

Dear editor,

We would again like to thank you and the reviewers for your input in the editing process of this manuscript. We agree with the majority of the further suggestions and have altered the manuscript to accommodate them.

The reviewer is correct in spotting that fluxes occur before the fertilisation plotted on the figure. This was due to the date of the fertilisation in the plot being wrong rather than early fluxes. This has been corrected for.

With regards to the issue with the reported detection limits, there is a bit of uncertainty over what the “detection limit” actually represents. The reviewer is correct in stating that the uncertainty in individual measurements reported in Cowan et al 2014 are 2 and 20 $\mu\text{gN}_2\text{O-N m}^{-2} \text{ h}^{-1}$, which roughly equals 0.02 and 0.2 $\text{nmol m}^{-2} \text{ s}^{-1}$, respectively. Investigating the detection limits further in Cowan et al 2014b we double this value (i.e. double the standard deviation of a zero flux measurement.) This is why the reported detection limits are larger than in the initial paper.

With regards to editing the figures, it is very difficult to contrast the black and grey colours in the plots without keeping them as they are. In Figure 5 the black dots stand out more and it is clear which of the measurements are eddy covariance and which are chambers. In Figure 6, the contrast between the GAM prediction and the measurements is more important. If the editor requests it, then we can try again to make the plots clearer with consistent colours/shapes; however for a relatively minor change, we hope this is not an issue.

I believe all other points in the reviewers replies have been edited and corrected for.

Sincerely,

Nick Cowan

1 **The influence of tillage on N₂O fluxes from an intensively managed grazed**
2 **grassland in Scotland**

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8
9 Keywords: plough, greenhouse gas, nitrous oxide, gap filling

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11

12 **Abstract**

13 Intensively managed grass production in high-rainfall temperate climate zones is a globally important source of
14 N₂O. Many of these grasslands are occasionally tilled to rejuvenate the sward, and this can lead to increased N₂O
15 emissions. This was investigated by comparing N₂O fluxes from two adjacent intensively managed grazed
16 grasslands in Scotland, one of which was tilled. A combination of eddy covariance, high-resolution dynamic
17 chamber and static chamber methods was used.

18 N₂O emissions from the tilled field increased significantly for several days immediately after ploughing and
19 remained elevated for approximately two months after the tillage event contributing to an estimated increase in
20 N₂O fluxes of 0.85 ± 0.11 kg N₂O-N ha⁻¹. However, any influence on N₂O emissions after this period appears to
21 be minimal. The cumulative N₂O emissions associated with the tillage event and a fertiliser application of 70 kg-
22 N ammonia nitrate from one field were not significantly different from the adjacent un-tilled field, in which two
23 fertiliser applications of 70 kg-N ammonia nitrate occurred during the same period. Total cumulative fluxes
24 calculated for the tilled and un-tilled fields over the entire 175 day measurement period were 2.14 ± 0.18 and 1.65
25 ± 1.02 kg N₂O-N ha⁻¹, respectively.

26 1 Introduction

27 Modern agriculture and intensive land management practices are believed to contribute over 39 % of total global
28 anthropogenic emissions of the greenhouse gas (GHG) nitrous oxide (N₂O) (IPCC, 2014). N₂O is a naturally
29 occurring GHG released into the atmosphere by the microbial processes of nitrification and denitrification which
30 occur in soils and aquatic systems (Davidson et al., 2000; Seitzinger et al., 2000). Human activities which alter
31 environmental conditions can have a significant impact on natural microbial processes, which in turn can increase
32 N₂O emissions. Agricultural activities such as the use of nitrogen fertilisers, livestock production and land use
33 changes are all important sources of anthropogenic N₂O from agricultural soils (Fowler et al., 2013).

34 There is still large uncertainty associated with the quantification of N₂O emissions released from
35 agricultural soils on a national and global scale, due to the large spatial and temporal variability of N₂O fluxes
36 (Cowan et al., 2015; Jahangir et al., 2011; Mathieu et al., 2006). Many past experiments have focussed on the
37 release of N₂O from soils after the application of nitrogen fertilisers - which is the main cause of the rise of in
38 N₂O emissions since pre-industrial times (e.g. Bouwman et al., 2002; Dobbie et al., 1999). Other factors affecting
39 N₂O emissions from agricultural soils, such as tillage and compaction, are less well documented, thus preventing
40 effective assessment of their role in controlling N₂O fluxes from the agricultural sector.

41 The addition of organic nitrogen in the form of decaying plant matter (crop residues) is a recognised
42 potential source of N₂O following tillage, but the phenomenon is not well quantified (Baggs et al., 2003; Mutegi
43 et al., 2010). Currently the IPCC emission inventories estimate that 1 % of all organic nitrogen applied to soils as
44 crop residues will be emitted in the form of N₂O (IPCC, 2006). However, the degree to which tillage induces a
45 change in N₂O emissions may be determined by several factors: the prior use of nitrogen fertilisers (Abdalla et
46 al., 2010; Yamulki and Jarvis, 2002), soil compaction (Ball et al., 2008; Yamulki and Jarvis, 2002) and the method
47 of tillage (Sheehy et al., 2013). Changes in the bulk density, water filled pore space (WFPS) and oxygen
48 availability in soils which can lead to an increase or decrease in nitrification and denitrification rates depending
49 on environmental conditions (Elmi et al., 2003; Palma et al., 1997).

50 The large number of variables which may alter microbiological processes in tilled soils can lead to a wide
51 range of results between experiments carried out at different field sites, under different meteorological conditions.
52 Some experiments have reported large increases in annual N₂O emissions varying from 0.89 to 3.37 kg N ha⁻¹
53 dependent on application of fertiliser post-tillage (i.e. Chatskikh and Olesen, 2007; Merbold et al., 2014; Omonode

54 et al., 2011; Pinto et al., 2004; Yamulki and Jarvis, 2002), whereas others have shown a zero (i.e. Boeckx et al.,
55 2011; Choudhary et al., 2002) or potentially negative effect of tillage ($-0.88 \text{ kg N ha}^{-1}$, Tan et al., 2009). There is
56 little consensus among these studies on the relative effect of different drivers of N_2O production. However, it is
57 commonly reported that factors influencing the aeration of the soil (such as WFPS and bulk density) are cited as
58 influential in most tillage studies.

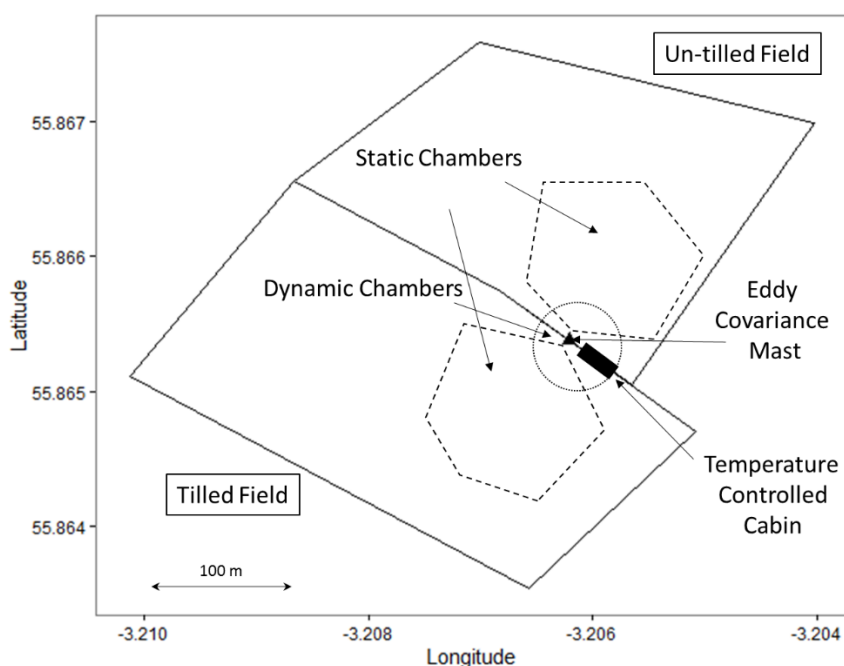
59 Improving our understanding of N_2O fluxes from tillage events is important, especially in countries such
60 as the UK, where agriculture accounts for approximately 70 % of the total land coverage (DEFRA, 2012) and
61 tillage is widely practiced. Improved grasslands alone account for 25 % of the total land coverage of the UK
62 (Morton et al., 2011). Tillage events occurs on rotational grasslands, for sward rejuvenation on permanent
63 grasslands, and in conversion to arable, and are a common enough occurrence that they could contribute
64 significantly to the total national inventory of anthropogenic N_2O emissions. However, few experiments have
65 been carried out on GHG emissions resulting from the tillage of grassland fields. The aim of this work was
66 therefore (i) to use multiple N_2O flux measurement methodologies to add to the understanding of the N_2O fluxes
67 from grasslands tilled for sward renewal, (ii) develop an improved statistical methodology which allows for
68 uncertainties in cumulative flux emissions to be calculated for these events, and (iii) compare our estimates with
69 those predicted using the current IPCC methodology.

70

71 2 **Materials and method**

72 2.1 **Field site**

73 Fluxes of N₂O were measured from an area of intensively managed, grazed grassland (Easter Bush, Scotland, 55°
74 51' 55.30"N, 3° 12' 22.17"W) before and after a tillage event on the 1st of May 2012, and were compared with
75 fluxes measured from an adjacent grassland which remained un-tilled (as described in Jones et al., 2011) (Figure
76 1). The climate is temperate maritime, with an average annual rainfall of 921 mm and average annual air
77 temperature of 9 °C (in the period 2001–2011). The two fields (each approximately 5.4 ha) have been managed
78 for intensive livestock production for at least twenty years, and since 2002 were predominately grazed by sheep.
79 The average stocking densities were 0.7 LSU ha⁻¹ (livestock units) and average N fertiliser application rates have
80 been approximately 200 kg N ha⁻¹ y⁻¹. Mainly NH₄NO₃ or NPK compound fertilisers were applied in three split
81 applications usually between March and July (Skiba et al., 2013).



82

83 **Figure 1** N₂O fluxes were measured from two adjacent grassland fields at the Easter Bush Farm (Penicuik,
84 Scotland). The north field remained un-tilled, while the south field was ploughed on the 1st of May 2012. An eddy
85 covariance mast was set up next to a permanent cabin positioned between the fields. Dynamic chamber
86 measurements were made within a 30 m radius of the cabin. Static chambers were located within the fetch of the
87 eddy covariance mast and moved periodically.

88 The soil in the fields is a clay loam with a sand/silt/clay texture of 52/20/28 and 57/19/24 for the top 30
 89 cm in the un-tilled and tilled fields, respectively with a pH of approximately 5.1 (in H₂O). They are classed as an
 90 imperfectly drained Macmerry soil of the Rowanhill association (eutric cambisol, FAO classification). A drainage
 91 system had been installed about 50 years ago, but is no longer functioning well, resulting in frequent occurrence
 92 of surface water during rainy periods. The fields had not been tilled for at least twenty years, and the farmer had
 93 reported reduced fertility and productivity. One field (also called the South Field in Jones et al., 2011) was
 94 therefore tilled in May 2012 (Table 1).

95 As standard practice, glyphosphate (1.5 l ha⁻¹) was applied to kill the grass three days prior ploughing
 96 on the 27th of April. The field was ploughed to a depth of 30 cm on the 1st of May 2012. Two days after ploughing,
 97 the field was harrowed, and then rolled and sown with ryegrass (*Lolium perenne L.*) on the third day after
 98 ploughing. The un-tilled field (also called the North field in Jones et al., 2011) was managed as usual and grazed
 99 by sheep (approximately 30 sheep ha⁻¹). Fertilisation events continued as normal on the un-tilled field which
 100 received two ammonium nitrate (Nitram) fertiliser applications of 70 kg-N ha⁻¹, one on the 28th of May and the
 101 second on the 9th of August. The tilled field only received a 70 kg-N ha⁻¹ Nitram application on the 9th of August,
 102 approximately four months after the tillage event.

103 **Table 1** Field management events for both the tilled and un-tilled fields in 2012.

Date	Tilled Field (South)	Un-Tilled Field (North)
16 th February 2012		Grazed by sheep (continuous)
27 th April 2012	Glyphosphate application (1.5 l ha ⁻¹)	
1 st May 2012	Ploughing at 30 cm depth	
3 rd May 2012	Harrowing, seeding & rolling	
28 th May 2012		70 kg-N ha ⁻¹ Nitram application
9 th August 2012	70 kg-N ha ⁻¹ Nitram application	70 kg-N ha ⁻¹ Nitram application
19 th September 2012	Grazed by sheep (continuous)	

104

105 Biomass samples were collected from the South Field prior to tillage in order to estimate the grass
 106 biomass that would be tilled into the soil. Twenty soil cores (12 cm deep and 5.8 cm diameter) were extracted
 107 from the field. At these points, all above-ground biomass was harvested and dried in an oven at 80 °C to constant

108 weight. Once dry, the above-ground biomass was weighed. The soil cores were broken up by hand and dried at
109 100°C until constant weight. After drying, the root material was separated from the soil by hand and weighed. Sub
110 samples of the dried plant materials were prepared for elemental analysis of total carbon and nitrogen contents
111 (vario EL cube, Elemaentar, Hanau, Germany).

112 Total (above- and below-ground) biomass on the tilled field before tillage averaged of $369 \pm 310 \text{ g m}^{-2}$,
113 with a root to shoot ratio of ~ 1.5 . The nitrogen content was 2.5 %. Based on these measurements it is estimated
114 that the tillage event added a total of 93.6 kg ha^{-1} of nitrogen to the field in the form of crop residues.

115

116 2.2 Flux Measurements

117 N_2O fluxes were measured from both tilled and un-tilled fields over a seven month period using three measurement
118 methodologies; eddy covariance, static chamber and dynamic chamber techniques. The mixture of methods were
119 used to try to obtain as many measurements as practically possible, both temporally and spatially, during the
120 experiment. Eddy covariance was the primary measurement methodology used. However, due to unpredictable
121 changes in wind direction at the site it was necessary to deploy manual chamber methodology to ensure that both
122 fields were measured periodically during the experiment. The dynamic chamber measurements were used as a
123 cost effective way to provide many (> 30) high-resolution N_2O fluxes on the days immediately after tillage without
124 the need for time consuming GC lab analysis required by static chambers.

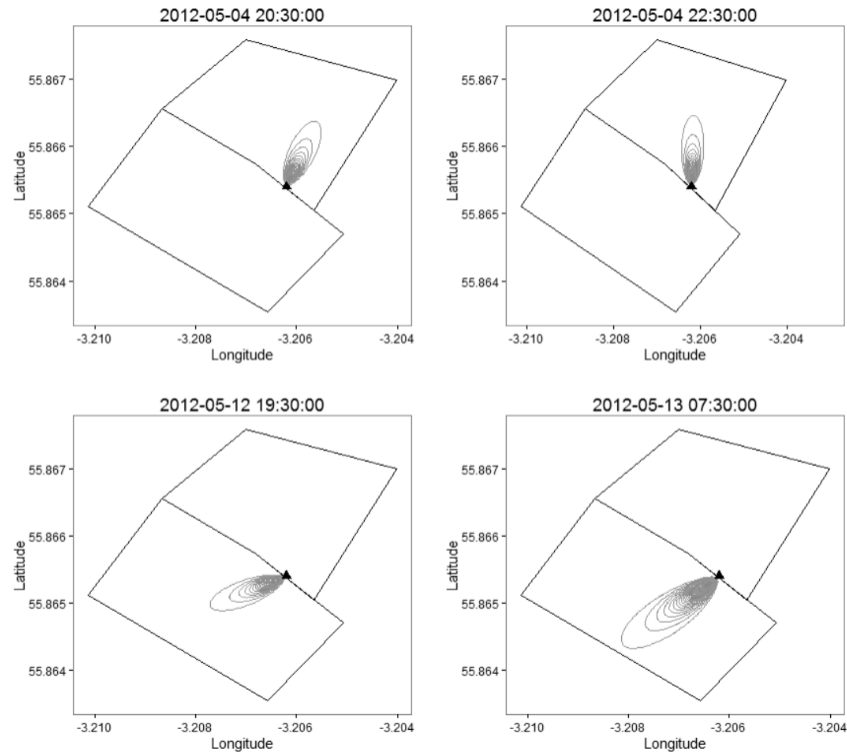
125 An eddy covariance system was installed on the 27th of March on the field boundary (Figure 1). An
126 ultra-sonic anemometer (WindMaster Pro 3-axis, Gill, Lymington, UK) mounted at 2.4 m was used to measure
127 fluctuations in 3-D wind components at a frequency of 10 Hz. Mixing ratios of N_2O , H_2O and CO_2 were measured
128 at 10 Hz by a quantum cascade laser (QCL) gas analyser (CW-QC-TILDAS-76-CS, Aerodyne Research Inc.,
129 Billerica, MA, USA), housed in a temperature controlled cabin. The inlet line to the QCL was a 13.5 m length of
130 Dekabon tubing (0.25 inch outer diameter), with a flow rate of approximately 13 l min^{-1} . Fluxes were calculated
131 at 30 min intervals using the EddyPro software (Version 5.2.1) (Li-Cor, Lincoln, NE, U.S.A.), based on the
132 covariance between the N_2O concentration (χ) and vertical wind speed (w):

$$133 \quad F_{\chi} = \overline{\chi'w'} \quad (\text{Eq. 1})$$

134 In the processing, we applied double coordinate rotation (vertical and crosswind), spike removal, block
135 averaging, and time lag removal by covariance maximisation. Correction for the frequency response of the system,
136 both high and low-frequency losses, were made using the method of Moncrieff et al., (1997). Corrections for
137 density fluctuations were applied on a half-hourly basis using the method of (Burba et al., 2012). The quality
138 control scheme of Foken et al., (2005), was used to remove poor quality flux measurements (their category 2).
139 Initially, fluxes measured with a mean wind direction between 180 and 270 degrees from north were classed as
140 from the tilled field; those measured at greater than 330 and less than 100 degrees were classed as from the un-
141 tilled field. The remaining data were disregarded due to obstruction of the wind by the cabin and fence line.

142 Further footprint analysis was carried out in which we visually checked individual footprint plots of each
143 30 min flux (Figure 2). Any flux footprints in which the majority of the contribution came from a distance less
144 than 10 m from the mast, or overlapped the two fields were removed from the dataset. Standard meteorological
145 variables (rainfall, air temperature and soil temperature) were recorded by a tipping bucket, thermometers (2 m
146 height & 10 cm depth) and TDR soil moisture probe at 10 cm depth. These measurements were made adjacent to
147 the flux tower at the site.

148



149

150 **Figure 2** Four example flux footprints, with contours showing the relative contribution to the measured eddy
 151 covariance flux, based on the model of Kormann and Meixner (2001). Half-hourly flux data were only included
 152 if 97.5 % of the measured flux was attributed to either the untilled (top) or tilled (bottom) fields.

153 N_2O fluxes were also measured from both fields using static chamber and dynamic chamber techniques.
 154 The static chambers consisted of a cylindrical polyvinyl chloride (PVC) plastic pipe of 38 cm inner diameter (ID)
 155 and 22 cm height. These chambers were inserted 5 cm into the soil, giving a headspace of approximately 20.4 l.
 156 Chambers were closed for 40 mins, during which time three 100 ml gas samples were collected via a syringe and
 157 a three-way tap fitted to the lid, at $t = 0, 20$ and 40 mins. After each measurement, chamber height was measured
 158 at five points to estimate the chamber volume. Gas samples were stored in 20 ml glass vials which were flushed
 159 with 100 ml of air in the syringe using a double needle. Samples were analysed using a Hewlett Packard 5890
 160 series II gas chromatograph (Agilent Technologies, Stockport, fitted with an electron capture detector) (Skiba et
 161 al., 2013).

162 Ten static chambers were positioned in each of the fields, within the estimated flux footprint of the eddy
 163 covariance system (10 to 200 m from the mast). Chambers in the fields were occasionally moved to prevent the
 164 effects of a micro-climate within the chambers that could bias measurements when compared to the surrounding

165 field area, and also to allow access to farm vehicles during the different stages of the tillage operation. Manual
166 chamber measurements were carried out between 9:00 and 15:00 on the measurement dates. Fluxes were
167 calculated as:

$$168 \quad F = \frac{dC}{dt} \cdot \frac{\rho V}{A} \quad (\text{Eq. 2})$$

169 where F is the gas flux from the soil ($\text{nmol m}^{-2} \text{s}^{-1}$), dC/dt is the rate of change in concentration with time in nmol
170 $\text{mol}^{-1} \text{s}^{-1}$ estimated by linear regression, ρ is the density of air in mol m^{-3} , V is the volume of the chamber in m^3
171 and A is the ground area enclosed by the chamber in m^2 . Static chamber measurements were made over a longer
172 period than shown in this paper and are discussed in relation to a second tillage event by Drewer et al. (2016).

173 Fluxes were also measured using the QCL in a closed, dynamic chamber system (Cowan et al., 2014a).
174 A chamber (39 cm inner diameter, 22 cm high) was placed onto a stainless steel collar inserted several cm into
175 the soil (on average 5 cm) at least 15 mins prior to measurement. Two 30 m lengths of 3/8 inch ID Tygon® tubing
176 connected the chamber to the inlet of the QCL and the outlet of a vacuum pump (SH-110, Varian Inc, CA, USA)
177 to form a closed system. This allowed a 30 m possible radius from the instrument cabin in which the chamber
178 could be placed (Figure 1). A flow rate of approximately 6 to 7 L min^{-1} was used, with a lag time of approximately
179 22 seconds between the chamber and analyser. Fluxes of N_2O were calculated with 1 Hz data over three minutes,
180 using both linear and non-linear asymptotic regression methods (Levy et al., 2011; Pedersen et al., 2010). Using
181 a mixture of goodness-of-fit statistics and visual inspection, the regression method that provided the best fit for
182 the time series of mixing ratios of N_2O was chosen for each individual measurement. The detection limit of
183 individual fluxes calculated by this method was approximately $0.04 \text{ nmol m}^{-2} \text{ s}^{-1}$ compared to $0.4 \text{ nmol m}^{-2} \text{ s}^{-1}$
184 when using the static chambers (Cowan et al., 2014a, 2014b).

185 In the first few days after the tillage event, the wind direction was north-easterly, meaning that the eddy
186 covariance system could not record fluxes from the tilled field (to the south-west). The dynamic chamber
187 measurements were primarily used to fill this gap in the eddy covariance time series with high precision chamber
188 measurements.

189

190 2.3 Gap filling

191 Because the eddy covariance system was placed on the field boundary, observations could only be made on a
192 single field at any given time. Furthermore, some data were missing because of instrument failure and some had
193 to be rejected according to the quality control criteria used. In order to estimate cumulative fluxes from both
194 fields, temporal interpolation of the missing data points was required. However, in the absence of a well-validated
195 process-based model for N₂O fluxes on which to base predictions, it is not obvious how this is best achieved. The
196 most common approach is to linearly interpolate in time between flux measurements. In this study, a general
197 additive model (GAM) was used as an alternative approach, which accounted for temporal patterns at a range of
198 time scales and nonlinear responses to environmental variables, implemented using the *mgcv* package in the R
199 software (Wood, 2006).

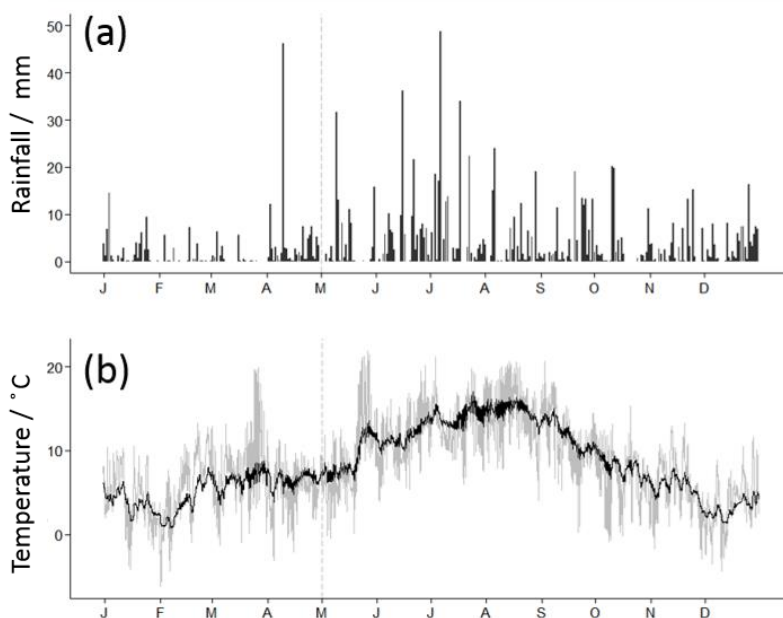
200 Fluxes measured by eddy covariance and both chamber methods from the tilled and un-tilled fields were
201 fitted to two separate GAMs using the same environmental terms for both fields. The environmental terms
202 included were air temperature, soil temperature, precipitation, and time. Additional terms for temperature and
203 precipitation aggregated over longer intervals (1, 6, 12, 24 and 48 hours preceding the flux measurement) were
204 examined and included where they improved the fit. The GAM allows for non-linearity by fitting a smooth
205 response with cubic splines. The degree of smoothing is optimised by the algorithm, but was also adjusted
206 subjectively, such that the model was not over-fitting to noise in the data. Observations from eddy covariance
207 and the two chamber methods were given equal weighting. Predictions from the GAM were used to fill gaps when
208 observations were not available. Uncertainty in predictions was estimated by simulating 2000 replicate time series
209 from the GAM, using the uncertainty in the fitted parameters, to estimate the posterior distribution. The quantiles
210 of this posterior distribution provided the 95 % credibility interval at each predicted 30-min interval time step. To
211 calculate cumulative fluxes, observed fluxes were used with their associated uncertainties (Finkelstein and Sims,
212 2001) when available; otherwise the GAM predictions were used.

213 3 Results

214 3.1 Meteorological data

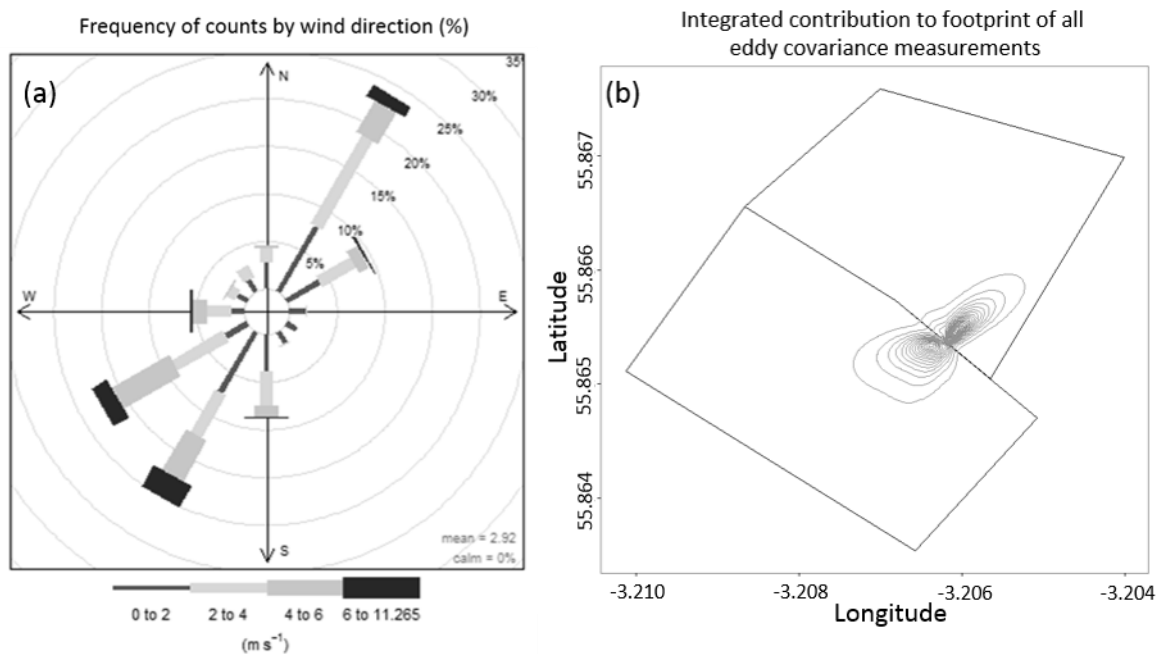
215 A total of 1191 mm of rain was recorded in 2012, higher than the average annual rainfall of 921 mm (2001 to
216 2011) for the Easter Bush area (Figure 3a). The annual variation in temperature was fairly typical of the field site

217 (Figure 3b). The wind direction at the field site is predominantly south-westerly (85 %). However, during the
218 measurement campaign, the wind direction was split fairly evenly between the tilled and un-tilled fields (Figure
219 4). This allowed a better basis for comparison of N₂O fluxes from the two fields, although data coverage for each
220 field was low, 34 % and 24 % for tilled and un-tilled respectively.



221
222 **Figure 3** (a) Accumulated daily rainfall at the Easter Bush Field site during the year 2012. (b) Air temperature at
223 height 3 m (grey) and soil temperature (black) recorded at the Easter Bush field site during the year 2012. Tillage
224 occurred on the 1st of May 2012 (grey dashed vertical line).

225



226

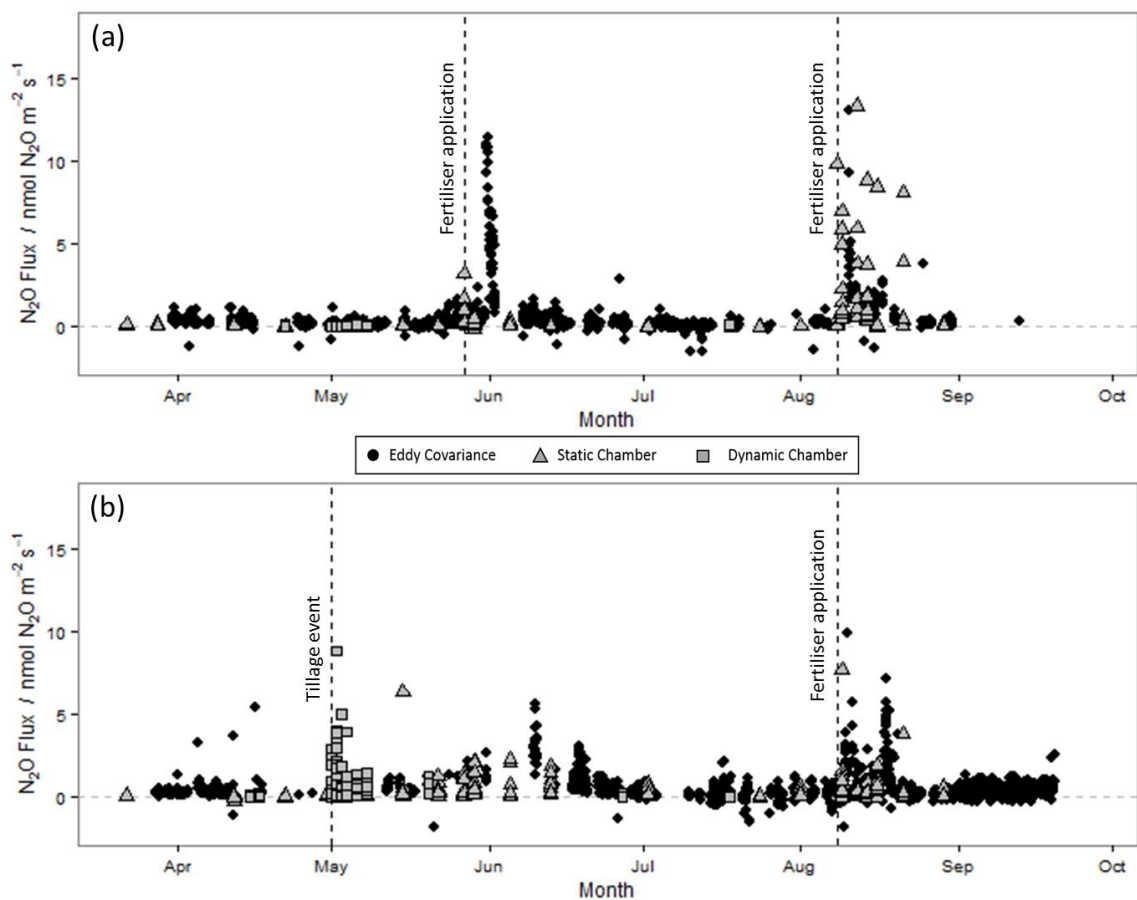
227 **Figure 4** (a) Wind rose plot for the Easter Bush field site during eddy covariance measurements (March – October
 228 2012). (b) Spatial distribution of the time-averaged flux footprint over the measurement period. The outer-most
 229 contour represents the area which, on average, contributed to 97.5 % of the measured half-hourly flux.

230 3.2 Comparison of N₂O fluxes measured from the un-tilled and tilled fields

231 Before the tillage event, N₂O fluxes were similar in the tilled and untilled fields. In both cases, around 90 % of
 232 measured fluxes were below 0.5 nmol m⁻² s⁻¹ (Figure 5). All three fertilisation events (the two fertiliser events in
 233 the untilled field and single fertiliser event in the tilled field) were characterised by an emission peak of 5-10 nmol
 234 m⁻² s⁻¹ lasting a few days, which declined over the following days and weeks, often with considerable variability
 235 and some apparent secondary peaks (Figure 5). Fluxes had returned to background levels (<0.5 nmol m⁻² s⁻¹)
 236 within 28 days of each of the fertilisation events. Fluxes measured by all methods agreed reasonably well in
 237 magnitude, and there is no strong evidence for a systematic bias, given the differences in the spatial and temporal
 238 sampling (for a more specific insight see e.g. Cowan et al., 2014a).

239 The tillage event also produced an increase in emissions, and although the peak was less clearly defined,
 240 the effect was more prolonged. Fluxes generally ranged from ~0 to 1.0 nmol m⁻² s⁻¹ in the days before tillage and
 241 ~0 to 8.8 nmol m⁻² s⁻¹ in the week immediately after tillage (Figure 5b). Three exceptionally high individual
 242 chamber measurements measured in the days immediately after the second fertilisation event in the un-tilled field
 243 which are included in the data analysis (19.5, 34.8 and 50 nmol m⁻² s⁻¹) are not included in Figures 5 or 7 in order

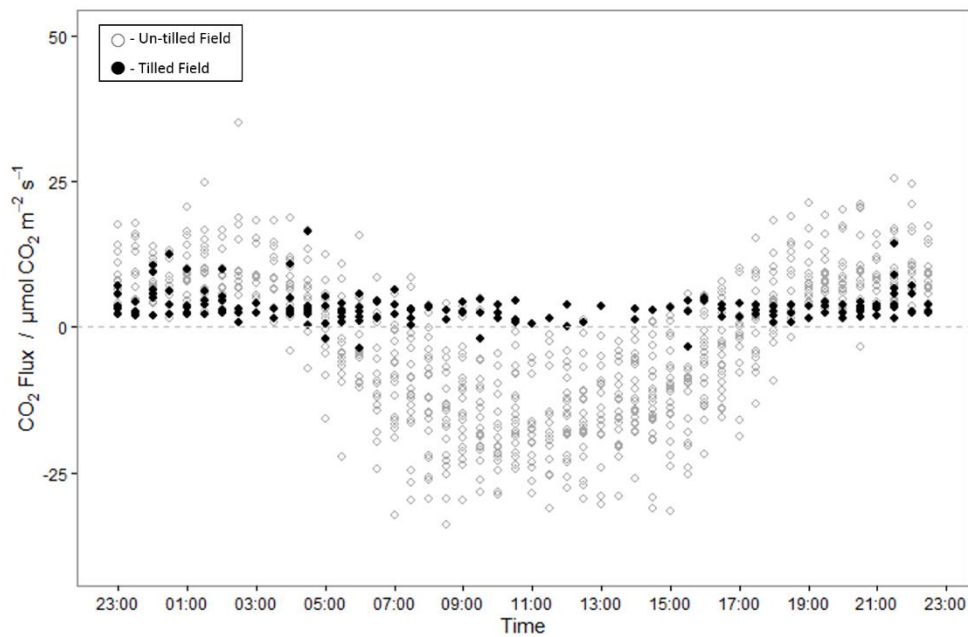
244 to keep the scale manageable. Fluxes from the tilled field from mid to late May were approximately $1 \text{ nmol m}^{-2} \text{ s}^{-1}$
 245 higher than from the untilled field (before the latter was fertilised). There followed an apparent increase in N_2O
 246 fluxes lasting approximately four weeks from the tilled field from late May to late June, peaking mid-June (Figure
 247 5b). Unfortunately, data coverage was rather low during this period due to changes in wind direction and a five
 248 day period in which the QCL was not operational. Because the tilled field had not been fertilised since the previous
 249 year, we infer that the increased fluxes were a result of the tillage event. Fluxes in the tilled field returned to pre-
 250 tillage magnitude during July. By July, a new sward of grass had grown in the tilled field, but sheep were not re-
 251 introduced into the field until September.



252

253 **Figure 5** Fluxes of N_2O from the (a) un-tilled and (b) tilled fields measured at the Easter Bush field site in 2012.
 254 Fertiliser was applied to the un-tilled field on the 28th of May and to both fields on 9th August (vertical dashed
 255 lines). Tillage began on 1st May. The Y-axis is limited to $15 \text{ nmol m}^{-2} \text{ s}^{-1}$ for better comparison between the
 256 fields. Only three static chamber measurements in the un-tilled field recorded fluxes above $15 \text{ nmol m}^{-2} \text{ s}^{-1}$ in the
 257 first few days after the August fertilisation.

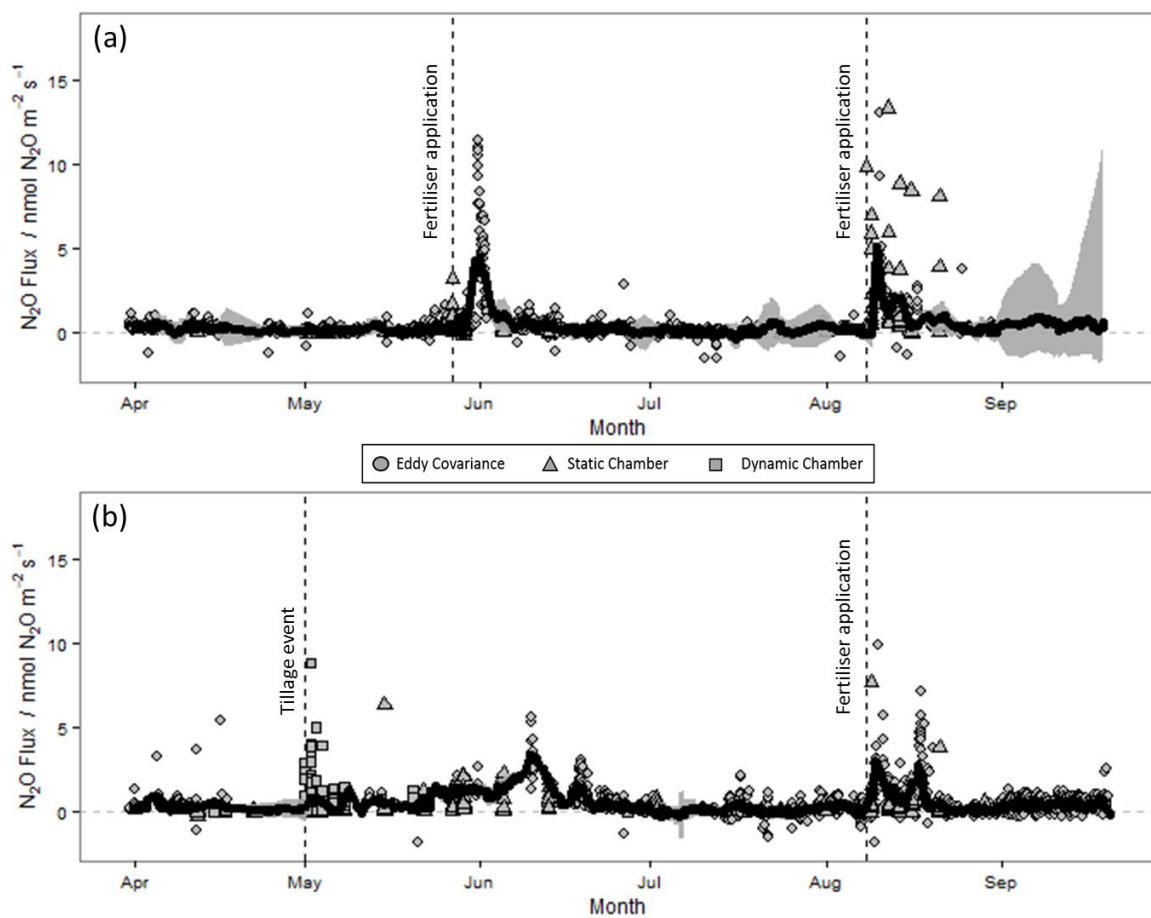
258 The relatively high N₂O fluxes measured from the tilled field in the weeks after tillage (May to July)
 259 occur in a similar timeframe to the fertilisation event in the un-tilled field (Figure 5). Beyond the analytical
 260 footprint analysis, we wanted to check that the high N₂O fluxes, which we attribute to the tillage, actually do come
 261 from the tilled field, and are not influenced by N₂O from fertilisation events on surrounding fields. The CO₂
 262 fluxes (measured by QCL instrument) provide a suitable tracer. We know that no significant photosynthesis took
 263 place on the tilled field between 1st of May and 17th of June, as there was no green foliage visible until after this
 264 period. Therefore, if the CO₂ fluxes showed no day-time uptake on the tilled field, we can be reasonably certain
 265 that the measured N₂O fluxes were also coming from the tilled field. Figure 6 shows that this was the case: in
 266 fluxes attributed to the tilled field, there was no day-time uptake of CO₂; in fluxes attributed to the un-tilled field,
 267 the normal diurnal cycle in CO₂ flux is seen. By inference, we can attribute the high N₂O emissions after tillage
 268 to the tilled field.



269
 270 **Figure 6** CO₂ flux measurements made from the un-tilled (grey) and tilled (black) fields between the 1st of May
 271 and the 17th of June. Uptake is denoted as a negative quantity. The results show a clear difference between the
 272 fields, with no day-time uptake on the tilled field. This implies that the high N₂O fluxes measured after the
 273 tillage event can also be attributed to the tilled field.

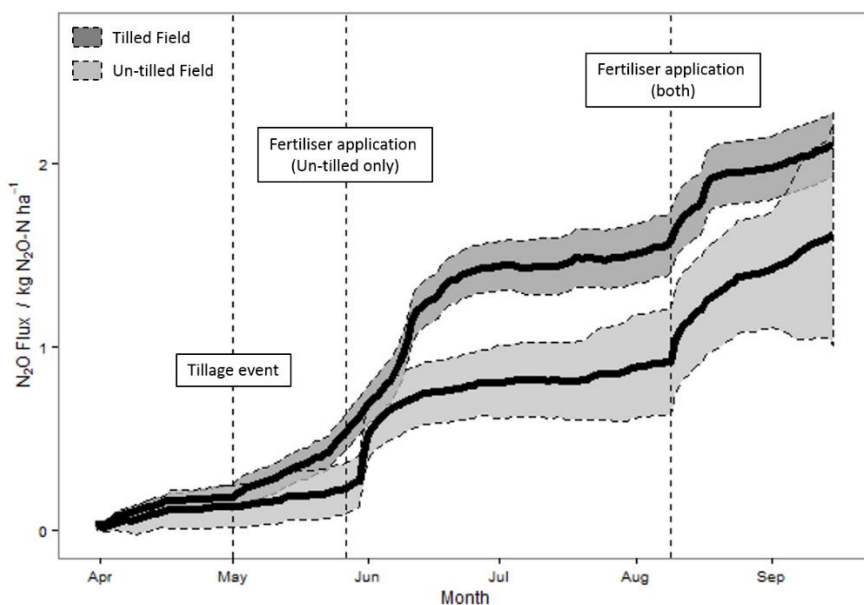
274 The GAM method was used to gap-fill flux data to calculate cumulative fluxes for both fields separately
 275 using the fluxes measured from each. (Figure 7). The total number of individual eddy covariance, dynamic

276 chamber and static chamber flux measurements used to fit the GAMs were 1563:273:234 and 1153:56:221 for the
277 tilled and un-tilled fields, respectively. Cumulative N₂O fluxes calculated for the tilled and un-tilled fields from
278 1st of April to the 16th of September were 2.14 ± 0.18 and 1.65 ± 1.02 kg N₂O-N ha⁻¹, respectively (Figure 8).
279 Uncertainty in the GAM prediction is particularly large when no measurements are available in which to fit the
280 model. There are sustained periods in which very few eddy covariance measurements were recorded from the un-
281 tilled field due to the wind direction being predominantly south westerly (Figure 7a). The uncertainty in predicted
282 flux becomes very large when compared to periods when measurements data is available and these uncertainties
283 propagate significantly in cumulative flux estimates (Figure 8).



284
285 **Figure 7** The GAM method (black line) provides an estimated N₂O flux which can be used to gap-fill
286 measurements from both the (a) un-tilled and (b) tilled fields at 30 min intervals. The 95 % confidence interval in
287 the estimated flux reported by the GAM is included (grey). Tillage and fertiliser dates are indicated (vertical lines).

288



289

290 **Figure 8** Cumulative flux is calculated for the tilled (dark grey) and un-tilled fields (light grey) using the gap-
 291 filled flux data. The cumulative 95 % confidence intervals are shown (grey areas). Fertiliser was applied to the
 292 un-tilled field on the 28th of May and to both fields on the 9th of August and tillage occurred on the 1st of May
 293 (black dashed vertical lines).

294 4 Discussion

295 4.1 The influence of tillage on N₂O fluxes

296 The comparison of pre-tillage and post-tillage fluxes from the tilled field suggests that the tillage event was
 297 directly responsible for an immediate increase in N₂O fluxes (Figures 5 & 8). N₂O fluxes significantly larger than
 298 those measured pre-tillage were observed from the tilled field over two separate periods during which no changes
 299 in N₂O fluxes were observed in the adjacent un-tilled field. The initial increase in N₂O flux from the tilled field is
 300 a short lived peak which occurs directly after the disturbance of the soil caused by ploughing and harrowing. The
 301 second is a sustained increase which is observed throughout May and June. In the two month period in which
 302 fluxes from the tilled field were elevated, a total of 1.26 ± 0.12 kg N₂O-N ha⁻¹ was estimated to have been released.
 303 Assuming fluxes in the tilled field had remained at approximately pre-tillage magnitude had the tillage event not
 304 taken place (~ 0.27 nmol m⁻² s⁻¹, based on an average of fluxes measurements before the tillage event), it can be
 305 concluded that the tillage event contributed to an additional 0.85 ± 0.11 kg N₂O-N ha⁻¹ emitted from the field over
 306 a two month period.

307 Increases in N₂O flux lasting up to two months after grassland tillage events have been observed before
308 in other studies using both static chamber and eddy covariance measurements (Chatskikh and Olesen, 2007;
309 Merbold et al., 2014). Reported fluxes can be relatively high over a sustained period of time (several days or
310 weeks) and similar in magnitude to those recorded after fertilisation events. The mechanisms driving these large
311 sustained fluxes are believed to be partly due to the mineralisation of organic materials in the soils (decaying grass
312 materials from the previous sward in tilled grasslands) (Baggs et al., 2003; Hellebrand, 1998; Pimentel et al.,
313 2015). The large quantities of decaying organic matter ploughed into the soils would have provided a gradual
314 release of carbon and nitrogen into the soils, which provide substrate for the microbial processes of nitrification
315 and denitrification (Pimentel et al., 2015; Seastedt et al., 1992). According to IPCC estimates, 1 % of N added to
316 soils in the form of crop residues can be expected to be released as N₂O (IPCC, 2006). Based on our pre-tillage
317 biomass measurements made prior to tillage (93.6 kg N ha⁻¹), we would expect to see N₂O fluxes of approximately
318 0.94 kg N₂O-N ha⁻¹ from the field. This estimated value is within the range of uncertainty of our calculated
319 cumulative fluxes in this study (0.85 ± 0.11 kg N₂O-N ha⁻¹). High emissions from crop residues tilled into arable
320 crops have been recorded in similar wet soils with high clay content (Ball, 1999) which may indicate a similar
321 process is occurring under these conditions at other field sites in the area.

322 Large N₂O fluxes (> 0.5 nmol m⁻² s⁻¹) are observed from both fields after fertilisation events. Elevated
323 fluxes recorded from the fields after fertilisation typically last three to four weeks with an occasional large spike
324 lasting 24 to 48 hours before returning to pre-fertilisation levels. This month long period in which the majority of
325 large fluxes occur after fertilisation is also generally observed by other similar studies from the local area (Skiba
326 et al., 2013; Smith et al., 2012). Assuming the majority of N₂O emitted after a fertilisation event occurs within a
327 28 day period after the fertiliser application, the 28 day cumulative flux emissions associated with the fertilisation
328 events on the 28th of May and 9th of August on the un-tilled field were 0.55 ± 0.05 and 0.76 ± 0.24 kg N₂O-N ha⁻¹,
329 respectively. This equates to 0.79 and 1.09 % of the total nitrogen applied, respectively. The 28 day cumulative
330 flux emissions associated with the fertilisation event on the tilled field was 0.77 ± 0.34 kg N₂O-N ha⁻¹, or 1.10 %
331 of the total nitrogen applied. Assuming the 28 day periods account well for the emission factors of the fertiliser
332 events, these results are well within the range of uncertainty of the generic 1 (0.3 to 3.0) % value reported by the
333 IPCC for N fertiliser events (IPCC, 2014).

334

335 4.2 Gap filling of N₂O fluxes

336 Gap-filling N₂O flux measurements is challenging due to the lack of reliable process-based models on which to
337 base predictions. N₂O fluxes are believed to be driven primarily by the availability of nitrogen compounds in the
338 soils (ammonium and nitrate) (Davidson et al., 2000) as well as physical properties of the soil such as WFPS,
339 aerobic extent, soil type, temperature and compaction (Ball et al., 2008; Butterbach-Bahl et al., 2013; Choudhary
340 et al., 2002; Davidson et al., 2000; Turner et al., 2008). The collection of these data on a temporal/spatial scale
341 which would allow these models to be applied is not often logistically possible or affordable. The GAM method
342 used in this study incorporates readily-available meteorological data with the temporal pattern in the data, to
343 provide an empirical but practical means of temporal interpolation, which makes use of more information than
344 simple linear interpolation. Although the GAM method has proved useful, we would also emphasise the dangers
345 of extrapolating to conditions beyond those to which the model was fitted. For example, as we have not measured
346 fluxes during the cold months in winter, the GAM is unable to reliably predict fluxes in temperatures lower than
347 those measured during the study. The method deals appropriately with the large uncertainties where measurement
348 data are unavailable, contributing considerably to the total uncertainty in cumulative flux estimates.

349 In this study, spatial variability was not explicitly accounted for in the cumulative flux uncertainty and
350 this remains a potentially large error if extrapolating to areas larger than the measurement footprint. Eddy
351 covariance is able to integrate over a large area of the field (several 100 m²) (Eugster and Merbold, 2015) but
352 these measurements are still subject to an element of spatial variability which is difficult to fully account for given
353 the spatially heterogeneous nature of N₂O fluxes. Any study which plans to report cumulative flux estimates
354 should consider how to minimise the uncertainties which arise when interpolating and/or extrapolating
355 measurements to larger temporal and spatial scales (e.g. from occasional chamber measurements to annual field-
356 scale emissions). Further studies may require more complex statistical analysis, using methods such as Bayesian
357 statistics, to properly quantify the uncertainty in estimates of cumulative fluxes over large areas.

358

359 5 Conclusion

360 N₂O emissions from the grassland field after the tillage event were relatively large and sustained, similar in
361 magnitude to a nitrogen fertilisation event. The tillage event in this study is estimated to be responsible for a
362 period of high and sustained N₂O emissions lasting over a two month period after tillage (0.85 ± 0.11 kg N₂O-N

363 ha⁻¹), with a cumulative flux value akin to an 85 Kg-N fertiliser application according to IPCC emission factor
364 estimates. Relatively little difference in N₂O fluxes were observed between the tilled and un-tilled fields after a
365 subsequent identical application of nitrogen fertiliser in August 2012. Our results agree with several other similar
366 studies that tillage and the resultant addition of crop residues into soils can result in significant emissions of N₂O,
367 similar in magnitude to 1 % of the nitrogen available in those residues (0.9 % in this study). This study highlights
368 that the tillage of grassland fields can potentially result in a short term but significant increase in emissions of
369 N₂O, with the potential to affect regional or national greenhouse gas budgets.

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375

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