relative importance of added NO_3^- and

50 NO_3^- derived from nitrified added NH_4^+.

51 Results show an important contribution of heterotrophic

52 nitrification (organic N oxidation to NO_3^-) which was highest at

53 the 55% water filled pore space (WFPS), decreasing in its

54 contribution to N-transformation processes with increasing

55 WFPS, while nitrification (NH_4^+ oxidation to NO_3^-) was

56 contributing the most at 70% WFPS. The contribution of

57 denitrification increased with increasing WFPS, but only

58 became dominant at 85% WFPS. While denitrification still

59 showed to be most important at high and nitrification at lower

60 WFPS, the actual % WFPS values were not as expected and

61 highlight the fact that WFPS is a contributor, but not the

62 sole/most important parameter determining the type of N-

63 transformation processes taking place.

64

65 **Keywords**

66   Nitrous oxide; denitrification; nitrification; heterotrophic

67   nitrification

1   Introduction

68   1   **Introduction**

69   Nitrous oxide (N_2O) is an important greenhouse gas (GHG)

70   accounting for approximately 6% of the current global warming

71   (WMO, 2018). The atmospheric N_2O concentration has been

72   increasing since the Industrial Revolution, with soils

73   representing its major source, making the understanding of its

74   sources and removal processes important for the development of

75   mitigation strategies.

76   Several processes have been studied to determine their

77   contribution to N_2O production in soils: (i) nitrification, which

78   has been reported as autotrophic (NH_4^+ oxidation) and

79   heterotrophic (organic N oxidation) (Zhang et al., 2015); (ii)

80   denitrification, due to the incomplete denitrification of nitrate

81   (NO_3^-) under anaerobic conditions (Attard et al., 2011); (iii)

82   nitrifier denitrification (Zhu et al., 2013); and (iv)

83   chemodenitrification as a non-biological process (Van Hecke et

84   al., 1990).

85   It has been found that N_2O is mainly produced via biological

86   processes and that emissions through nitrification and

87   denitrification produce up to 70% of the annual emitted N_2O

88   worldwide (Butterbach-Bahl et al., 2013). Several studies aimed

89   to distinguish the main pathway responsible for N_2O emissions

90 (Khalil et al., 2004; Bateman and Baggs, 2005), and identify a

91 predominant process under certain conditions. While

92 nitrification requires O_2, denitrification relies on its absence or

93 limitation and has been attributed to anoxic conditions (Khalil et

94 al., 2004). It is therefore generally agreed that water filled pore

95 space (WFPS) is one of the key factors affecting which process

96 dominates N_2O production. The higher the WFPS the more air

97 in pores is replaced by water, thereby removing O_2 from the soil.

98 However, it is also thought that several processes can occur

99 simultaneously in different microsites of the same soil (Arah,

100 1997) due to the generation of local differences in soil

101 aggregates.

102 It is well known that N_2O is produced by microorganisms who

103 are dependent on several factors, such as environmental

104 conditions, nutrient availability etc. (Saggar et al., 2013), which

105 suggests that it is also likely that the N_2O-source processes

106 themselves change over time due to changes in limiting factors

107 such as soil moisture and carbon availability, allowing newly

108 formed N-species to become new sources. As an example, in

109 addition to added NO_3^-, the native soil NO_3^- and that produced

110 from nitrification of applied or soil NH_4^+, can also be a source of

111 N_2O via denitrification following nitrification.

112 Different methods have been applied to identify the occurrence

113 and importance of different processes under different conditions.

114 Amongst those are ^{15}N-labelling techniques (Stark, 2000), as

well as isotopologue analyses of N_2O and O_2 ($^{15}N/^{18}O$) (Meijide et al., 2010; Bergstermann et al., 2011; Wu et al., 2016).

When aiming to determine how important different processes are under certain environmental conditions and management (e.g. soil moisture, C and N applications, etc), incubation experiments, where single factors and combinations of these can be manipulated, are the methodology of choice. Automated systems such as the denitrification incubation system, DENIS (Cárdenas et al., 2003) at Rothamsted have proven useful for process determination. In the DENIS, soil cores are incubated under an N_2-free atmosphere, allowing direct measurements of all emitted N gases (NO, N_2O and N_2) as well as CO_2. The transformation of N in soils and particularly the production of N_2O from different sources, such as fertilisers or animal excreta, has been studied through a series of laboratory incubation experiments (i.a. Meijide et al. (2010), Bergstermann et al. (2011), Loick et al. (2017)) using this system. The advantage of this system, when looking at N_2O source processes is, that under an N_2 free atmosphere it is possible to measure N_2 which, depending on the initial conditions, can only be produced via complete denitrification.

In order to fully investigate transformations leading to N_2O production and removal, quantifying their contributions and assessing the potential for change of processes, a combination of

139   laboratory experiments with models/analysis tools at the same

140   scale offer great potential.

141   One process model/analysis tool using ^{15}N distribution in the

142   data obtained from ^{15}N labelling experiments has been

143   developed by Müller et al. (2004; 2007). This analysis tool,

144   represents an improvement of the dilution model by Kirkham

145   and Bartholomew (1954), and includes soil nitrite and gaseous

146   compounds emitted. It traces ^{15}N applied to soil and quantifies

147   the gross N rates based on measurements of the partition of ^{15}N

148   in soil pools from dual or triple isotope labelling of the source.

149   The model determines the most suitable dynamics through the

150   best Akaike's Information Criterion (AIC). The objective of this

151   study is to show how N-transformation processes leading to N_2O

152   emissions change over time and how WFPS can influence the

153   initial dominance of certain processes but does not necessarily

154   determine a sole process. The advantage of the triple labelling

155   technique is that production of N_2O from an organic (unlabelled)

156   source outside the mineral N pools can be unambiguously

157   determined because if all relevant mineral N pools are labelled

158   then a dilution of the N_2O has to arrive from outside that system.

159   Also, for the parameter optimisation techniques it provides

160   additional observations which reduce the danger of over

161   parameterisation during parameter optimisation

162   To achieve this the triple labelling technique using Ammonium

163   Nitrate (NH_4NO_3) was applied as a substrate with the N being

164 labelled with ^{15}N in its different positions. Changes in soil N

165 (NO_2^-, NO_3^-, and NH_4^+) were measured to quantify the

166 underlying gross N transformation rates using the *Ntrace*

167 analysis tool (Müller et al., 2007) with the measured emissions

168 to then identify sub-rates based on the ^{15}N distribution in the

169 data. This was linked to gaseous N-emissions to identify the

170 production and consumption pathways and temporal dynamics

171 of N_2O. In order to determine the source of N_2O from the triple

172 labelling experiment, the DENIS was extended by connecting it

173 to a GC-MS to include continuous measurements of emitted ^{15}N-

174 N_2O.

175 We will test the following hypothesis: 1) that NO and N_2O losses

176 at different soil moisture levels will decrease at higher moisture

177 values due to easier diffusion and conversion to N_2; 2) that at the

178 highest soil moisture N_2O is mostly derived from NO_3^- whilst at

179 the low moisture from NH_4^+;   3) that nitrification and

180 denitrification are the main sources of N_2O at all moistures.

181

182 2   **Materials and Methods**

183 *2.1   Soil Preparation*

184 A clayey pelostagnogley soil of the Hallsworth series (Clayden

185 and Hollis, 1984) (44% clay, 40% silt, 15% sand (w/w), Table

186 1) was collected on the 26th of May 2015 from a typical

187 grassland in SW England, located at Rothamsted Research,

188   North Wyke, Devon, UK (50˚46'10''N, 3˚54'05''W). Spade-

189   squares (20 x 20 cm to a depth of 15 cm) of soil were taken from

190   12 locations along a 'W' line across a field of 600 m^2 size, which

191   had not had any grazing animals on it, nor received any fertiliser

192   input for over 20 years. After sampling, the soil was air dried to

193   ~30% H_2O (gravimetric moisture content), roots and plant

194   residues were removed, and the soil sieved to <2 mm and stored

195   at 4˚C before packing into cores and starting the incubation.

196   Initial soil characteristics are given in Table 1.

197

198   *2.2   Experimental Design*

199   The incubation experiment was carried out using the DENIS, a

200   specialized gas-flow-soil-core incubation system (Cárdenas et

201   al., 2003) in which environmental conditions can be tightly

202   controlled. The DENIS simultaneously incubates a maximum of

203   12 vessels containing one soil core each. Cores were packed to a

204   bulk density of 0.8 g cm^{-3} to reflect field conditions, to a height

205   of 75 mm into stainless steel sleeves of 141 mm diameter. Due

206   to the limited space within the DENIS and the requirement for

207   replication, three experiments (see below) were performed

208   directly one after another under the same tightly controlled

209   conditions (i.e. temperature, gas flow, amendment application).

210   All soil was kept in the fridge (4˚C) until needed and treated to

211   the same time scales to prevent any changes in soil

212   characteristics.

213 To promote nitrification-, denitrification- or a combination of

214 both, each experiment was performed at a different WFPS

215 (Bollmann and Conrad, 1998; Butterbach-Bahl et al., 2013). The

216 soil moisture was adjusted to 55%, 70% or 85% WFPS,

217 respectively, taking the amendment with nutrient solution into

218 account. To measure N_2 fluxes, the native N_2 was removed from

219 the soil and headspace without limiting O_2 levels that would be

220 present in air. This was achieved by using a helium-oxygen

221 mixture $He:O_2$ of 80:20. First the soil cores were flushed from

222 the bottom at a flow rate of 30 ml min^{-1} for 14 h. To measure

223 baseline emissions, flow rates were then decreased to 12 ml

224 min^{-1} and the flow re-directed over the surface of the soil core

225 for three days before amendment application and for the

226 remaining experimental period. The vessels were kept at 20°C

227 during flushing as well as for the 13-day incubation period after

228 amendment application.

229 Three incubations were needed to accommodate the different ^{15}N

230 treatments and soil moisture levels. Each incubation involved the

231 following three treatments of NH_4NO_3 (Sigma-Aldrich, St.

232 Louis, MO, USA), with three replicate vessels per treatment: i)

233 $^{15}NO_3$ = cores amended with single labelled $NH_4^{15}NO_3$ at 50

234 atom%; ii) $^{15}NH_4$ = cores amended with single labelled

235 $^{15}NH_4NO_3$ at 50 atom%; iii) $^{15}NO_3^{15}NH_4$ = cores amended with

236 double labelled $^{15}NH_4^{15}NO_3$ at 50 atom%. Considering the total

237 surface area of the vessel, N was applied at a rate of 75 kg N

238 ha^{-1}. The applied rate of N equates to 125 mg N kg^{-1} dry soil,

239 which was dissolved in 50 ml of H_2O before being applied to the

240 soil. To maintain the incubation conditions, the amendment was

241 applied to each of the three cores via a sealed amendment

242 container on top of the incubation vessel. Before amendment

243 application the headspace of the amendment vessel was flushed

244 with He to prevent any atmospheric N_2 entering the system.

245 Additionally, a parallel incubation only for destructive soil

246 sampling at 7 time-points after treatment application (5 h, days

247 1, 2, 3, 4, 7, 10) with 3 replicates of each was performed each

248 time. For logistical reasons smaller cores (4.5 cm diameter) had

249 to be used, which were packed with the same soil and to the same

250 specifications used for the DENIS incubation and kept under the

251 same controlled conditions. At the sampling time, soil was

252 analysed for extractable Ammonium (NH_4^+), Nitrate (NO_3^-),

253 Nitrite (NO_2^-) concentrations and ^{15}N-enrichment of those

254 molecules ($^{15}NH_4^+$, $^{15}NO_3^-$, $^{15}NO_2^-$).

255

256 **_2.3   Gas analyses_**

257 Gas samples were taken every four hours for each vessel from

258 the Denis system. Fluxes of N_2O and CO_2 were quantified using

259 a Perkin Elmer Clarus 500 gas chromatograph (Perkin Elmer

260 Instruments, Beaconsfield, UK) equipped with an electron

261 capture detector (ECD) for N_2O and CO_2. N_2 emissions were

262 measured by gas chromatography with a helium ionisation

263 detector (VICI AG International, Schenkon, Switzerland), while

264 NO concentrations were determined by chemiluminescence

265 (Sievers NOA280i, GE Instruments, Colorado, USA). All gas

266 concentrations were corrected for the surface area and flow rate

267 going through the vessel (measured daily). Fluxes were

268 calculated on a kg N or C ha^{-1} day^{-1} basis. Isotopic signatures

269 were determined via isotope ratio mass spectrometer (PDZ

270 Europa 20-20 Stable Isotope Analyser, Sercon, Crewe, UK)

271 linked to an ANCA-TGII gas preparation system (Sercon,

272 Crewe, UK).

273

274 *2.4   Soil analyses*

275 The initial soil N was measured at the start of each incubation by

276 randomly taking three 100 g samples from the bulk soil before

277 core packing and WFPS adjustment. This soil was analysed for

278 total extractable oxidised N (TO_xN, combined amount of NO_2^-

279 and NO_3^-) and NH_4^+. Soil samples (100 g) from the parallel

280 incubation were analysed for extractable NO_2^-, NO_3^- and NH_4^+

281 concentrations at each time point. WFPS was calculated from

282 soil moisture contents by drying a subsample (50 g) at 105°C

283 overnight. Soil extractable NO_2^-, NO_3^- and NH_4^+, concentrations

284 were analysed after blending the samples with 2M KCl at pH 8

285 following the method of Stevens and Laughlin (1995). The

286 extracts were analysed by colourimetry using a

287 Spectrophotometer (Cecil Instruments, Cambridge, UK) for the

288　　analysis of NO_2^-, or an Aquakem 250 discrete photometric

289　　analyser (Thermo Fisher Scientific, Hemel Hempsted, UK) for

290　　the analysis of NO_3^- and NH_4^+. The ^{15}N abundances of the NO_2^-

291　　, NO_3^- and NH_4^+ were determined by methods based on the

292　　generation of N_2O for isotope ratio mass spectrometry (IRMS).

293　　The production of N_2O from NO_2^- and NO_3^- is based on the

294　　reaction between NO_2^- and NH_2OH under acid conditions and

295　　the NO_3^- having been reduced to NO_2^- with Cd (Stevens and

296　　Laughlin, 1994). The production of N_2O from NH_4^+ consists of

297　　a diffusion stage where ammonia (NH_3) is absorbed into H_2SO_4

298　　followed by an oxidation step where recovered $(NH_4)_2SO_4$ is

299　　oxidised to N_2 by alkaline NaOBr, during which N_2O is

300　　produced as a by-product (Laughlin et al., 1997). In each case,

301　　the resulting N_2O was transferred to an Exetainer (Labco Ltd,

302　　Lampeter, Wales). The N_2O enrichment was determined using a

303　　Gilson Autosampler (Gilson UK, Dunstable, UK) by IRMS as

304　　described in the gas analyses section.

305

306　　*2.5　Statistical analysis*

307　　Statistical analysis was performed using GenStat 16th edition

308　　(VSN International Ltd). Prior to the statistical tests all data were

309　　analysed to proof their normal distribution (Kolmogorove-

310　　Smirnov test) and equality of variance (Levene test). Cumulative

311　　emissions of NO, N_2O, N_2 and CO_2 were calculated from the

312   area under the curve (time vs flux as shown in figure 2) after

313   linear interpolation between sampling points.

314

315   *2.6   Analysis of N_2O source contribution*

316   To determine the contribution of different sources to N_2O

317   emissions the *Ntrace*basic analysis tool by Müller et al. (2007)

318   was used. This analysis tool represents an extension of the

319   dilution approach of Kirkham and Bartholomew (1954) and

320   quantifies gross N rates based on measured data. To achieve this,

321   a model is used to quantify the individual gross rates, connecting

322   the various soil N pools by parameter optimization routines.

323   The gross N transformation rates quantified where:

324     $M_{Nrec,}$ mineralization of recalcitrant organic N to NH_4^+;

325     $M_{Nlab,}$ mineralization of labile organic N (e.g., monomolecular

326   organic N, amino acids, proteins) to NH_4^+;

327     $I_{NH4Nrec}$, immobilization of NH_4^+ to recalcitrant organic N;

328     $I_{NH4Nlab}$, immobilization of NH_4^+ to labile organic N;

329   A_{NH4}, adsorption of NH_4^+ on exchange sites;

330   R_{NH4a}, release of adsorbed NH_4^+;

331   O_{NH4}, oxidation of NH_4^+ to NO_3^-;

332   O_{Nrec}, oxidation of organic N to NO_3^-; (heterotrophic

333   nitrification)

334   as well as the following 4 rates, which were, however,

335   negligible:

336   I_{NO3}, immobilization of NO_3^- to recalcitrant organic N;

14

337   D_{NO3}, dissimilatory reduction of NO_3^- to NH_4^+;

338   A_{NO3}, adsorption of NO_3^- to labile organic N;

339    $R_{NO3,}$ release of adsorbed NO_3^-

340   One feature of *Ntrace* is to identify the simplest model structure

341   that is sufficient and adequate to explain the measured data.

342   Therefore, a range of different model versions (including/

343   excluding certain transformation rates) and/or kinetic setting are

344   tested. The most suitable model is then identified by comparing

345   the AIC of each model run which takes the goodness of fit and

346   the number of parameters used into account. Thus, this tool also

347   identifies rates which are not needed to explain the overall

348   dynamics (e.g. the mineralization of labile organic N in our

349   case). Figure 1 shows the full conceptual model according to

350   Müller et al. (2014) indicating the rates used based on the 2007

351   model (Müller et al., 2007) in the top left area.

352   Pathway specific N_2O emissions were determined by assuming

353   that N_2O originated from the NH_4^+, organic N and NO_3^- pool

354   (Fig. 1) (Stange et al., 2009; Müller et al., 2014). The

355   contributions of these three pools were calculated by the

356   parameter identification routine described by Rütting et al.

357   (2010):

358    $a_{N2O} = C_{NH4} \times a_{NH4} + C_{ON} \times a_{ON} + C_{NO3} \times a_{NO3}$ (1)

359    $C_{NH4} + C_{ON} + C_{NO3} = 1$ (2)

360   Where a_{N2O} is the ^{15}N abundance of N_2O produced during

361   incubation, a_{NH4}, a_{ON} and a_{NO3} are the ^{15}N abundance of NH_4^+,

362   organic N and NO_3^-, respectively, and C_{NH4}, C_{ON} and C_{NO3} are

363   the contributions from oxidation of NH_4^+ to NO_3^-, oxidation of

364   organic N to NO_3^- and reduction of NO_3^- to total N_2O

365   production, respectively.

366

367   3   **Results**

368   *3.1   Fluxes of N gases and CO$_2$*

369   Nitric oxide emissions increased in all treatments (Fig. 2a)

370   during the incubation period. At the highest moisture of 85%

371   WFPS, NO emissions reach a plateau after 6 days and start to

372   decrease after 10 days. For the 2 lower moisture levels emissions

373   were increasing over the whole course of the experiment.

374   Emissions increased significantly with WFPS, as shown.

375   Nitrous oxide emissions (Fig. 2b) were very low and near the

376   detection limit (N_2O: 0.5 ppm, equivalent to a flux of 0.00027 kg

377   N ha^{-1} h^{-1}) in the two lower WFPS treatments. In the 85% WFPS

378   treatment N_2O emissions were significantly higher ($p<0.05$) than

379   the other 2 treatments and showed a peak at day 1 of around 14

380   g N ha^{-1} h^{-1} after which emissions decreased to around 3 g N ha^{-}

381   ^{1} h^{-1} by the end of the experiment. At the lower WFPS of 55 and

382   70%, N_2O emissions were not significantly different between the

383   WFPS treatments.

384   Nitrogen gas emissions (Fig. 2c) were low in the 55% and 70%

385   WFPS treatments and did not show a peak. Higher N_2 emissions

were detected in the 85% WFPS treatment with a peak at around day 2. After day 5, N_2 emissions were low as in the other two treatments. Some N_2 was introduced into the system when the amendment was applied. This took about 1 day to disappear (see high soil moisture treatment) (see Fig. 2).

The total amounts of N emitted as NO, N_2O and N_2 show an increase with increasing WFPS (Tab.2). However, total amounts of NO-N were almost insignificant making up less than 0.04% of total N emissions. Total emissions of N_2O were low in the 55% and 70% WFPS treatment (<3% of total N emissions), but significantly higher at the highest WFPS of 85% (21.3% of total N emissions). N_2 emissions was only any significantly different at the high soil moisture. The N_2-N represented the largest component of the emitted N at least 80%. The N_2O-N to N_2-N ratios were smaller at the middle soil moisture (0.03) compared to 0.27 at 85% WFPS.

Carbon dioxide emissions (Fig. 2d) increased immediately after the application of NH_4NO_3 and showed a maximum on day 2 in the 55% and 85% WFPS treatments decreasing afterwards. In the 70% WFPS treatment emissions seem to have decreased in the first day to recover in day 2 which was followed by a steady decrease similarly to the other 2 treatments. Values for the 70% WFPS treatment were the lowest during all the incubation compared to the other 2 treatments.

3.2 Proportion of N_2O from added N

Results of the estimation of the proportion of N_2O derived from the applied treatments showed that initially, at 55% WFPS, very little N_2O emissions derived from added single-labelled NH_4^+ (Fig. 3a, ○). Larger amounts derived from added labelled ^{15}N, were found in the other ^{15}N-treatments within the first day (up to 50% from $^{15}NH_4^{15}NO_3$). Those rapidly decreased and became similar to the $^{15}NH_4^+$ treatment after 24 hours. For the rest of the incubation similar proportions of N_2O derived from all labelled amendments. Those proportions increased until day 12 when they reached about 10%.

The trends changed in the 70% moisture treatment (Fig. 3b), where the proportion of N_2O from the added ^{15}N initially increased for all ^{15}N amendments. After day 1 the proportion remained the same for the $^{15}NO_3^-$ amendment (▲) but kept increasing steadily for the other ^{15}N-amendments reaching 25 and 30% for $^{15}NH_4^+$ and $^{15}NH_4^{15}NO_3$, respectively.

For the highest moisture treatment (Fig. 3c), the proportion of N_2O from labelled N also increased on the first day for all treatments, however, with $^{15}NO_3$ and $^{15}NH_4^{15}NO_3$ the increase was significantly higher than with $^{15}NH_4$ (○; up to 50%). After this day, the contribution of the labelled amendment to N_2O emissions decreased for those amendments, reducing to 20 and 40% for $^{15}NO_3$ and $^{15}NH_4^{15}NO_3$, respectively on day 13. In the $^{15}NH_4$ treatment on the other hand, N_2O emissions decreased

436 slightly after the maximum in day 1 and then continued to

437 increase, reaching 20% on day 13.

438

439 ### *3.3 Soil N concentrations and ^{15}N enrichment*

440 Analysis of the soil N before each incubation and before core

441 packing showed the following values of TO_xN: 0.0681 ($\pm$0.001),

442 0.1335 ($\pm$0.0112) and 0.0844 ($\pm$0.0096) mg g^{-1} dry soil for 55,

443 70 and 85% WFPS-incubations, respectively. For NH_4^+, values

444 were 0.0869 ($\pm$0.0044), 0.0485 ($\pm$0.0010) and 0.0957 ($\pm$0.0017)

445 mg g^{-1} dry soil for 55, 70 and 85% WFPS-incubations,

446 respectively.

447

448 Figure 4 shows the dynamics of the analysed N forms in the soil

449 throughout the experiment. Soil NO_2^- was of the order of 0.1 μg

450 N g^{-1} dry soil during the incubation period and slightly higher in

451 the 85% WFPS treatment. Soil NH_4^+ and NO_3^- concentrations

452 were around 1000 times higher than NO_2^-, with more NO_3^- than

453 NH_4^+ in the 70% and 85% WFPS treatments, while no

454 differences in soil NH_4^+ and NO_3^- could be detected in the

455 55%WFPS treatment.

456 The 70 and 85% WFPS treatments showed larger changes in the

457 time series with soil NO_3^- increasing and NH_4^+ decreasing, while

458 those concentrations remained relatively constant and of similar

459   magnitude (around 0.15 mg N g^{-1} dry $soil^{-1}$) in the 55% moisture

460   treatment.

461

462   The ^{15}N-enrichment of soil NO_2^-, NO_3^- and NH_4 is shown in

463   Figure 5. The lowest ^{15}N-enrichment of soil NO_2^- and NO_3^- was

464   from the $^{15}NH_4$ amendment (●) (Fig. 5a and b) for all moisture

465   treatments while a higher enrichment of those two soil

466   components was found when $^{15}NO_3$ (▲) or $^{15}NH_4{}^{15}NO_3$ (■)

467   were applied (Fig. 5d,e,g and h). Values of enriched NO_2^- were

468   generally lower than those of enriched NO_3^- (5 vs. 20 atom%)

469   (Fig. 5a and b). Soil ^{15}N-enrichment of NO_3^- was generally in the

470   order 85%>55%>70% WFPS (*solid blue, dotted orange, dashed*

471   *green*) when the soil was amended with $^{15}NO_3$ or $^{15}NH_4{}^{15}NO_3$

472   (Fig. 5e and h).

473   The amendment with $^{15}NO_3$ (▲) resulted in lowest soil NH_4^+

474   enrichment (Fig. 5f) at 70 and 85% WFPS, while the opposite

475   was found for the initial 4 days when soil was at 55% WFPS.

476   Here treating the soil with $^{15}NO_3$ resulted in higher soil NH_4^+

477   enrichment than soil treated with $^{15}NH_4$ or $^{15}NH_4{}^{15}NO_3$. There

478   was no significant difference in the enrichment of the soil NH_4^+

479   depending on whether the soil was amended with $^{15}NH_4$ or

480     $^{15}NH_4{}^{15}NO_3$; enrichment was higher for the 70 and 85% WFPS

481   treatments than the 55% one (Fig. 5c and i).

482   As previously mentioned, compared to the other amendments the

483   addition of $^{15}NH_4$ resulted in significantly lower enrichment of

484 ^{15}N-labelled NO_2^- as well as NO_3^- for all WFPS treatments and a

485 significant decrease in $^{15}NH_4^+$ at the lower WFPS values of 55

486 and 70%.

487 When applying $^{15}NO_3$ the only significant changes in the

488 enrichment of ^{15}N-labelled compounds was found at 85% WFPS

489 where ^{15}N-labelled NO_3^- enrichment was significantly lower at

490 the end of the 10-day experiment and at 55% WFPS where ^{15}N-

491 labelled NH_4^+ enrichment was also significantly lower at the end

492 of the experimental period (Fig.5d-f).

493 Applying $^{15}NH_4^{15}NO_3$ did not result in any significant changes

494 in the enrichment of ^{15}N-labelled NO_2^- or NO_3^- at any of the

495 WFPSs. However, a significantly lower enrichment of ^{15}N-

496 labelled NH_4^+ between the beginning and end of the

497 experimental period was found for all WFPS values (Fig. 5g-i).

498

499 *3.4 Analysis of transformation rates*

500 The results of the *Ntrace* analysis tool (Fig. 1) showed that gross

501 transformation rates of NO_3^- and NH_4^+ and Mineralisation of

502 labile N to NH_4^+ were generally highest at 55% WFPS and

503 mostly decreased with increasing WFPS (Fig. 6a-c). Oxidation

504 of recalcitrant N to NO_3^-, however increased with increasing

505 WFPS (Fig. 6d). Desorption of adsorbed NH_4^+ as well as NO_3^-

506 was highest at 70% WFPS (Fig. 6e), although not statistically

507 significant, while the transformation of NH_4^+ to NO_3^- was

508 significantly lower at this WFPS than at the higher or lower

509 WFPS (Fig. 6a).

510

511 **_3.5 Apportioning of N_2O emissions_**

512 Figure 7 shows the resulting apportioning of the N_2O emissions

513 to the three different processes: heterotrophic nitrification,

514 denitrification and nitrification. At 55% WFPS, an initial large

515 contribution of denitrification is shown, which quickly

516 decreased in favour of heterotrophic nitrification (30%) by the

517 end of day 1. Heterotrophic nitrification remained the dominant

518 process throughout the incubation except on days 4 and 10, when

519 the sum of denitrification and autotrophic nitrification where

520 approximately 50%.

521 At 70% WFPS, heterotrophic nitrification dominated at the start

522 of the incubation vs denitrification (70 vs 30%) but decreased in

523 importance with time to almost zero at the end of the incubation,

524 when autotrophic nitrification became more dominant (65%).

525 At 85% WFPS, heterotrophic nitrification is only relevant on the

526 first day (80%); from then on, denitrification dominated (~100%

527 on days 1-2) and remained at about 60-80% with the rest of the

528 contribution coming from autotrophic nitrification.

529 The summary graph (Fig. 8) shows the average contribution of

530 each process to N_2O emissions as total amounts of N_2O-N

531 emitted, as well as percentage of N_2O emitted by each of the

532 three processes. With increasing soil moisture, an increase in the

533   contribution from denitrification to N_2O emissions was found,

534   whilst the contribution from heterotrophic nitrification

535   decreased. For autotrophic nitrification, however, the largest

536   contribution was at the intermediate soil moisture of 70% WFPS.

537

538   4   **Discussion**

539   In a recent literature review and meta-analysis, Barrat et al.

540   (2020) found that WFPS was a significant explanatory variable

541   for N_2O emissions and this was affected by the prior moisture

542   status of the soil. In our experiments, the soils were prepared in

543   a standard manner, so only the final moisture status at the start

544   of the incubation differed. Therefore in our study, we

545   investigated the relative differences between the 3 soil moisture

546   status (or WFPS) on N partitioning in the soil N compounds

547   and the N emitted compounds, and the apportioning of N_2O

548   emissions to different processes.

549   *4.1   Process dependent N-emissions at different WFPS*

550   Denitrification, if complete, transforms the produced N_2O into

551   N_2. Denitrification is commonly incomplete with N_2O not being

552   transformed to N_2 due to a lack of N_2O reductase (Nos) in the

553   microbial community, or due to a sufficient supply of NO_3^-

554   whose reduction is energetically more favourable than the

555   reduction of N_2O to N_2 (Saggar et al., 2013). Due to incomplete

556   denitrification, highest N_2O production is expected from

557 denitrification and consequently from soils with a relatively

558 higher WFPS. However, the importance and dominance of

559 certain processes ultimately depends on the microbial

560 community present in the soil and its activity which is influenced

561 by the soil conditions. In our study we used a grassland soil that,

562 has not had any fertiliser input, nor been grazed and therefore

563 has not received animal excrements as a nutrient source for over

564 20 years. We assume that due to the management of the field

565 lacking regular supply of nutrients, the microbial community

566 within the soil would have differed from those communities

567 found in other grasslands (Denef et al., 2009). This would have

568 had an influence on the N-transformation processes in this soil.

569 Additionally, it has been shown that soil moisture content

570 influences nutrient availability and movement through the soil

571 (Misra and Tyler, 1999) therefore influencing access of those

572 nutrients transported within a solution to the present microbial

573 community and subsequently influencing N transformation

574 processes.

575 In addition, the contributions observed from the treatments

576 applied to the emitted N_2O were generally less than 50%,

577 implying that the soil N pool was a larger contributor. We had

578 no zero N treatment in our experimental design to confirm this,

579 however, even if we had this, it is possible that the soil microbial

580 community was primed by added N (Müller and Clough, 2014),

581 <u>so more of the soil N would have been utilised in the N treated</u>

582 <u>soils, than in a zero N control.</u>

583 4.1.1 N-emission processes at 85% WFPS

584 In our study, the highest N_2O emissions were found at WFPS of

585 85% and these emissions decreased over time. At this high

586 WFPS the dual labelling analysis showed that more N_2O was

587 derived from the applied NO_3^- (Fig. 3c, initially $^{15}NO_3^-$

588 contributed over 50% while $^{15}NH_4^+$ contributed less than 5%),

589 indicating that denitrification was the dominant process in our

590 experiment. Over the course of the experiment at 85% WFPS,

591 the proportion of N_2O from the ^{15}N labelled NO_3^- decreased,

592 while that of NH_4^+ increased.

593 A possible explanation for the increased contribution of applied

594 $^{15}N-NH_4^+$ in N_2O emissions could be that the measured $^{15}N-N_2O$

595 derived from $^{15}NO_3^-$ which had previously been produced via

596 nitrification from the added $^{15}NH_4^+$. The results of soil NO_3^-

597 agree with this as there was an increase during the incubation

598 coinciding with a decrease in soil NH_4^+. The initial increase in

599 CO_2 reflects aerobic respiration after the treatments were applied

600 that settles at the end of the peak at about days 3-4. The N_2 fluxes

601 up till day 4 in the highest soil moisture treatment can be

602 explained by an increase in anaerobicity during this period

603 promoting denitrification. It is possible, that O_2 concentrations

604 recover with time, changing conditions from promoting

605   denitrification to promoting nitrification where N_2O is produced

606   from hydroxylamine NH_2OH. Nitrifying conditions might have

607   also developed at the surface by drying of the upper layers of the

608   soil. Though moisture contents of the soil cores used in this

609   experiment did not change significantly over time, it has been

610   shown in previous experiments that water can redistribute from

611   top to bottom creating more aerobic, nitrification promoting

612   conditions at the surface where gas exchange with the

613   atmosphere takes place (Loick et al., 2016). However, our results

614   suggest that most of the detected N_2O came from denitrification

615   of the NO_3^- produced via nitrification of the applied $^{15}NH_4^+$ due

616   to the increase in NO_3^- and a general decrease in NH_4^+ at 85%

617   WFPS (Fig. 4). Therefore, while nitrification is taking place even

618   under this high WFPS, denitrification is still the dominant

619   process producing N_2O. This is further supported by soil ^{15}N

620   analysis (Fig. 5), where results show a significant increase in soil

621     $^{15}NO_3^-$ in the $^{15}NH_4^+$ treatments, while the enrichment of $^{15}NH_4^+$

622   in the same treatment significantly decreased.

623   Emissions of other N-gases produced during N transformation

624   processes provide additional support that denitrification was

625   most important at the highest WFPS of 85%. Higher emissions

626   of N_2 (Fig. 2c), the final product of denitrification indicate that

627   complete denitrification had been achieved for some of the

628   available NO_3^-.

629 4.1.2 N-emission processes at 70% WFPS

630 At the intermediate WFPS of 70% it was expected that

631 nitrification and denitrification would be equally important. In

632 fact, the results of the *Ntrace* analysis tool show an equal

633 contribution of denitrification, nitrification and heterotrophic

634 nitrification at 70% WFPS. ^{15}N soil analysis also supports a near

635 equal distribution of nitrification and denitrification with $^{15}NH_4^+$

636 showing a decrease and $^{15}NO_3^-$ a corresponding increase when

637 $^{15}NH_4^+$ was added (Fig. 5b/c). The analysis of $^{15}N_2O$ (Fig. 3b)

638 revealed an approximately 3 times higher contribution of the

639 added $^{15}NO_3^-$ to N_2O emissions than that of added $^{15}NH_4^+$,

640 indicating that most of the emitted N_2O was produced via

641 denitrification. However, total amounts of N_2O were very small,

642 as were CO_2 emissions (Fig. 2d), both indicating that the

643 microbial N-transformation processes and denitrification in

644 particular were very slow/small under these conditions.

645 4.1.3. N-emission processes at 55% WFPS

646 The lowest WFPS of 55% was chosen to promote nitrification.

647 The results of the *Ntrace* analysis tool support that this was the

648 case with nitrification and heterotrophic nitrification

649 contributing to about 80% of N_2O emissions (Fig.8), while

650 denitrification only played a role at the very beginning of the

651 incubation after amendment was applied, which would have

652 temporarily increased the WFPS at the top of the core and

653 promoted anaerobic, denitrifying conditions prior to the

654　amendment solution percolating into the soil. This is supported

655　by the ^{15}N analysis of the emitted N_2O, which initially showed a

656　high contribution of added $^{15}NO_3^-$ to N_2O emissions, indicating

657　denitrification being the main process producing N_2O, which

658　quickly declined. By day 1 both, applied $^{15}NO_3^-$, as well as

659　$^{15}NH_4^+$, contributed equally to N_2O emissions. (Fig.3a).

660　Considering that N_2O is not an obligatory intermediate during

661　nitrification, but merely a potential by-product (Anderson,

662　1964), these results also indicate that nitrification processes

663　dominate over denitrification under these low moisture

664　conditions.

665

666　*4.2　Influence of WFPS on soil N-transformation*

667　　　*processes*

668　Our study demonstrates the influence of WFPS on soil N-

669　transformation processes. Generally, gross soil N transformation

670　rates associated with both NH_4^+ and NO_3^- turnover decreased

671　with increasing WFPS. The total contribution of nitrification to

672　soil N transformation processes was higher at low WFPS and

673　decreased with increasing WFPS. However, an interesting

674　observation was that the oxidation of organic N to NO_3^-

675　increased almost 5-fold from 70 to 85% WFPS which may

676　support the higher denitrification rate by supplying additional

677　electron acceptors. However, this increase was not paralleled by

678　an increase of N_2O emitted. This may be due to an increasing

679   reduction of N_2O to N_2 (i.e. increasing N_2:N_2O ratio or decrease

680   in N_2O:N_2 as described earlier) under increasing anaerobicity

681   (Butterbach-Bahl et al., 2013).

682   The optimal conditions for nitrification are said to occur between

683   30-60% WFPS (Medinets et al., 2015). Emissions of NO can

684   derive from nitrification as well as denitrification, though it has

685   been found that the rates of produced NO measured as emissions

686   are higher under drier conditions, where a lower WFPS leaves

687   more air-filled pores enabling NO to escape to the surface

688   (Pilegaard, 2013). At WFPS above 65% it is believed that

689   emissions of N_2O and N_2 increase due to an increase in

690   denitrification. NO, however, while it is being produced to a

691   larger extent at high soil moisture, is also reduced to N_2O due to

692   a longer residence time decreasing the amount emitted to the

693   surface (Pilegaard, 2013). In this study, the observed increase in

694   NO emissions with increasing moisture levels suggests

695   denitrification was the source. Loick et al. (2016) concluded that

696   up to 0.67% of the added N (from a nitrate source) was emitted

697   as NO from denitrification supporting our findings.

698   Our results did not confirm our first hypothesis that losses are

699   lower at higher moisture levels for NO and N_2O. In fact, for all

700   gases, losses were higher at the high soil moisture possibly

701   because the soil was not saturated enough to impede gas

702   diffusion. Our second hypothesis was partly proved, as at the

703   high soil moisture the proportion of N_2O from nitrate containing

704   amendments was higher. The results for the lower moisture level

705   did not agree with our hypothesis as the proportion of N_2O from

706   all the amendments was similar and not mainly from NH_4^+.

707   Overall, our results support the assumption that nitrification

708   (autotrophic as well as heterotrophic) plays a bigger part at lower

709   WFPS, when air filled pores increase aerobicity, while

710   denitrification becomes more important the higher the WFPS

711   and therefore the lower the aerobicity. With our ^{15}N tracing

712   approach we found that heterotrophic nitrification was the

713   dominant process at 55% WFPS disproving our third hypothesis

714   that nitrification and denitrification dominate at all moisture

715   levels, its contribution quickly decreased with increasing WFPS,

716   while nitrification contributed most at the intermediate WFPS of

717   70% and least at 55%. Heterotrophic nitrification has been

718   reported in previous studies as dependent on soil pH, C:N ratio

719   and land use and that it can contribute up to 85% of the total N_2O

720   flux in soils with pH values between 4.2 to 8.4 (Zhang et al.,

721   2015). This process converts organic N (although it is believed

722   it also happens with inorganic N sources (Zhang et al., 2014)) to

723   NO_3^-. It is believed this occurs particularly in acidic soils where

724   autotrophic nitrification can be inhibited. The soil used in this

725   study was of pH 5.6 (Table 1) placing it within the soils that can

726   potentially undergo this process. Müller et al. (2014) stated that

727   heterotrophic nitrification is a contributor to N_2O emissions in

728   grassland soils with high organic matter contents. This further

729 supports the finding that this process occurs in this study

730 (organic matter content 11.7% Table 1). In the study by Rütting

731 and Müller (2008) it was shown that heterotrophic nitrification

732 would carry out oxidation of organic N to NO_2^- (rather than NO_3^-

733). We also know that microbial consortia exist where a network

734 of metabolic activity is present (Butterbach-Bahl et al., 2013),

735 therefore it is likely that NO_2^- originating from the organic N pool

736 is directly reduced to N_2O (and not further oxidised to NO_3^-) by

737 the activity of denitrifying organisms. This also explains that

738 higher percentages of N_2O via the organic pathway occur under

739 higher WFPS values.

740 At the WFPS above 70% it has been shown that N_2O is produced

741 solely by denitrification (Bateman and Baggs, 2005). However,

742 in our case denitrification only became dominant at 85% WFPS,

743 and denitrification contributed about 70% of the N_2O emissions

744 at this WFPS (Fig. 7,8), while overall not much activity was

745 found at neither 50, nor 70% WFPS.

746 The lower N_2O emissions for the 2 lower moisture levels over

747 the course of the experiment could be due to a slower response

748 of the microbial community to the added N compared to the

749 highest soil moisture treatment where nutrient availability is

750 expected to be higher (Papendick and Camprell, 1981).

751 Emissions of CO_2 have been used as an indicator of microbial

752 respiration and activity (López-Aizpún et al., 2018). In this study

753 the results indicate that the microbial community was most

754 active at a WFPS of 85% in agreement with the above statement,

755 but this was followed by the driest treatment and the least active

756 was at the intermediate WFPS of 70% coinciding with the N_2O

757 trend. Other factors need to also be considered as N_2O

758 production and consumption from biogenic processes as well as

759 abiotic processes such as gas diffusion, are both dependant of

760 moisture in soil.

761

762 5   **Conclusions**

763 Our results highlight the variability in the effect of WFPS on the

764 dominance of different N transformation processes in soil.

765 Though the general assumption, that denitrification is more

766 important at high WFPS, is supported here, the actual percentage

767 of WFPS attributed to the different processes was not as

768 expected. Heterotrophic nitrification was found to be an

769 important source of N_2O especially under drier conditions while

770 nitrification plays a crucial role for N_2O emissions, directly but

771 also via nitrification coupled with denitrification under medium

772 and high WFPS.

773 Results obtained from the experiment performed at 85% WFPS

774 show the importance of nitrification even under high WFPS and

775 raise the question if and how much of the N_2O emissions could

776 have been mitigated by preventing nitrification supplying NO_3^-

32

777 for denitrification by e.g. using nitrification inhibitors (Owusu-

778 Twum et al., 2017; Wu et al., 2017a; Wu et al., 2017b).

779 Our study was performed under controlled conditions with a clay

780 soil that had not received any fertiliser or manure/slurry input for

781 few years. Under these conditions, we found a relatively equal

782 contribution of nitrification, denitrification and heterotrophic

783 nitrification to N_2O production at 70% WFPS. At the lower

784 WFPS of 55% the contribution of heterotrophic nitrification

785 dominated, while at the highest WFPS of 85% denitrification

786 contributed most of the measured N_2O. These results will not

787 necessarily apply to other soil types, particularly extreme high or

788 low organic matter soils. Further studies to understand how

789 carbon quality affect the fate of N in soils are needed.

790 However, the process that will be supported at a certain WFPS

791 will most likely depend on the type of soil including its natural

792 carbon and nutrient content, its history and the microbial

793 community present. Emissions are also influenced by abiotic

794 factors that are also dependant on soil moisture.

795

6   Acknowledgements

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Table 1. Soil characteristics (before amendment application).

Mean ± standard error ($n = 3$).

Parameter	Amount		
pH water [1:2.5]	5.6	±	0.27
BD (g cm^{-2})	0.8	±	0.0005
Available Magnesium (mg kg^{-1} dry soil)	100.4	±	4.81
Available Phosphorus (mg kg^{-1} dry soil)	10.4	±	1.10
Available Potassium (mg kg^{-1} dry soil)	97.5	±	12.83
Available Sulphate (mg kg^{-1} dry soil)	51.7	±	0.62
Total N (g kg^{-1} dry soil)	5.0	±	0.10
Total Extractable Oxidised N (mg kg^{-1} dry soil)	15.1	±	0.07
Ammonium N (mg kg^{-1} dry soil)	9.2	±	0.09
~~Organic Matter~~Total Organic Carbon (% w/w)	~~11.7~~6.79	±	~~0.29~~0.17

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967

Table 2. Average cumulative emissions of NO, N$_2$O over the experimental period and N$_2$ from day 2.6 (after flushing out of N$_2$ introduced with amendment) in kg N ha^{-1}

Mean ± standard error (n = 9). Different letters indicate significant differences in emissions between the WFPS treatments (p<0.05)

WFPS	NO-N				N$_2$O-N				N$_2$-N				total N				%N as NO-N	%N as N$_2$O-N	% N as N$_2$-N
55%	1.09E-04	±	6.28E-06	c	4.16E-03	±	2.35E-04	b	0.00	±	0.00	a	0.00	±	0.00		na	na	na
70%	1.41E-04	±	7.32E-07	b	2.69E-03	±	4.28E-05	a	0.08	±	0.08	a	0.09	±	0.08		0.16	3.0	89
85%	1.61E-04	±	5.71E-06	a	8.51E-02	±	3.52E-03	c	0.32	±	0.30	a	0.40	±	0.31		0.04	21.2	80

968

Dear editor and reviewers:

We would like to thank you for your comments.

Reviewer #2: The authors have unfortunately only responded to a small part of my comments on your manuscript. Possibly this is due to the change in the person of the corresponding author. Remaining are above all deficiencies concerning the reliability of the methods used and the interpretation of the presented results. Only when these have been corrected can I agree to the publication of the manuscript. However, the revision will certainly contribute to a much better recognition of the novelty value of the presented research approach.

In detail, this involves the following issues.

In general, a critical discussion of the reliability of the results is missing. This concerns first of all the measurement of gas fluxes with the DENIS system. Thus, given the low N2O fluxes at 55% and 70% WFPS and the absence of N2O in the He-O2 mixture, it is possible that negative N2O fluxes could not be detected. Furthermore, the rather surprising occurrence of N2-fluxes at 55% and 70% could well be due to leaks of the incubation system and/or a low detection sensitivity of the helium ionization detector.

Previous answer: We thank the reviewer for the points raised. We would only be able to detect concentrations that are within DLs. For consumption of N2O to occur, we would have to have N2O in the headspace which we don't have. However, there is consumption of N2O from the added N, which we measure as N2. Emissions of N2 were negligible in the 55 and 70% moisture treatments, had there been leaks in the system, all the vessels would have had high values.
R: the gas measurements are in a dynamic chamber, so there is a flow of gas continuously supplied to the headspace. We have not seen negative fluxes in this system in 20 years. Normally negative fluxes are observed in static chambers, but even in these there is controversy about negative fluxes and whether they are real.
As figure 2c shows, only the highest moisture treatment showed significant N2 fluxes, the lower moisture treatments didn't.

Another disadvantage in this context is that the investigations by DENIS did not include an unfertilized variant. The comparison of the N2O release from the control with the fertilized variant would certainly have provided clear indications as to whether the relatively high proportion of soil N in the N2O formation at 55% and 70% WFPS is also plausible. Please also address this issue briefly in the discussion.
Previous answer: We have a limited number of treatments that we can include in 1 incubation. As we needed 3 treatments (3 labelling) we sacrificed the controls for an extra replicate for the treatments (3 treatments x 4 replicates) to make experiments more robust. We were interested in the relative difference between treatments which resulted in this design, but we agree with the reviewer a control could have given us other insights.
R: we understand that the reviewer suggests that soil N might not be a large contributor to emissions. Unfortunately, even if we had included a non-fertiliser treatment, we would not be able to prove this, as adding N to the soil might have primed the microbes to take up more soil N, which might have not been taken otherwise. However, we have included some text in the discussion regarding this.

A discussion is also necessary regarding the results of the 15N-based investigations. In view of the much lower oxidation intensity of Nrec compared to the NH4 and NO3 transformations, it must be critically questioned whether the calculated high percentages of heterotrophic nitrification, especially at 55% and 70% WFPS, are at all plausible.

R: In an earlier study (Rütting, T., Müller, C., 2008. Process-specific analysis of nitrite dynamics in a permanent grassland soil by using a Monte Carlo sampling technique. European Journal of Soil Science 59, 208-215) it was shown that heterotrophic nitrification would carry out oxidation of Norg to nitrite (rather than nitrate). We also know that microbial consortia exist where a network of metabolic activity is present (see Butterbach-Bahl, K., Baggs, E.M., Dannenmann, M., Kiese, R., Zechmeister-Boltenstern, S., 2013. Nitrous oxide emissions from soils, how well do we understand the processes and their controls. Philosophical Transactions of the Royal Society London B368, 16-21.) therefore it is likely that nitrite originating from the Norg pool is directly reduced to N2O (and not further oxidised to nitrate) by the activity of denitrifying organisms. This also explains that higher percentages of N2O via the organic pathway occur under higher WFPS values (i.e. more reducing conditions).

Especially serious is the lack of a comparative consideration of the results from both methods. However, this is very important because only then it becomes clear whether the coupling of different methods actually leads to synergy effects in the acquisition of new knowledge, as indicated in the introduction. Particular attention must be paid to the clarification of apparent contradictions. For example, the extremely high shares of N2, which presumably originates from denitrification, in the total amount of gaseous N losses, especially at 55% and 70% (Table 2), contradict the relatively low share of denitrification in N2O formation due to the 15N studies (Figures 7 and 8).

R: we can clarify this, as the reviewer might be misunderstanding the difference between the 2 datasets:

The fluxes from the Denis system represent the total fluxes from the amended soils, including: soil N + amendment N sources.

The isotope data, are tracing the pathway of the amendment N through the soil and out as gas. So whilst Table 2 gives the total fluxes emitted, the figures (7, 8) are using the enrichment of the emitted N_2O to apportion to the different sources.

So a small flux can have a large proportion of that flux attributed to a particular source. The two methods are not comparable but supplementary.

For the generalizability of the results, it is also important to deal with the limited suitability of the WFPS for characterizing the real gas dynamics in soils (e.g. Farquharson & Baldock (2008), Plant and Soil 309, 147-16). Not least because the very low bulk density of 0.8 g soil cm-3 used here means that the absolute volume of aerated pores is still very large even at high WFPS. On arable sites with usually much higher bulk densities this is of course quite different.

R: although we agree with the reviewer using WFPS can be limited, it is still a good indicator of source processes. We have just published a literature review (Barrat et al., attached) demonstrating this and have included text in the discussion with this reference.

We also agree that even at high WFPS we can still see processes such as nitrification, and this is shown in Fig. 7 and 8. So the isotope tracing tool has helped us determine the contributions of the processes as stated in the manuscript (ie denitrification, nitrification, etc).

Minor deficits

In the section Material and Methods it must be explicitly clarified which N2O was used for the 15N analyses. With high probability, not the N2O from the DENIS facility, but from soil cores of the parallel incubation (lines 282-294).

R: we used the N_2O from the Denis system, as we have continuous data. We have added this in the methods to make it clear (2.3).

Table 1: Please indicate total organic C instead of organic matter.

R: we have converted OM to TOC as requested.

Highlights

- heterotrophic nitrification is an important source of N_2O at low WFPS
- similar contributions of nitrification, heterotrophic nitrification and denitrification occurred at medium WFPS level
- high N_2O emissions result via nitrification coupled denitrification at medium & high WFPS
- abiotic factors need to be considered as well as biotic factors to understand the cause of emissions

1   **Application of a triple ^{15}N tracing technique to elucidate N**

2   **transformations in a UK grassland soil**

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27

Abstract

29   To identify the production and consumption pathways and

30   temporal dynamics of N_2O emitted from soil, this study uses ^{15}N-

31   labelled substrate-N to quantify the underlying gross N

32   transformation rates using the *Ntrace* analysis tool and link them

33   to N-emissions. In three experiments twelve soil cores each were

34   incubated in a lab incubation system to measure gaseous

35   emissions, while parallel incubations under the same conditions

36   were set up for destructive soil sampling at 7 time points. Using

37   the triple labelling technique (applying NH_4NO_3 with either the

38   NH_4^+-N or the NO_3^--N, or both being ^{15}N labelled), this study

39   investigated the effects of 55, 70 and 85% water filled pore

40   space (deemed to promote nitrification, both nitrification and

41   denitrification, and denitrification, respectively) in a clay soil on

42 gaseous N emissions and investigates the source and processes

43 leading to N_2O emissions.

44 To assess the utilisation of applied NO_3^- vs. nitrified NO_3^- from

45 applied NH_4^+, the ^{15}N tracing tool *Ntrace* was used to quantify

46 the rates of immobilisation of NO_3^- and NH_4^+, oxidation of NH_4^+,

47 mineralisation of organic N and subsequent nitrification by the

48 analysis of the ^{15}N in the soil. Gross transformation rates were

49 calculated, indicating the relative importance of added NO_3^- and

50 NO_3^- derived from nitrified added NH_4^+.

51 Results show an important contribution of heterotrophic

52 nitrification (organic N oxidation to NO_3^-) which was highest at

53 the 55% water filled pore space (WFPS), decreasing in its

54 contribution to N-transformation processes with increasing

55 WFPS, while nitrification (NH_4^+ oxidation to NO_3^-) was

56 contributing the most at 70% WFPS. The contribution of

57 denitrification increased with increasing WFPS, but only

58 became dominant at 85% WFPS. While denitrification still

59 showed to be most important at high and nitrification at lower

60 WFPS, the actual % WFPS values were not as expected and

61 highlight the fact that WFPS is a contributor, but not the

62 sole/most important parameter determining the type of N-

63 transformation processes taking place.

64

65 **Keywords**

66   Nitrous oxide; denitrification; nitrification; heterotrophic

67   nitrification

68   1   **Introduction**

69   Nitrous oxide (N_2O) is an important greenhouse gas (GHG)

70   accounting for approximately 6% of the current global warming

71   (WMO, 2018). The atmospheric N_2O concentration has been

72   increasing since the Industrial Revolution, with soils

73   representing its major source, making the understanding of its

74   sources and removal processes important for the development of

75   mitigation strategies.

76   Several processes have been studied to determine their

77   contribution to N_2O production in soils: (i) nitrification, which

78   has been reported as autotrophic (NH_4^+ oxidation) and

79   heterotrophic (organic N oxidation) (Zhang et al., 2015); (ii)

80   denitrification, due to the incomplete denitrification of nitrate

81   (NO_3^-) under anaerobic conditions (Attard et al., 2011); (iii)

82   nitrifier denitrification (Zhu et al., 2013); and (iv)

83   chemodenitrification as a non-biological process (Van Hecke et

84   al., 1990).

85   It has been found that N_2O is mainly produced via biological

86   processes and that emissions through nitrification and

87   denitrification produce up to 70% of the annual emitted N_2O

88   worldwide (Butterbach-Bahl et al., 2013). Several studies aimed

89   to distinguish the main pathway responsible for N_2O emissions

90   (Khalil et al., 2004; Bateman and Baggs, 2005), and identify a

91   predominant process under certain conditions. While

92   nitrification requires O_2, denitrification relies on its absence or

93   limitation and has been attributed to anoxic conditions (Khalil et

94   al., 2004). It is therefore generally agreed that water filled pore

95   space (WFPS) is one of the key factors affecting which process

96   dominates N_2O production. The higher the WFPS the more air

97   in pores is replaced by water, thereby removing O_2 from the soil.

98   However, it is also thought that several processes can occur

99   simultaneously in different microsites of the same soil (Arah,

100   1997) due to the generation of local differences in soil

101   aggregates.

102   It is well known that N_2O is produced by microorganisms who

103   are dependent on several factors, such as environmental

104   conditions, nutrient availability etc. (Saggar et al., 2013), which

105   suggests that it is also likely that the N_2O-source processes

106   themselves change over time due to changes in limiting factors

107   such as soil moisture and carbon availability, allowing newly

108   formed N-species to become new sources. As an example, in

109   addition to added NO_3^-, the native soil NO_3^- and that produced

110   from nitrification of applied or soil NH_4^+, can also be a source of

111   N_2O via denitrification following nitrification.

112   Different methods have been applied to identify the occurrence

113   and importance of different processes under different conditions.

114   Amongst those are ^{15}N-labelling techniques (Stark, 2000), as

well as isotopologue analyses of N_2O and O_2 ($^{15}N/^{18}O$) (Meijide et al., 2010; Bergstermann et al., 2011; Wu et al., 2016).

When aiming to determine how important different processes are under certain environmental conditions and management (e.g. soil moisture, C and N applications, etc), incubation experiments, where single factors and combinations of these can be manipulated, are the methodology of choice. Automated systems such as the denitrification incubation system, DENIS (Cárdenas et al., 2003) at Rothamsted have proven useful for process determination. In the DENIS, soil cores are incubated under an N_2-free atmosphere, allowing direct measurements of all emitted N gases (NO, N_2O and N_2) as well as CO_2. The transformation of N in soils and particularly the production of N_2O from different sources, such as fertilisers or animal excreta, has been studied through a series of laboratory incubation experiments (i.a. Meijide et al. (2010), Bergstermann et al. (2011), Loick et al. (2017)) using this system. The advantage of this system, when looking at N_2O source processes is, that under an N_2 free atmosphere it is possible to measure N_2 which, depending on the initial conditions, can only be produced via complete denitrification.

In order to fully investigate transformations leading to N_2O production and removal, quantifying their contributions and assessing the potential for change of processes, a combination of

139   laboratory experiments with models/analysis tools at the same

140   scale offer great potential.

141   One process model/analysis tool using ^{15}N distribution in the

142   data obtained from ^{15}N labelling experiments has been

143   developed by Müller et al. (2004; 2007). This analysis tool,

144   represents an improvement of the dilution model by Kirkham

145   and Bartholomew (1954), and includes soil nitrite and gaseous

146   compounds emitted. It traces ^{15}N applied to soil and quantifies

147   the gross N rates based on measurements of the partition of ^{15}N

148   in soil pools from dual or triple isotope labelling of the source.

149   The model determines the most suitable dynamics through the

150   best Akaike's Information Criterion (AIC). The objective of this

151   study is to show how N-transformation processes leading to N_2O

152   emissions change over time and how WFPS can influence the

153   initial dominance of certain processes but does not necessarily

154   determine a sole process. The advantage of the triple labelling

155   technique is that production of N_2O from an organic (unlabelled)

156   source outside the mineral N pools can be unambiguously

157   determined because if all relevant mineral N pools are labelled

158   then a dilution of the N_2O has to arrive from outside that system.

159   Also, for the parameter optimisation techniques it provides

160   additional observations which reduce the danger of over

161   parameterisation during parameter optimisation

162   To achieve this the triple labelling technique using Ammonium

163   Nitrate (NH_4NO_3) was applied as a substrate with the N being

164 labelled with ^{15}N in its different positions. Changes in soil N

165 (NO_2^-, NO_3^-, and NH_4^+) were measured to quantify the

166 underlying gross N transformation rates using the *Ntrace*

167 analysis tool (Müller et al., 2007) with the measured emissions

168 to then identify sub-rates based on the ^{15}N distribution in the

169 data. This was linked to gaseous N-emissions to identify the

170 production and consumption pathways and temporal dynamics

171 of N_2O. In order to determine the source of N_2O from the triple

172 labelling experiment, the DENIS was extended by connecting it

173 to a GC-MS to include continuous measurements of emitted ^{15}N-

174 N_2O.

175 We will test the following hypothesis: 1) that NO and N_2O losses

176 at different soil moisture levels will decrease at higher moisture

177 values due to easier diffusion and conversion to N_2; 2) that at the

178 highest soil moisture N_2O is mostly derived from NO_3^- whilst at

179 the low moisture from NH_4^+; 3) that nitrification and

180 denitrification are the main sources of N_2O at all moistures.

181

182 2   **Materials and Methods**

183   *2.1   Soil Preparation*

184 A clayey pelostagnogley soil of the Hallsworth series (Clayden

185 and Hollis, 1984) (44% clay, 40% silt, 15% sand (w/w), Table

186 1) was collected on the 26th of May 2015 from a typical

187 grassland in SW England, located at Rothamsted Research,

188 North Wyke, Devon, UK (50°46'10''N, 3°54'05''W). Spade-

189 squares (20 x 20 cm to a depth of 15 cm) of soil were taken from

190 12 locations along a 'W' line across a field of 600 m^2 size, which

191 had not had any grazing animals on it, nor received any fertiliser

192 input for over 20 years. After sampling, the soil was air dried to

193 ~30% H$_2$O (gravimetric moisture content), roots and plant

194 residues were removed, and the soil sieved to <2 mm and stored

195 at 4°C before packing into cores and starting the incubation.

196 Initial soil characteristics are given in Table 1.

197

198 ## *2.2 Experimental Design*

199 The incubation experiment was carried out using the DENIS, a

200 specialized gas-flow-soil-core incubation system (Cárdenas et

201 al., 2003) in which environmental conditions can be tightly

202 controlled. The DENIS simultaneously incubates a maximum of

203 12 vessels containing one soil core each. Cores were packed to a

204 bulk density of 0.8 g cm^{-3} to reflect field conditions, to a height

205 of 75 mm into stainless steel sleeves of 141 mm diameter. Due

206 to the limited space within the DENIS and the requirement for

207 replication, three experiments (see below) were performed

208 directly one after another under the same tightly controlled

209 conditions (i.e. temperature, gas flow, amendment application).

210 All soil was kept in the fridge (4°C) until needed and treated to

211 the same time scales to prevent any changes in soil

212 characteristics.

213   To promote nitrification-, denitrification- or a combination of

214   both, each experiment was performed at a different WFPS

215   (Bollmann and Conrad, 1998; Butterbach-Bahl et al., 2013). The

216   soil moisture was adjusted to 55%, 70% or 85% WFPS,

217   respectively, taking the amendment with nutrient solution into

218   account. To measure N_2 fluxes, the native N_2 was removed from

219   the soil and headspace without limiting O_2 levels that would be

220   present in air. This was achieved by using a helium-oxygen

221   mixture $He{:}O_2$ of 80:20. First the soil cores were flushed from

222   the bottom at a flow rate of 30 ml min^{-1} for 14 h. To measure

223   baseline emissions, flow rates were then decreased to 12 ml

224   min^{-1} and the flow re-directed over the surface of the soil core

225   for three days before amendment application and for the

226   remaining experimental period. The vessels were kept at 20°C

227   during flushing as well as for the 13-day incubation period after

228   amendment application.

229   Three incubations were needed to accommodate the different ^{15}N

230   treatments and soil moisture levels. Each incubation involved the

231   following three treatments of NH_4NO_3 (Sigma-Aldrich, St.

232   Louis, MO, USA), with three replicate vessels per treatment: i)

233    $^{15}NO_3$ = cores amended with single labelled $NH_4{}^{15}NO_3$ at 50

234   atom%; ii) $^{15}NH_4$ = cores amended with single labelled

235    $^{15}NH_4NO_3$ at 50 atom%; iii) $^{15}NO_3{}^{15}NH_4$ = cores amended with

236   double labelled $^{15}NH_4{}^{15}NO_3$ at 50 atom%. Considering the total

237   surface area of the vessel, N was applied at a rate of 75 kg N

238 ha^{-1}. The applied rate of N equates to 125 mg N kg^{-1} dry soil,

239 which was dissolved in 50 ml of H_2O before being applied to the

240 soil. To maintain the incubation conditions, the amendment was

241 applied to each of the three cores via a sealed amendment

242 container on top of the incubation vessel. Before amendment

243 application the headspace of the amendment vessel was flushed

244 with He to prevent any atmospheric N_2 entering the system.

245 Additionally, a parallel incubation only for destructive soil

246 sampling at 7 time-points after treatment application (5 h, days

247 1, 2, 3, 4, 7, 10) with 3 replicates of each was performed each

248 time. For logistical reasons smaller cores (4.5 cm diameter) had

249 to be used, which were packed with the same soil and to the same

250 specifications used for the DENIS incubation and kept under the

251 same controlled conditions. At the sampling time, soil was

252 analysed for extractable Ammonium (NH_4^+), Nitrate (NO_3^-),

253 Nitrite (NO_2^-) concentrations and ^{15}N-enrichment of those

254 molecules ($^{15}NH_4^+$, $^{15}NO_3^-$, $^{15}NO_2^-$).

255

256 *2.3 Gas analyses*

257 Gas samples were taken every four hours for each vessel from

258 the Denis system. Fluxes of N_2O and CO_2 were quantified using

259 a Perkin Elmer Clarus 500 gas chromatograph (Perkin Elmer

260 Instruments, Beaconsfield, UK) equipped with an electron

261 capture detector (ECD) for N_2O and CO_2. N_2 emissions were

262 measured by gas chromatography with a helium ionisation

11

263 detector (VICI AG International, Schenkon, Switzerland), while

264 NO concentrations were determined by chemiluminescence

265 (Sievers NOA280i, GE Instruments, Colorado, USA). All gas

266 concentrations were corrected for the surface area and flow rate

267 going through the vessel (measured daily). Fluxes were

268 calculated on a kg N or C ha^{-1} day^{-1} basis. Isotopic signatures

269 were determined via isotope ratio mass spectrometer (PDZ

270 Europa 20-20 Stable Isotope Analyser, Sercon, Crewe, UK)

271 linked to an ANCA-TGII gas preparation system (Sercon,

272 Crewe, UK).

273

274 *2.4   Soil analyses*

275 The initial soil N was measured at the start of each incubation by

276 randomly taking three 100 g samples from the bulk soil before

277 core packing and WFPS adjustment. This soil was analysed for

278 total extractable oxidised N (TO$_x$N, combined amount of NO$_2^-$

279 and NO$_3^-$) and NH$_4^+$. Soil samples (100 g) from the parallel

280 incubation were analysed for extractable NO$_2^-$, NO$_3^-$ and NH$_4^+$

281 concentrations at each time point. WFPS was calculated from

282 soil moisture contents by drying a subsample (50 g) at 105°C

283 overnight. Soil extractable NO$_2^-$, NO$_3^-$ and NH$_4^+$, concentrations

284 were analysed after blending the samples with 2M KCl at pH 8

285 following the method of Stevens and Laughlin (1995). The

286 extracts   were   analysed   by   colourimetry   using   a

287 Spectrophotometer (Cecil Instruments, Cambridge, UK) for the

288   analysis of NO_2^-, or an Aquakem 250 discrete photometric

289   analyser (Thermo Fisher Scientific, Hemel Hempsted, UK) for

290   the analysis of NO_3^- and NH_4^+. The ^{15}N abundances of the NO_2^-

291   , NO_3^- and NH_4^+ were determined by methods based on the

292   generation of N_2O for isotope ratio mass spectrometry (IRMS).

293   The production of N_2O from NO_2^- and NO_3^- is based on the

294   reaction between NO_2^- and NH_2OH under acid conditions and

295   the NO_3^- having been reduced to NO_2^- with Cd (Stevens and

296   Laughlin, 1994). The production of N_2O from NH_4^+ consists of

297   a diffusion stage where ammonia (NH_3) is absorbed into H_2SO_4

298   followed by an oxidation step where recovered $(NH_4)_2SO_4$ is

299   oxidised to N_2 by alkaline NaOBr, during which N_2O is

300   produced as a by-product (Laughlin et al., 1997). In each case,

301   the resulting N_2O was transferred to an Exetainer (Labco Ltd,

302   Lampeter, Wales). The N_2O enrichment was determined using a

303   Gilson Autosampler (Gilson UK, Dunstable, UK) by IRMS as

304   described in the gas analyses section.

305

306   *2.5   Statistical analysis*

307   Statistical analysis was performed using GenStat 16th edition

308   (VSN International Ltd). Prior to the statistical tests all data were

309   analysed to proof their normal distribution (Kolmogorove-

310   Smirnov test) and equality of variance (Levene test). Cumulative

311   emissions of NO, N_2O, N_2 and CO_2 were calculated from the

312   area under the curve (time vs flux as shown in figure 2) after

313   linear interpolation between sampling points.

314

2.6   *Analysis of N₂O source contribution*

316   To determine the contribution of different sources to N_2O

317   emissions the *Ntrace*basic analysis tool by Müller et al. (2007)

318   was used. This analysis tool represents an extension of the

319   dilution approach of Kirkham and Bartholomew (1954) and

320   quantifies gross N rates based on measured data. To achieve this,

321   a model is used to quantify the individual gross rates, connecting

322   the various soil N pools by parameter optimization routines.

323   The gross N transformation rates quantified where:

324    $M_{Nrec,}$ mineralization of recalcitrant organic N to NH_4^+;

325    $M_{Nlab,}$ mineralization of labile organic N (e.g., monomolecular

326   organic N, amino acids, proteins) to NH_4^+;

327    $I_{NH4Nrec}$, immobilization of NH_4^+ to recalcitrant organic N;

328    $I_{NH4Nlab}$, immobilization of NH_4^+ to labile organic N;

329   A_{NH4}, adsorption of NH_4^+ on exchange sites;

330   R_{NH4a}, release of adsorbed NH_4^+;

331   O_{NH4}, oxidation of NH_4^+ to NO_3^-;

332   O_{Nrec}, oxidation of organic N to NO_3^-; (heterotrophic

333   nitrification)

334   as well as the following 4 rates, which were, however,

335   negligible:

336   I_{NO3}, immobilization of NO_3^- to recalcitrant organic N;

337   D_{NO3}, dissimilatory reduction of NO_3^- to NH_4^+;

338   A_{NO3}, adsorption of NO_3^- to labile organic N;

339   R_{NO3}, release of adsorbed NO_3^-

340   One feature of *Ntrace* is to identify the simplest model structure

341   that is sufficient and adequate to explain the measured data.

342   Therefore, a range of different model versions (including/

343   excluding certain transformation rates) and/or kinetic setting are

344   tested. The most suitable model is then identified by comparing

345   the AIC of each model run which takes the goodness of fit and

346   the number of parameters used into account. Thus, this tool also

347   identifies rates which are not needed to explain the overall

348   dynamics (e.g. the mineralization of labile organic N in our

349   case). Figure 1 shows the full conceptual model according to

350   Müller et al. (2014) indicating the rates used based on the 2007

351   model (Müller et al., 2007) in the top left area.

352   Pathway specific N_2O emissions were determined by assuming

353   that N_2O originated from the NH_4^+, organic N and NO_3^- pool

354   (Fig. 1) (Stange et al., 2009; Müller et al., 2014). The

355   contributions of these three pools were calculated by the

356   parameter identification routine described by Rütting et al.

357   (2010):

358   $$a_{N2O} = C_{NH4} \times a_{NH4} + C_{ON} \times a_{ON} + C_{NO3} \times a_{NO3} \quad (1)$$

359   $$C_{NH4} + C_{ON} + C_{NO3} = 1 \quad (2)$$

360   Where a_{N2O} is the ^{15}N abundance of N_2O produced during

361   incubation, a_{NH4}, a_{ON} and a_{NO3} are the ^{15}N abundance of NH_4^+,

362 organic N and NO_3^-, respectively, and C_{NH4}, C_{ON} and C_{NO3} are

363 the contributions from oxidation of NH_4^+ to NO_3^-, oxidation of

364 organic N to NO_3^- and reduction of NO_3^- to total N_2O

365 production, respectively.

366

367 3 **Results**

368 *3.1 Fluxes of N gases and CO_2*

369 Nitric oxide emissions increased in all treatments (Fig. 2a)

370 during the incubation period. At the highest moisture of 85%

371 WFPS, NO emissions reach a plateau after 6 days and start to

372 decrease after 10 days. For the 2 lower moisture levels emissions

373 were increasing over the whole course of the experiment.

374 Emissions increased significantly with WFPS, as shown.

375 Nitrous oxide emissions (Fig. 2b) were very low and near the

376 detection limit (N_2O: 0.5 ppm, equivalent to a flux of 0.00027 kg

377 N ha^{-1} h^{-1}) in the two lower WFPS treatments. In the 85% WFPS

378 treatment N_2O emissions were significantly higher ($p<0.05$) than

379 the other 2 treatments and showed a peak at day 1 of around 14

380 g N ha^{-1} h^{-1} after which emissions decreased to around 3 g N ha$^-$

381 ^{1} h^{-1} by the end of the experiment. At the lower WFPS of 55 and

382 70%, N_2O emissions were not significantly different between the

383 WFPS treatments.

384 Nitrogen gas emissions (Fig. 2c) were low in the 55% and 70%

385 WFPS treatments and did not show a peak. Higher N_2 emissions

386   were detected in the 85% WFPS treatment with a peak at around

387   day 2. After day 5, N_2 emissions were low as in the other two

388   treatments. Some N_2 was introduced into the system when the

389   amendment was applied. This took about 1 day to disappear (see

390   high soil moisture treatment) (see Fig. 2).

391   The total amounts of N emitted as NO, N_2O and N_2 show an

392   increase with increasing WFPS (Tab.2). However, total amounts

393   of NO-N were almost insignificant making up less than 0.04%

394   of total N emissions. Total emissions of N_2O were low in the

395   55% and 70% WFPS treatment (<3% of total N emissions), but

396   significantly higher at the highest WFPS of 85% (21.3% of total

397   N emissions). N_2 emissions was only any significantly different

398   at the high soil moisture. The N_2-N represented the largest

399   component of the emitted N at least 80%. The N_2O-N to N_2-N

400   ratios were smaller at the middle soil moisture (0.03) compared

401   to 0.27 at 85% WFPS.

402   Carbon dioxide emissions (Fig. 2d) increased immediately after

403   the application of NH_4NO_3 and showed a maximum on day 2 in

404   the 55% and 85% WFPS treatments decreasing afterwards. In

405   the 70% WFPS treatment emissions seem to have decreased in

406   the first day to recover in day 2 which was followed by a steady

407   decrease similarly to the other 2 treatments. Values for the 70%

408   WFPS treatment were the lowest during all the incubation

409   compared to the other 2 treatments.

410

3.2   *Proportion of N_2O from added N*

Results of the estimation of the proportion of N_2O derived from the applied treatments showed that initially, at 55% WFPS, very little N_2O emissions derived from added single-labelled NH_4^+ (Fig. 3a, ○). Larger amounts derived from added labelled ^{15}N, were found in the other ^{15}N-treatments within the first day (up to 50% from $^{15}NH_4^{15}NO_3$). Those rapidly decreased and became similar to the $^{15}NH_4^+$ treatment after 24 hours. For the rest of the incubation similar proportions of N_2O derived from all labelled amendments. Those proportions increased until day 12 when they reached about 10%.

The trends changed in the 70% moisture treatment (Fig. 3b), where the proportion of N_2O from the added ^{15}N initially increased for all ^{15}N amendments. After day 1 the proportion remained the same for the $^{15}NO_3^-$ amendment (▲) but kept increasing steadily for the other ^{15}N-amendments reaching 25 and 30% for $^{15}NH_4^+$ and $^{15}NH_4^{15}NO_3$, respectively.

For the highest moisture treatment (Fig. 3c), the proportion of N_2O from labelled N also increased on the first day for all treatments, however, with $^{15}NO_3$ and $^{15}NH_4^{15}NO_3$ the increase was significantly higher than with $^{15}NH_4$ (○; up to 50%). After this day, the contribution of the labelled amendment to N_2O emissions decreased for those amendments, reducing to 20 and 40% for $^{15}NO_3$ and $^{15}NH_4^{15}NO_3$, respectively on day 13. In the $^{15}NH_4$ treatment on the other hand, N_2O emissions decreased

436 slightly after the maximum in day 1 and then continued to

437 increase, reaching 20% on day 13.

438

439 ### *3.3 Soil N concentrations and ^{15}N enrichment*

440 Analysis of the soil N before each incubation and before core

441 packing showed the following values of TO_xN: 0.0681 (±0.001),

442 0.1335 (±0.0112) and 0.0844 (±0.0096) mg g^{-1} dry soil for 55,

443 70 and 85% WFPS-incubations, respectively. For NH_4^+, values

444 were 0.0869 (±0.0044), 0.0485 (±0.0010) and 0.0957 (±0.0017)

445 mg g^{-1} dry soil for 55, 70 and 85% WFPS-incubations,

446 respectively.

447

448 Figure 4 shows the dynamics of the analysed N forms in the soil

449 throughout the experiment. Soil NO_2^- was of the order of 0.1 μg

450 N g^{-1} dry soil during the incubation period and slightly higher in

451 the 85% WFPS treatment. Soil NH_4^+ and NO_3^- concentrations

452 were around 1000 times higher than NO_2^-, with more NO_3^- than

453 NH_4^+ in the 70% and 85% WFPS treatments, while no

454 differences in soil NH_4^+ and NO_3^- could be detected in the

455 55%WFPS treatment.

456 The 70 and 85% WFPS treatments showed larger changes in the

457 time series with soil NO_3^- increasing and NH_4^+ decreasing, while

458 those concentrations remained relatively constant and of similar

459   magnitude (around 0.15 mg N g^{-1} dry $soil^{-1}$) in the 55% moisture

460   treatment.

461

462   The ^{15}N-enrichment of soil NO_2^-, NO_3^- and NH_4 is shown in

463   Figure 5. The lowest ^{15}N-enrichment of soil NO_2^- and NO_3^- was

464   from the $^{15}NH_4$ amendment (●) (Fig. 5a and b) for all moisture

465   treatments while a higher enrichment of those two soil

466   components was found when $^{15}NO_3$ (▲) or $^{15}NH_4^{15}NO_3$ (■)

467   were applied (Fig. 5d,e,g and h). Values of enriched NO_2^- were

468   generally lower than those of enriched NO_3^- (5 vs. 20 atom%)

469   (Fig. 5a and b). Soil ^{15}N-enrichment of NO_3^- was generally in the

470   order 85%>55%>70% WFPS (*solid blue, dotted orange, dashed*

471   *green*) when the soil was amended with $^{15}NO_3$ or $^{15}NH_4^{15}NO_3$

472   (Fig. 5e and h).

473   The amendment with $^{15}NO_3$ (▲) resulted in lowest soil NH_4^+

474   enrichment (Fig. 5f) at 70 and 85% WFPS, while the opposite

475   was found for the initial 4 days when soil was at 55% WFPS.

476   Here treating the soil with $^{15}NO_3$ resulted in higher soil NH_4^+

477   enrichment than soil treated with $^{15}NH_4$ or $^{15}NH_4^{15}NO_3$. There

478   was no significant difference in the enrichment of the soil NH_4^+

479   depending on whether the soil was amended with $^{15}NH_4$ or

480    $^{15}NH_4^{15}NO_3$; enrichment was higher for the 70 and 85% WFPS

481   treatments than the 55% one (Fig. 5c and i).

482   As previously mentioned, compared to the other amendments the

483   addition of $^{15}NH_4$ resulted in significantly lower enrichment of

484 ^{15}N-labelled NO_2^- as well as NO_3^- for all WFPS treatments and a

485 significant decrease in $^{15}NH_4^+$ at the lower WFPS values of 55

486 and 70%.

487 When applying $^{15}NO_3$ the only significant changes in the

488 enrichment of ^{15}N-labelled compounds was found at 85% WFPS

489 where ^{15}N-labelled NO_3^- enrichment was significantly lower at

490 the end of the 10-day experiment and at 55% WFPS where ^{15}N-

491 labelled NH_4^+ enrichment was also significantly lower at the end

492 of the experimental period (Fig.5d-f).

493 Applying $^{15}NH_4^{15}NO_3$ did not result in any significant changes

494 in the enrichment of ^{15}N-labelled NO_2^- or NO_3^- at any of the

495 WFPSs. However, a significantly lower enrichment of ^{15}N-

496 labelled NH_4^+ between the beginning and end of the

497 experimental period was found for all WFPS values (Fig. 5g-i).

498

499   *3.4   Analysis of transformation rates*

500 The results of the *Ntrace* analysis tool (Fig. 1) showed that gross

501 transformation rates of NO_3^- and NH_4^+ and Mineralisation of

502 labile N to NH_4^+ were generally highest at 55% WFPS and

503 mostly decreased with increasing WFPS (Fig. 6a-c). Oxidation

504 of recalcitrant N to NO_3^-, however increased with increasing

505 WFPS (Fig. 6d). Desorption of adsorbed NH_4^+ as well as NO_3^-

506 was highest at 70% WFPS (Fig. 6e), although not statistically

507 significant, while the transformation of NH_4^+ to NO_3^- was

508 significantly lower at this WFPS than at the higher or lower

509 WFPS (Fig. 6a).

510

511 **_3.5   Apportioning of N_2O emissions_**

512 Figure 7 shows the resulting apportioning of the N_2O emissions

513 to the three different processes: heterotrophic nitrification,

514 denitrification and nitrification. At 55% WFPS, an initial large

515 contribution of denitrification is shown, which quickly

516 decreased in favour of heterotrophic nitrification (30%) by the

517 end of day 1. Heterotrophic nitrification remained the dominant

518 process throughout the incubation except on days 4 and 10, when

519 the sum of denitrification and autotrophic nitrification where

520 approximately 50%.

521 At 70% WFPS, heterotrophic nitrification dominated at the start

522 of the incubation vs denitrification (70 vs 30%) but decreased in

523 importance with time to almost zero at the end of the incubation,

524 when autotrophic nitrification became more dominant (65%).

525 At 85% WFPS, heterotrophic nitrification is only relevant on the

526 first day (80%); from then on, denitrification dominated (~100%

527 on days 1-2) and remained at about 60-80% with the rest of the

528 contribution coming from autotrophic nitrification.

529 The summary graph (Fig. 8) shows the average contribution of

530 each process to N_2O emissions as total amounts of N_2O-N

531 emitted, as well as percentage of N_2O emitted by each of the

532 three processes. With increasing soil moisture, an increase in the

22

533 contribution from denitrification to N_2O emissions was found,

534 whilst the contribution from heterotrophic nitrification

535 decreased. For autotrophic nitrification, however, the largest

536 contribution was at the intermediate soil moisture of 70% WFPS.

537

538 4 **Discussion**

539 In a recent literature review and meta-analysis, Barrat et al.

540 (2020) found that WFPS was a significant explanatory variable

541 for N_2O emissions and this was affected by the prior moisture

542 status of the soil. In our experiments, the soils were prepared in

543 a standard manner, so only the final moisture status at the start

544 of the incubation differed. Therefore in our study, we

545 investigated the relative differences between the 3 soil moisture

546 status (or WFPS) on N partitioning in the soil N compounds

547 and the N emitted compounds, and the apportioning of N_2O

548 emissions to different processes.

549 *4.1 Process dependent N-emissions at different WFPS*

550 Denitrification, if complete, transforms the produced N_2O into

551 N_2. Denitrification is commonly incomplete with N_2O not being

552 transformed to N_2 due to a lack of N_2O reductase (Nos) in the

553 microbial community, or due to a sufficient supply of NO_3^-

554 whose reduction is energetically more favourable than the

555 reduction of N_2O to N_2 (Saggar et al., 2013). Due to incomplete

556 denitrification, highest N_2O production is expected from

557 denitrification and consequently from soils with a relatively

558 higher WFPS. However, the importance and dominance of

559 certain processes ultimately depends on the microbial

560 community present in the soil and its activity which is influenced

561 by the soil conditions. In our study we used a grassland soil that,

562 has not had any fertiliser input, nor been grazed and therefore

563 has not received animal excrements as a nutrient source for over

564 20 years. We assume that due to the management of the field

565 lacking regular supply of nutrients, the microbial community

566 within the soil would have differed from those communities

567 found in other grasslands (Denef et al., 2009). This would have

568 had an influence on the N-transformation processes in this soil.

569 Additionally, it has been shown that soil moisture content

570 influences nutrient availability and movement through the soil

571 (Misra and Tyler, 1999) therefore influencing access of those

572 nutrients transported within a solution to the present microbial

573 community and subsequently influencing N transformation

574 processes.

575 In addition, the contributions observed from the treatments

576 applied to the emitted N_2O were generally less than 50%,

577 implying that the soil N pool was a larger contributor. We had

578 no zero N treatment in our experimental design to confirm this,

579 however, even if we had this, it is possible that the soil microbial

580 community was primed by added N (Müller and Clough, 2014),

581   so more of the soil N would have been utilised in the N treated

582   soils, than in a zero N control.

583   4.1.1   N-emission processes at 85% WFPS

584   In our study, the highest N_2O emissions were found at WFPS of

585   85% and these emissions decreased over time. At this high

586   WFPS the dual labelling analysis showed that more N_2O was

587   derived from the applied NO_3^- (Fig. 3c, initially $^{15}NO_3^-$

588   contributed over 50% while $^{15}NH_4^+$ contributed less than 5%),

589   indicating that denitrification was the dominant process in our

590   experiment. Over the course of the experiment at 85% WFPS,

591   the proportion of N_2O from the ^{15}N labelled NO_3^- decreased,

592   while that of NH_4^+ increased.

593   A possible explanation for the increased contribution of applied

594   ^{15}N-NH_4^+ in N_2O emissions could be that the measured ^{15}N-N_2O

595   derived from $^{15}NO_3^-$ which had previously been produced via

596   nitrification from the added $^{15}NH_4^+$. The results of soil NO_3^-

597   agree with this as there was an increase during the incubation

598   coinciding with a decrease in soil NH_4^+. The initial increase in

599   CO_2 reflects aerobic respiration after the treatments were applied

600   that settles at the end of the peak at about days 3-4. The N_2 fluxes

601   up till day 4 in the highest soil moisture treatment can be

602   explained by an increase in anaerobicity during this period

603   promoting denitrification. It is possible, that O_2 concentrations

604   recover with time, changing conditions from promoting

605 denitrification to promoting nitrification where N_2O is produced

606 from hydroxylamine NH_2OH. Nitrifying conditions might have

607 also developed at the surface by drying of the upper layers of the

608 soil. Though moisture contents of the soil cores used in this

609 experiment did not change significantly over time, it has been

610 shown in previous experiments that water can redistribute from

611 top to bottom creating more aerobic, nitrification promoting

612 conditions at the surface where gas exchange with the

613 atmosphere takes place (Loick et al., 2016). However, our results

614 suggest that most of the detected N_2O came from denitrification

615 of the NO_3^- produced via nitrification of the applied $^{15}NH_4^+$ due

616 to the increase in NO_3^- and a general decrease in NH_4^+ at 85%

617 WFPS (Fig. 4). Therefore, while nitrification is taking place even

618 under this high WFPS, denitrification is still the dominant

619 process producing N_2O. This is further supported by soil ^{15}N

620 analysis (Fig. 5), where results show a significant increase in soil

621 $^{15}NO_3^-$ in the $^{15}NH_4^+$ treatments, while the enrichment of $^{15}NH_4^+$

622 in the same treatment significantly decreased.

623 Emissions of other N-gases produced during N transformation

624 processes provide additional support that denitrification was

625 most important at the highest WFPS of 85%. Higher emissions

626 of N_2 (Fig. 2c), the final product of denitrification indicate that

627 complete denitrification had been achieved for some of the

628 available NO_3^-.

629   4.1.2   N-emission processes at 70% WFPS

630   At the intermediate WFPS of 70% it was expected that

631   nitrification and denitrification would be equally important. In

632   fact, the results of the *Ntrace* analysis tool show an equal

633   contribution of denitrification, nitrification and heterotrophic

634   nitrification at 70% WFPS. ^{15}N soil analysis also supports a near

635   equal distribution of nitrification and denitrification with $^{15}NH_4^+$

636   showing a decrease and $^{15}NO_3^-$ a corresponding increase when

637     $^{15}NH_4^+$ was added (Fig. 5b/c). The analysis of $^{15}N_2O$ (Fig. 3b)

638   revealed an approximately 3 times higher contribution of the

639   added $^{15}NO_3^-$ to N_2O emissions than that of added $^{15}NH_4^+$,

640   indicating that most of the emitted N_2O was produced via

641   denitrification. However, total amounts of N_2O were very small,

642   as were CO_2 emissions (Fig. 2d), both indicating that the

643   microbial N-transformation processes and denitrification in

644   particular were very slow/small under these conditions.

645   4.1.3. N-emission processes at 55% WFPS

646   The lowest WFPS of 55% was chosen to promote nitrification.

647   The results of the *Ntrace* analysis tool support that this was the

648   case with nitrification and heterotrophic nitrification

649   contributing to about 80% of N_2O emissions (Fig.8), while

650   denitrification only played a role at the very beginning of the

651   incubation after amendment was applied, which would have

652   temporarily increased the WFPS at the top of the core and

653   promoted anaerobic, denitrifying conditions prior to the

654 amendment solution percolating into the soil. This is supported

655 by the ^{15}N analysis of the emitted N_2O, which initially showed a

656 high contribution of added $^{15}NO_3^-$ to N_2O emissions, indicating

657 denitrification being the main process producing N_2O, which

658 quickly declined. By day 1 both, applied $^{15}NO_3^-$, as well as

659 $^{15}NH_4^+$, contributed equally to N_2O emissions. (Fig.3a).

660 Considering that N_2O is not an obligatory intermediate during

661 nitrification, but merely a potential by-product (Anderson,

662 1964), these results also indicate that nitrification processes

663 dominate over denitrification under these low moisture

664 conditions.

665

4.2   Influence of WFPS on soil N-transformation

        processes

668 Our study demonstrates the influence of WFPS on soil N-

669 transformation processes. Generally, gross soil N transformation

670 rates associated with both NH_4^+ and NO_3^- turnover decreased

671 with increasing WFPS. The total contribution of nitrification to

672 soil N transformation processes was higher at low WFPS and

673 decreased with increasing WFPS. However, an interesting

674 observation was that the oxidation of organic N to NO_3^-

675 increased almost 5-fold from 70 to 85% WFPS which may

676 support the higher denitrification rate by supplying additional

677 electron acceptors. However, this increase was not paralleled by

678 an increase of N_2O emitted. This may be due to an increasing

679 reduction of N_2O to N_2 (i.e. increasing N_2:N_2O ratio or decrease

680 in N_2O:N_2 as described earlier) under increasing anaerobicity

681 (Butterbach-Bahl et al., 2013).

682 The optimal conditions for nitrification are said to occur between

683 30-60% WFPS (Medinets et al., 2015). Emissions of NO can

684 derive from nitrification as well as denitrification, though it has

685 been found that the rates of produced NO measured as emissions

686 are higher under drier conditions, where a lower WFPS leaves

687 more air-filled pores enabling NO to escape to the surface

688 (Pilegaard, 2013). At WFPS above 65% it is believed that

689 emissions of N_2O and N_2 increase due to an increase in

690 denitrification. NO, however, while it is being produced to a

691 larger extent at high soil moisture, is also reduced to N_2O due to

692 a longer residence time decreasing the amount emitted to the

693 surface (Pilegaard, 2013). In this study, the observed increase in

694 NO emissions with increasing moisture levels suggests

695 denitrification was the source. Loick et al. (2016) concluded that

696 up to 0.67% of the added N (from a nitrate source) was emitted

697 as NO from denitrification supporting our findings.

698 Our results did not confirm our first hypothesis that losses are

699 lower at higher moisture levels for NO and N_2O. In fact, for all

700 gases, losses were higher at the high soil moisture possibly

701 because the soil was not saturated enough to impede gas

702 diffusion. Our second hypothesis was partly proved, as at the

703 high soil moisture the proportion of N_2O from nitrate containing

704 amendments was higher. The results for the lower moisture level

705 did not agree with our hypothesis as the proportion of N_2O from

706 all the amendments was similar and not mainly from NH_4^+.

707 Overall, our results support the assumption that nitrification

708 (autotrophic as well as heterotrophic) plays a bigger part at lower

709 WFPS, when air filled pores increase aerobicity, while

710 denitrification becomes more important the higher the WFPS

711 and therefore the lower the aerobicity. With our ^{15}N tracing

712 approach we found that heterotrophic nitrification was the

713 dominant process at 55% WFPS disproving our third hypothesis

714 that nitrification and denitrification dominate at all moisture

715 levels, its contribution quickly decreased with increasing WFPS,

716 while nitrification contributed most at the intermediate WFPS of

717 70% and least at 55%. Heterotrophic nitrification has been

718 reported in previous studies as dependent on soil pH, C:N ratio

719 and land use and that it can contribute up to 85% of the total N_2O

720 flux in soils with pH values between 4.2 to 8.4 (Zhang et al.,

721 2015). This process converts organic N (although it is believed

722 it also happens with inorganic N sources (Zhang et al., 2014)) to

723 NO_3^-. It is believed this occurs particularly in acidic soils where

724 autotrophic nitrification can be inhibited. The soil used in this

725 study was of pH 5.6 (Table 1) placing it within the soils that can

726 potentially undergo this process. Müller et al. (2014) stated that

727 heterotrophic nitrification is a contributor to N_2O emissions in

728 grassland soils with high organic matter contents. This further

729   supports the finding that this process occurs in this study

730   (organic matter content 11.7% Table 1). In the study by Rütting

731   and Müller (2008) it was shown that heterotrophic nitrification

732   would carry out oxidation of organic N to NO_2^- (rather than NO_3^-

733   ). We also know that microbial consortia exist where a network

734   of metabolic activity is present (Butterbach-Bahl et al., 2013),

735   therefore it is likely that NO_2^- originating from the organic N pool

736   is directly reduced to N_2O (and not further oxidised to NO_3^-) by

737   the activity of denitrifying organisms. This also explains that

738   higher percentages of N_2O via the organic pathway occur under

739   higher WFPS values.

740   At the WFPS above 70% it has been shown that N_2O is produced

741   solely by denitrification (Bateman and Baggs, 2005). However,

742   in our case denitrification only became dominant at 85% WFPS,

743   and denitrification contributed about 70% of the N_2O emissions

744   at this WFPS (Fig. 7,8), while overall not much activity was

745   found at neither 50, nor 70% WFPS.

746   The lower N_2O emissions for the 2 lower moisture levels over

747   the course of the experiment could be due to a slower response

748   of the microbial community to the added N compared to the

749   highest soil moisture treatment where nutrient availability is

750   expected to be higher (Papendick and Camprell, 1981).

751   Emissions of CO_2 have been used as an indicator of microbial

752   respiration and activity (López-Aizpún et al., 2018). In this study

753   the results indicate that the microbial community was most

754 active at a WFPS of 85% in agreement with the above statement,

755 but this was followed by the driest treatment and the least active

756 was at the intermediate WFPS of 70% coinciding with the N_2O

757 trend. Other factors need to also be considered as N_2O

758 production and consumption from biogenic processes as well as

759 abiotic processes such as gas diffusion, are both dependant of

760 moisture in soil.

761

762 5   **Conclusions**

763 Our results highlight the variability in the effect of WFPS on the

764 dominance of different N transformation processes in soil.

765 Though the general assumption, that denitrification is more

766 important at high WFPS, is supported here, the actual percentage

767 of WFPS attributed to the different processes was not as

768 expected. Heterotrophic nitrification was found to be an

769 important source of N_2O especially under drier conditions while

770 nitrification plays a crucial role for N_2O emissions, directly but

771 also via nitrification coupled with denitrification under medium

772 and high WFPS.

773 Results obtained from the experiment performed at 85% WFPS

774 show the importance of nitrification even under high WFPS and

775 raise the question if and how much of the N_2O emissions could

776 have been mitigated by preventing nitrification supplying NO_3^-

777 for denitrification by e.g. using nitrification inhibitors (Owusu-

778 Twum et al., 2017; Wu et al., 2017a; Wu et al., 2017b).

779 Our study was performed under controlled conditions with a clay

780 soil that had not received any fertiliser or manure/slurry input for

781 few years. Under these conditions, we found a relatively equal

782 contribution of nitrification, denitrification and heterotrophic

783 nitrification to N_2O production at 70% WFPS. At the lower

784 WFPS of 55% the contribution of heterotrophic nitrification

785 dominated, while at the highest WFPS of 85% denitrification

786 contributed most of the measured N_2O. These results will not

787 necessarily apply to other soil types, particularly extreme high or

788 low organic matter soils. Further studies to understand how

789 carbon quality affect the fate of N in soils are needed.

790 However, the process that will be supported at a certain WFPS

791 will most likely depend on the type of soil including its natural

792 carbon and nutrient content, its history and the microbial

793 community present. Emissions are also influenced by abiotic

794 factors that are also dependant on soil moisture.

795

796 **6   Acknowledgements**

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963

964

Table 1. Soil characteristics (before amendment application).

Mean $\pm$ standard error ($n = 3$).

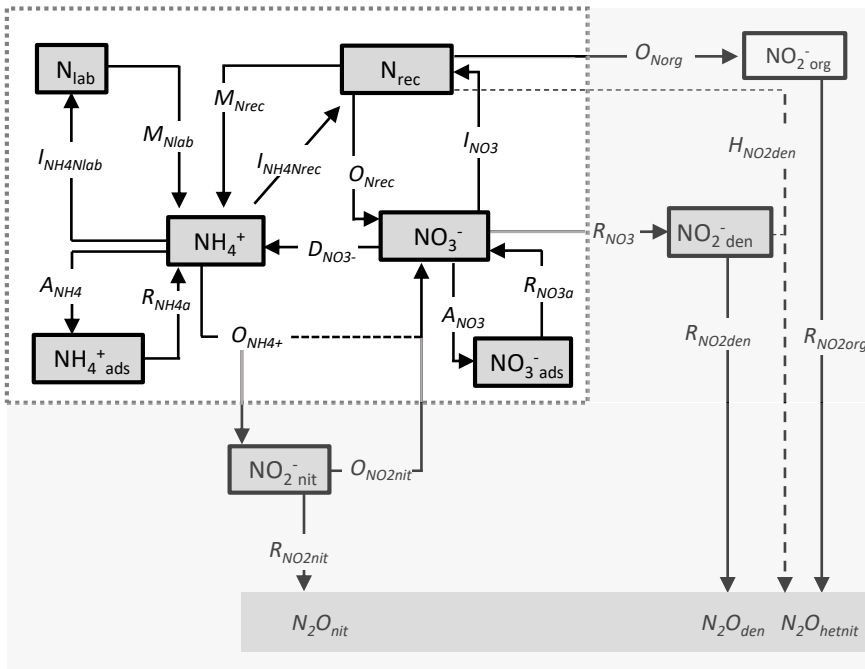| Parameter	Amount		
pH water [1:2.5]	5.6	$\pm$	0.27
BD (g cm^{-2})	0.8	$\pm$	0.0005
Available Magnesium (mg kg^{-1} dry soil)	100.4	$\pm$	4.81
Available Phosphorus (mg kg^{-1} dry soil)	10.4	$\pm$	1.10
Available Potassium (mg kg^{-1} dry soil)	97.5	$\pm$	12.83
Available Sulphate (mg kg^{-1} dry soil)	51.7	$\pm$	0.62
Total N (g kg^{-1} dry soil)	5.0	$\pm$	0.10
Total Extractable Oxidised N (mg kg^{-1} dry soil)	15.1	$\pm$	0.07
Ammonium N (mg kg^{-1} dry soil)	9.2	$\pm$	0.09
Total Organic Carbon (% w/w)	6.79	$\pm$	0.17

966

967

Table 2. Average cumulative emissions of NO, N$_2$O over the experimental period and N$_2$ from day 2.6 (after flushing out of N$_2$ introduced with amendment) in kg N ha^{-1}

Mean ± standard error (n = 9). Different letters indicate significant differences in emissions between the WFPS treatments ($p<0.05$)

WFPS	NO-N			N$_2$O-N			N$_2$-N			total N			%N as NO-N	%N as N$_2$O-N	% N as N$_2$-N
55%	1.09E-04	±	6.28E-06 [c]	4.16E-03	±	2.35E-04 [b]	0.00	±	0.00 [a]	0.00	±	0.00	na	na	na
70%	1.41E-04	±	7.32E-07 [b]	2.69E-03	±	4.28E-05 [a]	0.08	±	0.08 [a]	0.09	±	0.08	0.16	3.0	89
85%	1.61E-04	±	5.71E-06 [a]	8.51E-02	±	3.52E-03 [c]	0.32	±	0.30 [a]	0.40	±	0.31	0.04	21.2	80

968

Fig1

Figure 1

Parameter Discription	
Parameter	Description
M_x	Mineralisation of X
I_x	Immobilisation of X
O_x	Oxidation X
R_x	Release of X
D_x	Dissimilatory reduction of X
H_x	X transformation by Heterotrophs
A_x	Adsorption of X
X_{org}	Organic X
X_{lab}	Labile X
X_{rec}	Recalcitrant X
X_a	Adsorbed X
X_{nit}	X from Nitrification
X_{den}	X from Denitrification
X_{hetnit}	X from heterotrophic nitrification

Fig2
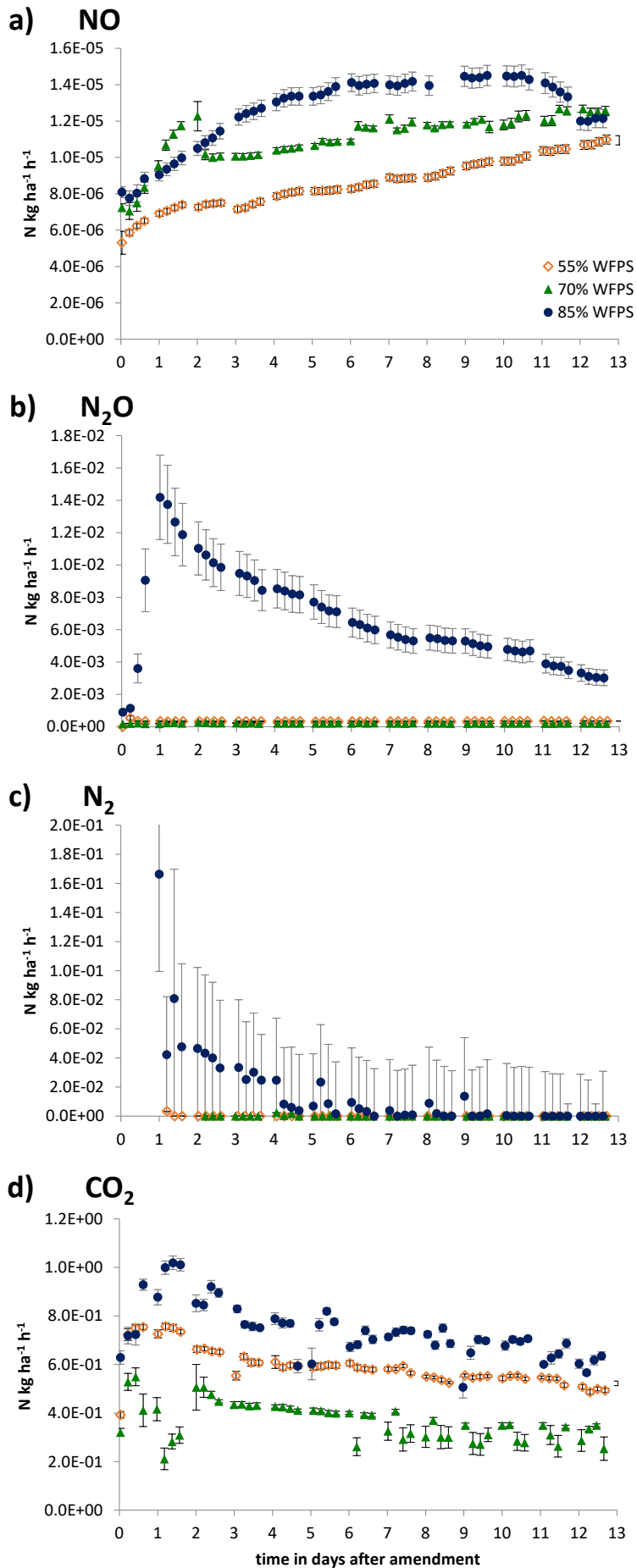

Figure 2

Fig3

Figure 3

a) **55% WFPS**

b) **70% WFPS**

c) **85% WFPS**

Fig4
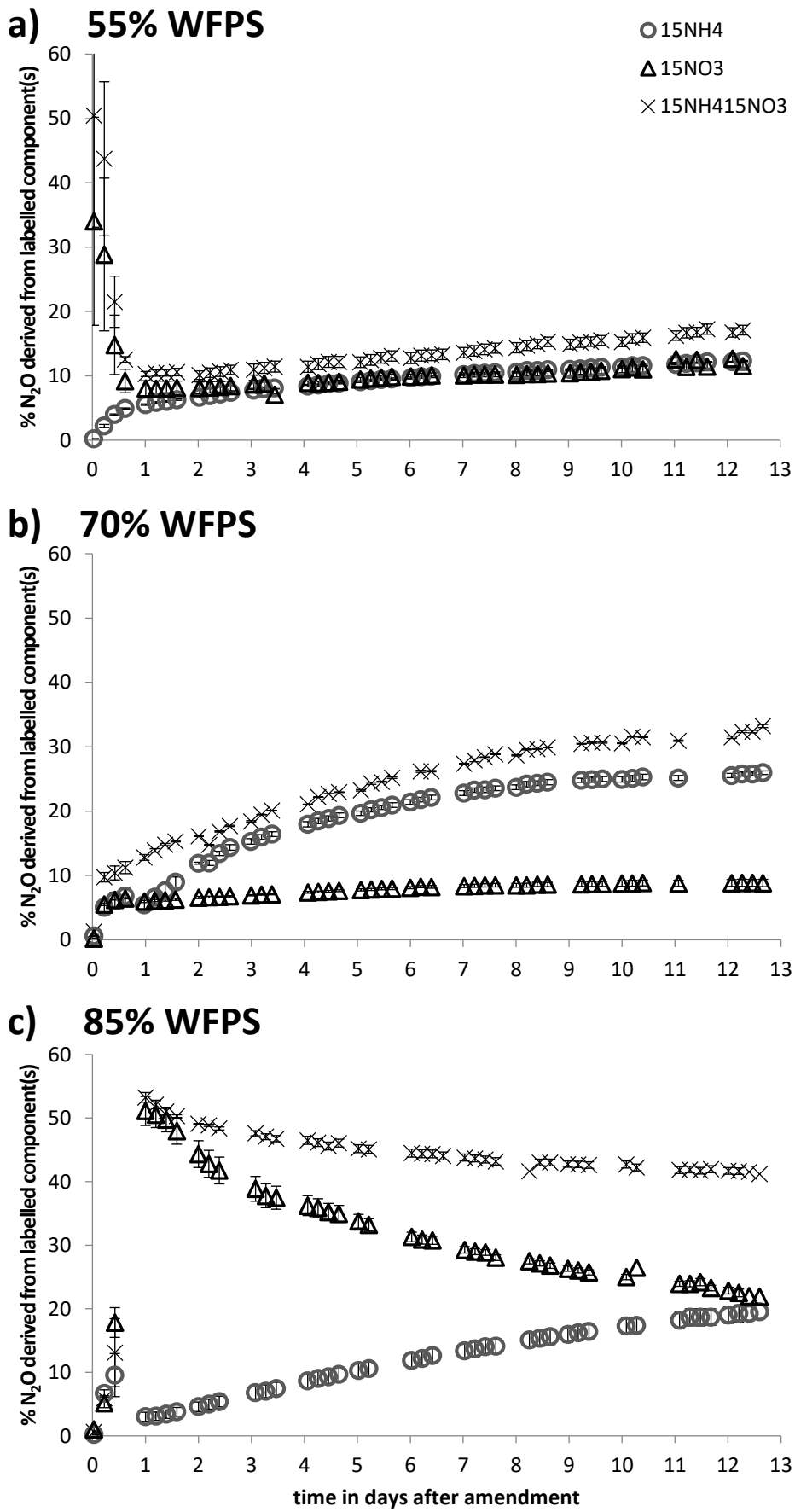
Figure 4

Soil N concentrations

Fig5

Figure 5
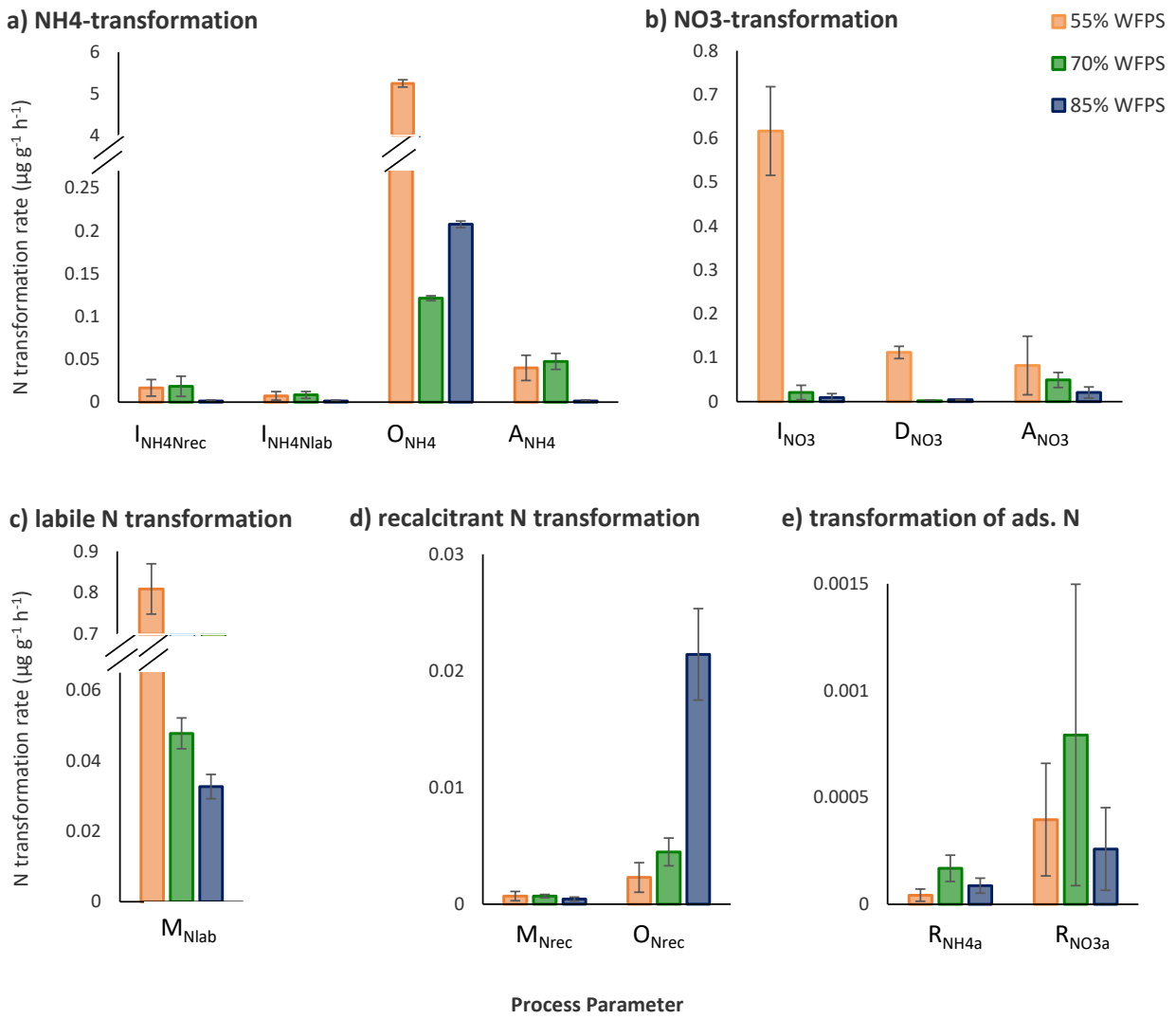

after $^{15}NH_4$ amendment

after $^{15}NO_3$ amendment

after $^{15}NO_3$$^{15}NH_4$ amendment

soil $^{15}NO_2$

a) $^{15}NO_2$ from $^{15}NH_4$

d) $^{15}NO_2$ from $^{15}NO_3$

g) $^{15}NO_2$ from $^{15}NO_3$$^{15}NH_4$

soil $^{15}NO_3$

b) $^{15}NO_3$ from $^{15}NH_4$

e) $^{15}NO_3$ from $^{15}NO_3$

h) $^{15}NO_3$ from $^{15}NO_3$$^{15}NH_4$

soil $^{15}NH_4$

c) $^{15}NH_4$ from $^{15}NH_4$

f) $^{15}NH_4$ from $^{15}NO_3$

i) $^{15}NH_4$ from $^{15}NO_3$$^{15}NH_4$

^{15}N atom% enrichment

time in days after amendment application

Fig6

Figure 6

a) NH4-transformation

N transformation rate ($\mu g\ g^{-1}\ h^{-1}$)

Axis values: 6, 5, 4, 0.25, 0.2, 0.15, 0.1, 0.05, 0

Categories: $I_{NH4Nrec}$, $I_{NH4Nlab}$, O_{NH4}, A_{NH4}

b) NO3-transformation

Axis values: 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1, 0

Categories: I_{NO3}, D_{NO3}, A_{NO3}

Legend: 55% WFPS, 70% WFPS, 85% WFPS

c) labile N transformation

N transformation rate ($\mu g\ g^{-1}\ h^{-1}$)

Axis values: 0.9, 0.8, 0.7, 0.06, 0.04, 0.02, 0

Category: M_{Nlab}

d) recalcitrant N transformation

Axis values: 0.03, 0.02, 0.01, 0

Categories: M_{Nrec}, O_{Nrec}

e) transformation of ads. N

Axis values: 0.0015, 0.001, 0.0005, 0

Categories: R_{NH4a}, R_{NO3a}

Process Parameter

Fig7
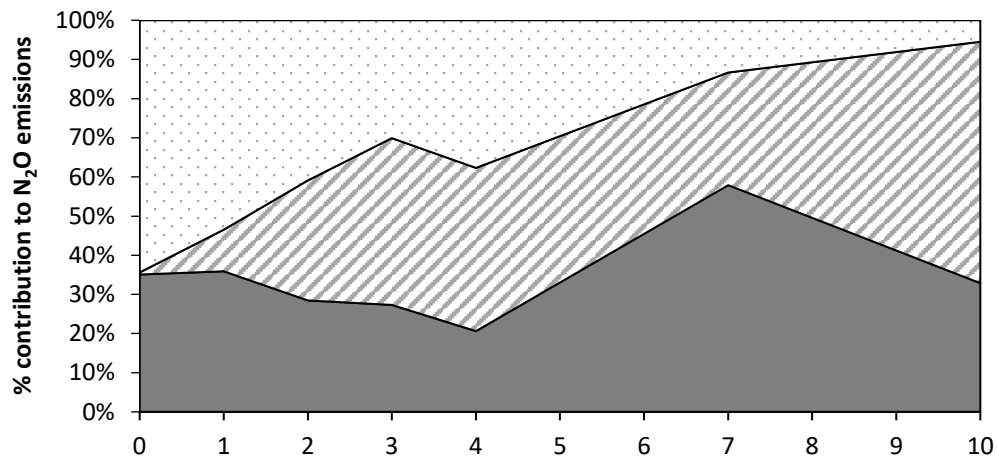
Figure 7

a) 55% WFPS
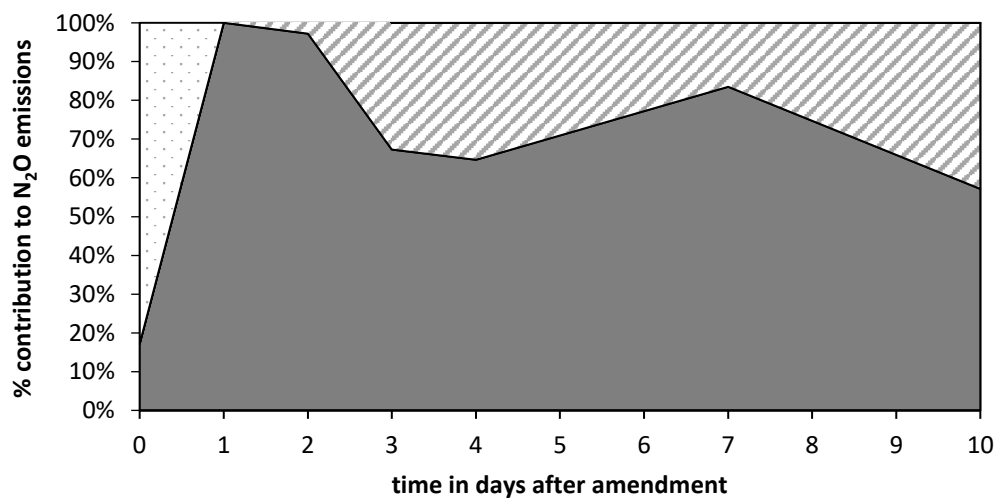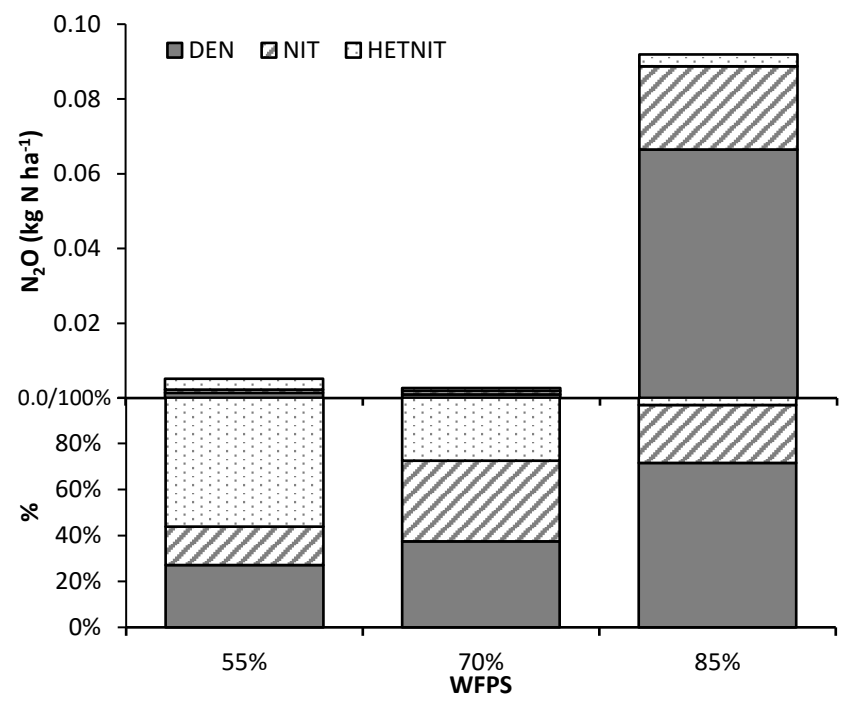
b) 70% WFPS

c) 85% WFPS

Fig8

Figure 8

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

'Declarations of interest: none'