

Scotland's Rural College

## The impact of ploughing intensively managed temperate grasslands on N<sub>2</sub>O, CH<sub>4</sub> and CO<sub>2</sub> fluxes

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1 *GHG fluxes after ploughing*

2 **The impact of ploughing intensively managed temperate grasslands on**  
3 **N<sub>2</sub>O, CH<sub>4</sub> and CO<sub>2</sub> fluxes**

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18

19 **Keywords**

20 Ploughing, N<sub>2</sub>O, CH<sub>4</sub>, CO<sub>2</sub>, grassland, temperate climate, tillage

21 **Abstract**

22

23 *Background and aims*

24 Temperate grasslands are a globally important component of agricultural production systems  
25 and a major contributor to the exchange of greenhouse gases (GHG) between the biosphere  
26 and atmosphere. Many intensively managed grazed grasslands in NW Europe are ploughed  
27 and reseeded occasionally in order to improve their productivity. Here, we examined the  
28 impact of ploughing on the emission of GHGs a grassland.

29 *Methods*

30 To study these interactions we measured soil GHG fluxes using the static chamber method in  
31 addition to the net ecosystem exchange (NEE) of CO<sub>2</sub> by eddy covariance from two adjacent  
32 fields. Until ploughing one field in 2012 and the other in 2014, management of these  
33 intensively grazed grasslands was almost the same and typical for the study region.

34 *Results*

35 The effect on N<sub>2</sub>O is small, but distinguishable from the effects of N fertilisation, soil  
36 temperature and soil moisture. Tillage-induced N<sub>2</sub>O fluxes were close to expectations based  
37 on the IPCC default methodology. By far the dominant effect on the GHG balance was the  
38 temporary reduction in GPP.

39 *Conclusions*

40 Ploughing and reseeded can substantially influence short-term GHG emissions. Therefore  
41 tillage-induced fluxes ought to be considered when estimating greenhouse gas fluxes or  
42 budgets from grasslands that are periodically ploughed.

43

## 44 **Introduction**

45 Grasslands rank among the world's most extensive ecosystems and are used for forage  
46 production and animal grazing (Campbell and Stafford Smith 2000). They cover 22% of the  
47 EU-25 land area, accounting for 80 million ha (EEA 2005). Managed grasslands are major  
48 source of emissions of N<sub>2</sub>O, CO<sub>2</sub> and CH<sub>4</sub>, if grazed by ruminants. Emission rates depend on  
49 soil management, soil type, climate and interannual climate variability (Skiba et al, 2012,  
50 Jones et al. 2005).

51

52 In order to maintain high harvest yields and optimal grass growth for grazing, renovation  
53 activities, such as ploughing and harrowing, are periodically carried out on intensively  
54 managed grasslands. To maximise productivity, these grasslands are heavily fertilised and  
55 therefore known large sources of N<sub>2</sub>O (Davies et al. 2001, Soussana et al, 2007).

56

57 Tillage is defined as the mechanical manipulation of soil conditions to support crop  
58 production, including ploughing and harrowing operations (Brady and Weil 2002).  
59 Depending on local soil properties and weather patterns, grassland tillage can increase grass  
60 yield and improve soil structure and aeration through drainage, which is often necessary in  
61 order to maintain productivity. On the other hand, this mechanical agitation is known to  
62 change soil properties and thereby can affect the net GHG exchange of grasslands (Ball et al.  
63 2014).

64

65 Pagliai et al. (2004) showed that soil porosity can decrease under conventionally tilled loam  
66 soils, and by reducing the size and the continuity of pores, water conductivity decreases.  
67 Conventional tillage (particularly in wet soils) can increase subsoil compaction, promoting  
68 conditions that are associated with increased rates of denitrification (Uchida et al. 2008). On

69 the other hand, conventional tillage can be beneficial for certain soil types, such as poorly  
70 drained and compactable soils (Ball et al. 1999). Other studies reported that long-term  
71 ploughing practices resulted in soil organic matter (SOM) losses (Eriksen and Jensen 2001),  
72 microbial biomass and water-stable aggregation decrease as well as lower potentially  
73 mineralisable N (Karlen et al. 2013). Generally, the impact on ploughing on soil properties  
74 depends on the soil type and weather conditions, thus resulting in many contrasting reports in  
75 the literature (Soane et al. 2012). Ball et al. (1999) reported that high rates of N<sub>2</sub>O emissions  
76 were mainly associated with rainfall patterns and compact arable soils, and no strong  
77 correlation between soil tillage and N<sub>2</sub>O emissions was found. In contrast, Kessavalou et al.  
78 (1998) found a 100% increase in N<sub>2</sub>O emissions from a loam soil after a tillage event during  
79 fallow, which agrees with other studies (Estavillo et al. 2002). For poorly drained grasslands,  
80 conventional tillage can be used as a mitigation method to increase soil porosity and water  
81 infiltration. As a consequence, denitrification rates can decrease and N<sub>2</sub>O emissions are  
82 reduced (MacDonald et al. 2011).

83

84 We report detailed data which allowed comparison of the effect of ploughing on GHG  
85 exchange at the long-term field study site, Easter Bush, South East Scotland. Two adjacent,  
86 predominately sheep grazed grasslands under the same management, were ploughed two  
87 years apart and thereby provided the opportunity to evaluate the magnitude of ploughing-  
88 induced GHG fluxes. This was not a designed experiment, but reflects common farming  
89 practice in this region, and can therefore provide useful information directly relevant to this  
90 kind of land management.

91 Our questions were:

92 (1) Do ploughing and associated management operations increase N<sub>2</sub>O, CH<sub>4</sub> and CO<sub>2</sub>  
93 fluxes?

94 (2) How variable are ploughing-induced emissions?

95

## 96 **Materials and Methods**

### 97 *Site description*

98 The study site is located at Easter Bush, 10 km south of Edinburgh, Scotland, in a  
99 mesothermal maritime climate (latitude 55°52'N, longitude 3°2'W). The two adjacent fields  
100 (North Field (NF) and South Field (SF)) are managed grasslands (>90% *Lolium perenne*).  
101 The soil is an imperfectly drained sandy clay loam (FAO classification: eutric cambisol) with  
102 a clay content varying from 20 - 26% and a pH varying from 5 to 6 (in H<sub>2</sub>O), depending  
103 when the soil was last limed. Soils were limed prior to the ploughing in 2012 and the soil pH  
104 was 6.1 (in H<sub>2</sub>O) during the 2012 - 2014 study period. During extended periods of rain, these  
105 fields tend to have localised waterlogging due to an insufficient drainage system. A  
106 meteorological station positioned between these two fields provides continuous  
107 measurements, with data averaged over 30 min periods. Rainfall amount is measured using a  
108 tipping bucket and air temperature at a height of 1.5 m above ground. The 10 year mean (1  
109 Jan 2004 – 31 Dec 2014) air temperature was 8.8 °C and rainfall 958 mm with a variation of  
110 less than 100 mm from the 10-year mean.

111

112 Agronomic management of both fields was very similar. In the 10 years prior to the  
113 ploughing experiment, the fields were predominately grazed by sheep and occasionally, for  
114 short periods, by cattle in 2004-2006, and on the NF in August and September 2012.  
115 Livestock was sporadically removed from the fields for periods of several days up to several  
116 weeks. The 10 year average livestock density was 0.84 LSU ha<sup>-1</sup> y<sup>-1</sup>, cattle contributed only  
117 with 0.05 LSU ha<sup>-1</sup> y<sup>-1</sup>. In order to maintain high grass yields, the fields receive mineral N  
118 fertiliser, mainly as ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub>), but occasionally as NPK compound

119 fertiliser or urea. The 10 year average nitrogen (N) fertiliser application rate was 194 kg N ha<sup>-1</sup>  
120 y<sup>-1</sup>, usually split across three applications during spring and early summer months (Skiba et  
121 al. 2013).

122

123 Foregoing ploughing and reseeded the grass was killed using Glyphosate (Table 1). The  
124 South field (SF) and the North field (NF) were ploughed on 1<sup>st</sup> May 2012 and on 20<sup>th</sup> May  
125 2014, respectively, with a mouldboard plough to a depth of 30 cm. The fields were harrowed,  
126 reseeded and rolled a few days after both ploughing events. All management operations and  
127 fertiliser applications during the study periods in both years are summarised in Table 1 and  
128 the management operations were essentially identical for the two years. It is common practice  
129 not to apply N fertiliser until the grass is well established. Therefore only the NF received N  
130 fertiliser on the 28<sup>th</sup> of May 2012, and only the SF was fertilised on the 9<sup>th</sup> May 2014. In  
131 2012 GHG flux measurements were made for 39 days before ploughing and 142 days after  
132 ploughing and in 2014 a shorter study provided the same measurements for 67 days before  
133 ploughing and 34 days after ploughing.

134

#### 135 *Measurements of soil N<sub>2</sub>O, CH<sub>4</sub> and CO<sub>2</sub> fluxes*

136 The static chamber method (Clayton et al. 1994) was used for N<sub>2</sub>O and CH<sub>4</sub> flux  
137 measurements. Round static chambers (diameter = 40 cm) consisting of opaque  
138 polypropylene bases, were installed on each field; 20 (10 in each field) in 2012 and 10 (5 in  
139 each field) in 2014, respectively. The bases of 10 cm height were inserted into the ground to a  
140 depth of approximately 5 cm for the entire study period to allow free grazing. Lids of 20 cm  
141 height, were fastened onto the bases using four strong clips, only during the 60 minute  
142 measurement periods. A strip of commercially available draft excluder glued onto flange of  
143 the lid provided a gas tight seal between chamber and lid. The lids were fitted with a pressure

144 compensation plug to maintain ambient pressure in the chambers during and after sample  
145 removal. Gas samples were taken at regular intervals over one hour (0, 30, 60 min in 2012  
146 and 0, 20, 40, 60 min in 2014) for each chamber. A three way tap was used for gas sample  
147 removal using a 100 ml syringe. 20 ml glass vials were filled with a double needle system to  
148 flush the vials with five times their volume. The samples and three sets of four certified  
149 standard concentrations (N<sub>2</sub>O, CH<sub>4</sub>, CO<sub>2</sub> in N<sub>2</sub> with 20% O<sub>2</sub>) were analysed at CEH on an  
150 HP5890 Series II gas chromatograph (Hewlett Packard (Agilent Technologies) UK Ltd.,  
151 Stockport, UK) with electron capture detector (ECD) for N<sub>2</sub>O analysis and flame ionization  
152 detector (FID) for CH<sub>4</sub> analysis. These detectors were setup in parallel allowing the analysis  
153 of the two GHGs at the same time. Limit of detection was 7 ppb for N<sub>2</sub>O and 0.07 ppm for  
154 CH<sub>4</sub>. Peak integration was carried out with Clarity chromatography software (DataApex,  
155 Prague, Czech Republic). The flux  $F$  ( $\mu\text{g m}^{-2} \text{s}^{-1}$ ) for each sequence of gas samples from the  
156 different chambers was calculated according to Equation 1:

157

$$158 \quad F = \frac{dC}{dt} \times \frac{\rho V}{A} \quad (\text{Equation 1})$$

159

160 Where  $\frac{dC}{dt}$  is the concentration ( $C$ ,  $\mu\text{mol mol}^{-1}$ ) change over time ( $t$ , in s), which was  
161 calculated by linear regression.

162  $\frac{\rho V}{A}$  is the number of molecules in the enclosure volume to ground surface ratio, where  $\rho$  is the  
163 density of air ( $\text{mol m}^{-3}$ ),

164  $V$  ( $\text{m}^3$ ) is the air volume in the chamber and

165  $A$  ( $\text{m}^2$ ) is the surface area in the chamber (Levy et al. 2012).

166

167 In addition, ecosystem CO<sub>2</sub> respiration rates, which is the sum of soil and vegetation CO<sub>2</sub>  
168 respiration, were measured close to each chamber location using an opaque closed dynamic



169 chamber (volume: 0.001171 m<sup>3</sup>) covering 0.0078 m<sup>2</sup> of soil for 120 s with an EGM-4  
170 infrared gas analyser (IRGA: InfraRed Gas Analyser) (PP Systems; Hitchin, Hertfordshire,  
171 England). Taking into account the soil temperature, fluxes were calculated based on the linear  
172 increase of CO<sub>2</sub> concentrations. In 2012, the short-term physical release of CO<sub>2</sub> immediately  
173 after ploughing the SF was investigated from 4 random locations. First soil respiration  
174 measurements were made within 10 – 19 minutes after the plough turned the soil over and  
175 were repeated at intervals up to almost 3 hours. Thereafter CO<sub>2</sub> respiration rates (bulk soil  
176 and vegetation), were always measured at approximately the same time and adjacent to the  
177 chambers used for N<sub>2</sub>O and CH<sub>4</sub> flux measurements, both in 2012 and 2014.

178

#### 179 *Auxiliary physical and chemical soil measurements*

180 Other environmental parameters were measured during time of chamber enclosure as possible  
181 explanatory variables for correlation with recorded GHG fluxes. Soil temperature was  
182 measured with a handheld Omega HH370 temperature probe (Omega Engineering UK Ltd.,  
183 Manchester, UK) for each chamber location at a depth of 10 cm. Volumetric soil moisture  
184 content (VSM) was measured at a depth of 7 cm with a handheld Theta probe HH 2 moisture  
185 meter (Delta T-Devices, Cambridge, UK) horizontally inserted at four points around each  
186 chamber. Gravimetric moisture content (GWC) was occasionally measured to calibrate the  
187 Theta probes. In order to determine bulk density, total C/N, ammonium (NH<sub>4</sub><sup>+</sup>) and nitrate  
188 (NO<sub>3</sub><sup>-</sup>) concentrations, soil cores were taken around each of the chamber locations. Soil  
189 samples for determination of bulk density were collected using a galvanised iron ring (98.17  
190 cm<sup>3</sup>) with a sharp edge that was inserted in the upper soil layer with a hammer to 5 cm depth  
191 without compaction. Samples were oven-dried at 105 °C until constant weight (usually 48  
192 hours) and bulk density (g cm<sup>-3</sup>) was calculated based on the dry weight occupying the  
193 volume of the ring.

194

195 For  $\text{NH}_4^+$  and  $\text{NO}_3^-$  analysis 15 g of fresh soil was mixed in plastic flasks with 50 ml of 1 M  
196 KCl solution made up with deionised water. The flasks were put on a Stuart Orbital Shaker  
197 SSL1 (Barloworld Scientific Ltd., Stone, UK) set to 100 rpm for 1 hour. The extract was  
198 filtered with Whatman 42 filter papers and poured into vials that were stored frozen  
199 thereafter. Defrosted samples were then analysed with a SAN++ Automated Wet Chemistry  
200 Analyzer (Skalar Analytical B.V., Breda, Netherlands). To determine total soil C and N,  
201 samples were oven-dried at 105°C and ground with a mixer mill MM200 (Retsch GmbH &  
202 Co. KG, Haan, Germany) at CEH. Between 10 and 20 mg of each soil sample was transferred  
203 to tin capsules and analysed together with four standards of aspartic acid with a Flash 2000  
204 Elemental Analyzer (Thermo Fisher Scientific, Cambridge, UK).

205

#### 206 *Net ecosystem exchange of $\text{CO}_2$*

207 In addition to the above described ecosystem respiration rates, we measured the net  
208 ecosystem exchange of  $\text{CO}_2$ . In order to measure from the ploughed and unploughed fields  
209 simultaneously we installed a mobile eddy covariance (EC) system in addition to our  
210 permanent, long-term system, in both years.

211

#### 212 *Long-term eddy-covariance system*

213 Fluxes of  $\text{CO}_2$  have been measured continuously by eddy-covariance (EC) at Easter Bush  
214 since 2002. The EC mast is located along the fence line which separates the NF from the SF  
215 (Figure 1). The EC system consists of a Gill WindmasterPro ultrasonic anemometer for the  
216 measurement of 3D wind vector components and sonic temperature (20 Hz data), and of a  
217 LICOR 7000 closed-path infrared gas analyser (IRGA) operating at 10 Hz for the  
218 simultaneous measurement of  $\text{CO}_2$  and  $\text{H}_2\text{O}$  mole fractions. Air is sampled at  $10 \text{ l min}^{-1}$ , 20

219 cm below the mid-point between the anemometer's transducers (effective measurement  
220 height of 2.5 m) through a 10 m long Dekabon© line (OD ¼"). Data is captured and  
221 processed offline into half-hourly fluxes using in-house software written in LabView™  
222 (National Instruments). Data capture was high in the period 9<sup>th</sup> May - 20<sup>th</sup> Aug 2012 (85%),  
223 with a 52% to 48% split between measured fluxes originating from the SF and the NF  
224 respectively. The extent of the flux footprint of the long-term EC system during the 2012  
225 measurement period relevant to the ploughing experiment is shown in Figure 1. The footprint  
226 statistics used for this figure were obtained with the analytical Kormann-Meixner footprint  
227 model for non-neutral stratification (Kormann and Meixner 2001). In 2014, total data capture  
228 after filtering was 84% for the long-term EC system with a 71% to 29% split between  
229 measured fluxes originating from the SF and the NF respectively.

230

#### 231 *Mobile eddy covariance system in 2012*

232 The prevailing wind direction pre- and post-ploughing was from the N/NW and not the usual  
233 S/SE. This means that the long-term EC system mainly measured CO<sub>2</sub> fluxes from the  
234 unploughed grassland in the NF. Therefore a temporary mast was erected in the SF in April  
235 2012 (Figure 1) to achieve the direct temporal comparison of F<sub>CO<sub>2</sub></sub> from the ploughed and  
236 unploughed field for wind directions in the range ~ N-NW to N-NE. The SF system was a  
237 Campbell Scientific EC150 open-path infrared gas analyser for CO<sub>2</sub> and H<sub>2</sub>O combined with  
238 a Campbell CSAT3 ultrasonic anemometer, with effective measurement height of 1.90 m.  
239 Data were logged at 20 Hz to a Campbell Scientific CR3000 data logger and processed  
240 offline. The SF system provided 3245 half-hourly average flux in total in the period 9<sup>th</sup> May -  
241 20<sup>th</sup> Aug 2012 (66% of possible half-hourly data points during this measuring period), of  
242 which 926 (28%) corresponded to wind directions in the range ~ N-NW to N-NE. Low  
243 turbulence ( $u_* < 0.1 \text{ m s}^{-1}$ ) and periods of rain accounted for over 95% of missing data.

244

245 *Mobile eddy covariance system in 2014*

246 The prevailing wind direction was SE and the above mentioned long-term eddy covariance  
247 system provided the measurements for the SF (which in 2014 was the newly established grass  
248 sward, after ploughing in 2012). A mobile system, different to the mobile system used in  
249 2012, was erected in the NF in May 2014 prior to the ploughing of the field on 20<sup>th</sup> May  
250 2014 (Figure 1) and was removed on 4<sup>th</sup> Aug 2014. The EC system consisted of a Metek  
251 USA-1 ultrasonic anemometer operating at 20 Hz and a Licor 7000 closed-path infrared gas  
252 analysed measuring CO<sub>2</sub> and H<sub>2</sub>O mole fractions at 10 Hz. Air was sampled 20 cm below the  
253 mid-point between the anemometer's transducers (effective measurement height of 2.3 m) at  
254 8 l min<sup>-1</sup> through a 1.5 m long piece of Dekabon© tubing (OD ¼"). Data was logged by a  
255 laptop running an in-house data acquisition software written in LabView<sup>TM</sup> and were  
256 processed offline. Data capture was 58% with 47% of available data points attributable to the  
257 North field. After standard filtering and quality control (Helfter et al. 2015), there remained  
258 25% of high quality data (19% daytime and 6% night time data). The IRGA was run with a  
259 scrubbing column (1:1 mixture of soda lime and drierite) in front of the reference cell rather  
260 than a supply of N<sub>2</sub>; exhaustion of the chemicals was the greatest cause of data loss (> 80%).

261

262 *Data analysis*

263 For comparing soil properties before and after the ploughing event, paired t-tests were carried  
264 out and results with p<0.05 regarded as significant.

265 In an attempt to separate the effects of fertilisation and ploughing on N<sub>2</sub>O flux, we used a  
266 simple model which describes the expected response to fertilisation. The N<sub>2</sub>O flux was  
267 expected to increase to a peak value some time after the date of fertilisation, and show an  
268 exponential decline thereafter. We used the lognormal density function to represent this

269 pattern in time. Using data from all fertilisation events, we fitted two parameters,  $\mu$  and  
270  $\sigma$ . Conventionally, these represent the mean and standard deviation of the log-  
271 transformed data. However, in this context,  $\mu$  represents the time delay between fertilisation  
272 and the peak flux occurring, and  $\sigma$  represents a decay rate parameter. By expressing the  
273 flux data appropriately, these parameters can be found as the mean and standard deviation of  
274 a transformed data set, so numerical optimisation is not required. A scaling coefficient was  
275 derived by linear regression of these predictions on the observations. In this way, we found  
276 the best fit to the observations, given a lognormal-shape pattern following fertilisation. This  
277 procedure was applied only to  $\text{N}_2\text{O}$  fluxes, as there was no similar a priori expectation of a  
278 response of  $\text{CH}_4$  or  $\text{CO}_2$  fluxes to fertilisation.

279

280 We statistically analysed whether  $\text{N}_2\text{O}$  fluxes were related to ploughing using a mixed-effects  
281 model (Pinheiro and Bates, 2004). This expressed the  $\text{N}_2\text{O}$  flux in terms of four fixed effects:  
282 soil temperature, soil moisture, the predicted response following fertilisation, and whether  
283 ploughing had recently taken place or not. We also included two nested random effects,  
284 accounting for repeated measurements on individual chambers, which were nested within the  
285 two fields. For  $\text{CH}_4$  and  $\text{CO}_2$ , we could fit a simpler model with the same random effects, but  
286 only the three fixed effects of soil temperature, soil moisture, and ploughing. All analyses  
287 were performed on log-transformed fluxes, so that the data met normality assumptions. To  
288 allow for negative values, an offset of 50 was added to  $\text{CH}_4$  fluxes.

289

## 290 **Results**

### 291 *Rainfall, Temperature and soil moisture*

292 The rainfall patterns in 2012 and 2014 were similar. Cumulative rainfall over the two months  
293 prior to ploughing in 2012 was 118 mm, compared with 136 mm in 2014 (Figure 2c,d). Both

294 ploughing events were followed by a similarly wet period: 100 mm for the month of May  
295 2012, and 116 mm during the post-ploughing month in 2014, around twice the long-term  
296 mean for May.

297

298 In 2012, the average air and soil temperatures in the two weeks before ploughing and one  
299 week after ploughing stayed below 10 °C (Figure 2a, 3a). The air temperature only increased  
300 to double figures (15 °C) on the 21 May, and stayed between 12 and 18 °C until the end of  
301 the measurement period. There was no significant rainfall the week before and the week after  
302 ploughing, but from the 31 May (i.e. almost one month after ploughing) rainfall frequency  
303 and amount increased (Figure 2c). Because of these cold, dry conditions, germination was  
304 very slow.

305

306 In 2014, the soil temperature was around 5 °C warmer at the time of ploughing, compared  
307 with 2012 (Figure 3a). Soil temperature rose fairly steadily from 12 °C to 20 °C over the  
308 study period following ploughing. In both years, soil temperature increased after ploughing,  
309 and the increase was greater in the ploughed field than in the unploughed field (Figure 3a).  
310 Unlike in 2012, there was no rainfall in the week before ploughing and reseeded in 2014  
311 (Figure 2d), but frequent rain showers within two weeks of the ploughing event together with  
312 the warmer temperatures facilitated fast germination and almost complete canopy closure by  
313 the end of this much shorter study period.

314 Volumetric soil moisture (VSM) content in 2012 was larger in the NF than the SF  
315 irrespective of the ploughing (Figure 3b). In 2014 the VSM in the NF decreased from 70-90%  
316 to <30%. The downward trend was stronger after ploughing. The unploughed SF did not  
317 show this trend and even showed a slight increase in VSM in June to a maximum of around  
318 60% from averages around 40% previously (Figure 3b).

319

320 *Soil properties*

321 Bulk density, total C and N, and KCl extractable  $\text{NH}_4^+$  and  $\text{NO}_3^-$  for the top 10 cm were  
322 measured one week before and one and five weeks after ploughing from both ploughed and  
323 non-ploughed fields in both years (Table 2). Both ploughing events significantly increased  
324 the soil bulk density of the top 5 cm by 37%, from  $0.75 \text{ g cm}^{-3}$  to  $1.19 \text{ g cm}^{-3}$ . The small  
325 differences in bulk densities between 2012 and 2014 shown in Table 2 are not significant.  
326 Total C/N ratio was lower in 2012 than 2014 for both fields, none of the differences between  
327 years and fields were significant. In 2012 and 2014 differences in  $\text{NH}_4^+$  and  $\text{NO}_3^-$   
328 concentrations were not significant for the two fields before ploughing. After ploughing the  
329  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentrations were larger from the ploughed fields compared to the  
330 unploughed field, both 1 and 5 weeks after ploughing. These differences were significant for  
331  $\text{NH}_4^+$  on both post-ploughing dates in 2012 ( $p < 0.001$ ), and for  $\text{NO}_3^-$  1 week after ploughing in  
332 both years ( $p < 0.05$ ). In 2012 SF and NF  $\text{NH}_4^+$  and  $\text{NO}_3^-$  increased with time between pre-  
333 ploughing and 1 week later, and also between the 1 week and 5 week measurements.  
334 Differences were significant at  $p < 0.05$  and above for all, except for  $\text{NO}_3^-$  concentrations from  
335 the SF 1 and 5 weeks after ploughing and the NF pre and 1 week after ploughing. In 2014  
336 there was no significant change in  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentrations from the unploughed SF.

337

338 *N<sub>2</sub>O fluxes*

339 Background mean fluxes in early spring in both years were  $< 5 \mu\text{g m}^{-2} \text{ h}^{-1} \text{ N}_2\text{O-N}$  (Figure 4a).  
340 After fertilisation events,  $\text{N}_2\text{O}$  fluxes generally showed a peak followed by a decline, and the  
341 lognormal density function approximates this pattern in the data reasonably well (fitted lines  
342 in Figure 4a). After both ploughing events,  $\text{N}_2\text{O}$  fluxes showed a strong deviation from the  
343 pattern expected from fertilisation alone, and increased approximately linearly over the

344 following month, up to around  $200 \mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$  in 2012, and to  $1300 \mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$   
345 in 2014 (red points in Figure 4a). However, soil temperatures also increased over both  
346 periods, so we cannot interpret this simply as a response to ploughing. To separate the effects  
347 of fertilisation, temperature and soil moisture from that of ploughing, we used the mixed-  
348 effects model analysis. This shows a strong indication that  $\text{N}_2\text{O}$  fluxes were higher after  
349 ploughing, after accounting for the effect of fertilisation, temperature and soil moisture  
350 (Table 3). Because the mixed-effects model is fitted to the log-transformed flux, the  
351 interpretation of the coefficients is not as straight-forward as in the normal case. The  
352 exponentiated coefficients are interpreted as the proportional change in flux for a unit change  
353 in the independent variable. To translate these into more meaningful units, we calculate the  
354 absolute effect size as the difference in the fitted mixed model predictions with and without  
355 ploughing, at the mean level of all other inputs (Table 4). This predicts that fluxes were  
356 higher after ploughing by  $14.1 \mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$  in 2012, and  $49.9 \mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$  in 2014.  
357 By comparison with the average magnitude of fluxes after fertilisation events, we would  
358 expect fluxes to be on average  $96 \mu\text{g m}^{-2} \text{ h}^{-1}$  higher, if 1% of  $70 \text{ kg N ha}^{-1}$  were released as  
359  $\text{N}_2\text{O}$  in the 30 days following fertiliser application (although we would expect this to follow  
360 the lognormal pattern in time described previously). We thus estimate that ploughing has an  
361 effect which is ~14 - 52 % of that of typical N fertilisation.

362

### 363 *CH<sub>4</sub> fluxes*

364 Both positive and negative  $\text{CH}_4$  fluxes were measured in both years. In early spring in both  
365 years on both fields, background fluxes ranged from uptake of a few tens of  $\mu\text{g CH}_4\text{-C m}^{-2} \text{ h}^{-1}$   
366 to positive emission fluxes of a few tens of  $\mu\text{g CH}_4\text{-C m}^{-2} \text{ h}^{-1}$ . After ploughing in May 2012,  
367 fluxes from the SF increased to a few hundreds of  $\mu\text{g CH}_4\text{-C m}^{-2} \text{ h}^{-1}$  (Figure 4b, red points)  
368 whilst fluxes from the unploughed NF remained in the order of a few tens of  $\mu\text{g CH}_4\text{-C m}^{-2} \text{ h}^{-1}$



369 <sup>1</sup>. After ploughing of the NF in 2014, CH<sub>4</sub> fluxes increased to >5000 μg CH<sub>4</sub>-C m<sup>-2</sup> h<sup>-1</sup>.  
370 Fluxes also increased from the SF but only to about 500 μg CH<sub>4</sub>-C m<sup>-2</sup> h<sup>-1</sup>. Again, we used  
371 the mixed-effects model to separate the effect of ploughing from the effects of temperature  
372 and soil moisture (Table 3). This showed a strong effect of temperature, a weak effect of soil  
373 moisture, and a variable response to ploughing. Ploughing decreased CH<sub>4</sub> fluxes by 11 μg m<sup>-2</sup>  
374 h<sup>-1</sup> in 2012, and increased them by 36.5 μg m<sup>-2</sup> h<sup>-1</sup> in 2014 (Table 4). In the absence of  
375 ploughing, fertilisation in May in 2012 appeared to increase CH<sub>4</sub> fluxes, and to a lesser extent  
376 in August 2012, but there was no effect apparent in 2014 (Figure 4b).

377

### 378 *Ecosystem respiration rates*

379 Although variable, all 4 random locations on the ploughing day on 2012 demonstrated the  
380 immediate increase in CO<sub>2</sub> respiration within the first 30 min after the plough passed that  
381 particular area (Figure 5). This physical release of CO<sub>2</sub> remained for at least 3 hours, and  
382 fluxes then returned to near- background levels after around 80-90 min.

383

384 Ecosystem respiration rates in 2012 were on average around 250 mg CO<sub>2</sub>-C m<sup>-2</sup> h<sup>-1</sup> in early  
385 spring for both fields (Figure 4c). Results from the mixed-model analysis show that  
386 ploughing decreased ecosystem respiration quite consistently, as well as showing a strong  
387 positive response to temperature (Table 3). The net effect of ploughing was to decrease  
388 ecosystem respiration by 71-85 mg CO<sub>2</sub>-C m<sup>-2</sup> h<sup>-1</sup> (Table 4). An effect of fertilisation separate  
389 from that of temperature was not easily discernible.

390

### 391 *Net ecosystem exchange of CO<sub>2</sub> measured by eddy covariance*

392 There was a greater than usual occurrence of wind from the N-NW in the summer of 2012  
393 which resulted in the near 50:50 split of data collected from NF and SF (Figure 1). The 70:30  
394 split in favour of winds blowing from the SW observed in 2014 is more typical for the site.

395

396 The two ploughing events in 2012 and 2014 exhibited multiple similarities in terms of NEE  
397 (Figure 6). Daytime uptake of CO<sub>2</sub> by the ploughed field ceased after ploughing and fluxes  
398 remained positive for approximately 40 days after the event (Figure 6a and c). This is most  
399 obvious at ploughing of the NF in 2014 with highest coverage of eddy covariance data  
400 (Figure 6c). After ca. 40 days, CO<sub>2</sub> uptake in the ploughed and re-sown field was comparable  
401 to the non-ploughed field in each year; however, the variability in daytime NEE in the two  
402 fields was large (2-3 times larger in 2014 than in 2012; Figure 6a and c). Night time fluxes of  
403 CO<sub>2</sub> were not statistically different between fields in 2012 (Figure 6b) and the temporal  
404 variability was consistent with variations in soil temperature (weak positive correlation of  
405 fluxes with soil temperature which peaked in both fields ca. 27 days after ploughing; Figure  
406 3a). Night time fluxes in the ploughed NF also followed the upward trend in soil temperature  
407 observed in 2014 (Figure 6d and Figure 3a). In contrast, night time respiration in the SF was  
408 larger than in the ploughed NF, it was more scattered and did not exhibit a clear correlation  
409 with soil temperature. Ploughing had a transient effect on CO<sub>2</sub> fluxes at Easter Bush, with a  
410 full recovery of the sink strength observed within 1.5 to 2 months after ploughing and re-  
411 sowing.

412

413 Daytime and night time CO<sub>2</sub> fluxes measured by EC increased sharply from the day of  
414 ploughing in 2014 and peaked 3 days later (Figure 6c and d) which we attribute to the  
415 combined effects of the physical removal of the CO<sub>2</sub> sink and the release of CO<sub>2</sub> from  
416 upturned soil layers.

417 Ploughing caused a net release of carbon of the order of  $120 \text{ g CO}_2\text{-C m}^{-2}$  (95% confidence  
418 interval range 87 to  $153 \text{ g CO}_2\text{-C m}^{-2}$ ) during the month following the 2014 ploughing event.  
419 Data coverage for the ploughed SF during the month following the 2012 ploughing event was  
420 too sparse for the calculation of reliable cumulative fluxes. However, in light of Figure 6 it  
421 seems reasonable to assume that the net carbon loss in 2012 would be of similar magnitude as  
422 that observed in 2014 under similar meteorological conditions.

423

## 424 **Discussion**

425 Our results show that ploughing increased  $\text{N}_2\text{O}$  emissions, decreased ecosystem respiration,  
426 and had a mixed effect on  $\text{CH}_4$  fluxes. We can estimate the total impact of ploughing by  
427 adding the increase in  $\text{N}_2\text{O}$  emissions, accounting for their relative global warming potential,  
428 to the net release of carbon following ploughing. We assume the net effect on  $\text{CH}_4$  is small  
429 enough to be negligible. If  $\text{N}_2\text{O}$  emissions are increased by  $14\text{-}50 \mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$  over the  
430 month following ploughing, converting this to total mass of  $\text{N}_2\text{O}$  and  $\text{CO}_2$  equivalent units  
431 using a global warming potential of 298 (IPCC 2014), we obtain values of  $4\text{-}17 \text{ g CO}_2\text{-eq m}^{-2}$ .  
432 This is small compared to the  $440 \text{ g CO}_2 \text{ m}^{-2}$  released as  $\text{CO}_2$  in the month following  
433 ploughing, and gives a total of  $444 - 457 \text{ g CO}_2\text{-eq m}^{-2}$ . To put this into context, this  
434 represents 55% of the average harvest yield at this site when managed for hay or silage rather  
435 than grazing (Jones et al., submitted). Alternatively, the ploughing loss represents 7% of  
436 average GPP at the site. The purpose of ploughing is to increase sward productivity, so GPP  
437 would be expected to be larger in subsequent months and years. Whether the ploughing  
438 operation is GHG-neutral depends on the magnitude and duration of this longer-term effect  
439 on GPP, as this determines when/whether the increased carbon uptake offsets the short-term  
440 net source induced by the ploughing operation. This is difficult to discern without a longer  
441 term study.

442

443 The ploughing-induced increases in N<sub>2</sub>O emissions were rather different in 2012 and 2014, at  
444 14 and 50 µg N<sub>2</sub>O-N m<sup>-2</sup> h<sup>-1</sup>, respectively. Because we have accounted for the effects of  
445 temperature and soil moisture in the analysis, it is not likely that this is due to differences in  
446 weather conditions. The difference between years may be related to different N contents in  
447 the vegetation at the time of ploughing. The increase in N<sub>2</sub>O emissions following ploughing  
448 is most likely due to increases in nitrogen inputs from mineralisation of the organic N in plant  
449 litter. In 2014, the ploughing took place six weeks after a fertilisation event, so the N stock in  
450 the vegetation was presumably higher than in 2012, when the field had not been fertilised that  
451 year. However, the difference in N<sub>2</sub>O emission between ploughing events is not clearly  
452 reflected in the measured ammonium and nitrate concentrations (Table 2). Similar short lived  
453 N<sub>2</sub>O emissions after tillage events on managed grassland were measured by other authors  
454 (Davies et al. 2001; Velthof et al. 2010; Merbold et al. 2014) and (Ball et al. 1997; Estavillo  
455 et al. 2002) linked these to increases in soil NO<sub>3</sub><sup>-</sup> concentrations, following mineralisation of  
456 the organic N in plant litter. An analysis of 39 studies in Europe concluded that incorporation  
457 of crop residue into the soil by ploughing resulted in a 6 fold increase in soil respiration rates  
458 and 12 fold increase in N<sub>2</sub>O emissions (Lethinen et al., 2014). The IPCC default inventory  
459 methodology for incorporation of crop residue (De Klein 2013) would predict an N<sub>2</sub>O  
460 emission of around 50 µg N<sub>2</sub>O-N m<sup>-2</sup> h<sup>-1</sup> for our site, based on estimates of biomass and plant  
461 N content from Jones et al (submitted), a shoot:root ratio of 1.5, using the 1% default  
462 emission factor, and assuming this were emitted over a month. This is very close to our  
463 higher value, obtained in 2014. The average fertiliser-induced N<sub>2</sub>O emission over the 3 weeks  
464 after fertilisation for the whole study period ranged from 0.29% to 2.94%.

465

466 Mineral agricultural soils tend to be only small sources and sinks for CH<sub>4</sub>, unless irrigated.  
467 This is also the case for the Easter Bush fields, for which the average annual CH<sub>4</sub> fluxes were  
468 3.4 µg CH<sub>4</sub>-C m<sup>-2</sup> h<sup>-1</sup> for the period 2007 – 2010 (Skiba et al, 2013). On the ploughed field  
469 an additional CH<sub>4</sub> source was the decomposition of the ploughed under grass turf, which  
470 provided the labile carbon compounds and anaerobicity required for methanogenesis, and  
471 possibly was responsible for the slightly larger CH<sub>4</sub> emissions (Figure 4b). In 2014, CH<sub>4</sub>  
472 emissions were much larger from the ploughed NF, than the unploughed SF (Figure 4b). It is  
473 likely that under these warmer conditions, the main CH<sub>4</sub> source was the decomposition of the  
474 grass turf (Yamulki and Jarvis 2002).

475

476 A number of studies reported no conclusive evidence of tillage impacting soil microbial  
477 respiration rates in the long term (Yamulki and Jarvis, 2002, Jones et al. 2005, Ball et al,  
478 1999). Our observations show a small but consistent decrease in ecosystem respiration rate  
479 following ploughing. However, it is important to make the distinction between soil  
480 respiration rate and ecosystem respiration rate (ie. including the above-ground plants), as the  
481 system definitions are different. When comparing ecosystem respiration rate before and after  
482 ploughing, the total biomass is initially the same, except the plants are over-turned, mostly  
483 dead and no longer respiring. The ecosystem respiration rate will therefore generally  
484 decrease. When comparing soil respiration rate before and after ploughing, the total biomass  
485 is generally increased after ploughing, as the above-ground plant material is now  
486 incorporated in to the soil. The soil respiration rate will therefore generally decrease. The  
487 physical release of CO<sub>2</sub> trapped in soil air for several days immediately after the ploughing of  
488 grassland soils (Kessavalou et al. 1998) and arable soils (Reicosky 1997; Vinten et al. 2002,  
489 Omonde et al. 2007) only makes a small contribution to the overall CO<sub>2</sub> emissions.

490

491 Our estimated net release of  $4.0 \text{ g CO}_2\text{-C m}^{-2} \text{ d}^{-1}$  (95% confidence interval range 2.9 to 5.1 g  
492  $\text{CO}_2\text{-C m}^{-2} \text{ d}^{-1}$ ) following the 2014 ploughing event is consistent with other European studies  
493 (e.g. Merbold et al. (2014):  $2.8 \text{ g CO}_2\text{-C m}^{-2} \text{ d}^{-1}$  for a restored grassland in Switzerland;  
494 Willems et al. (2011):  $3.1 \pm 1.2 \text{ g CO}_2\text{-C m}^{-2} \text{ d}^{-1}$  for a grassland in Ireland). In contrast, the  
495 unploughed SF had a net flux of  $-0.9 \text{ g CO}_2\text{-C m}^{-2} \text{ d}^{-1}$  (95% confidence interval range -2.7 to  
496  $0.9 \text{ g CO}_2\text{-C m}^{-2} \text{ d}^{-1}$ ) for the same 2014 time period.

497

498 The tillage management considerably changed the soil physical and chemical properties, broadly in  
499 the same manner on both fields in both years. Both tillage events, increased the bulk density in the top  
500 5 cm soil depth from  $0.77 \text{ g cm}^{-3}$  to  $1.22 \text{ g cm}^{-3}$  (Table 2). The ploughing induced increase in bulk  
501 density is caused by the mechanically disruption of stable soil aggregates and mixing lighter more  
502 organic top soil with heavier mineral soil from the deeper layers. After the soil is rolled, the newly  
503 arranged soil aggregates are compacted and porosity and conductivity between pores decrease in the  
504 upper top soil layer (Ball, 2013). The reduction of soil aggregation increases evaporation (Six et  
505 al., 1998) and explains our observed reduction in soil moisture content after ploughing from  
506 both fields (Figure 3b). Average volumetric soil moisture content from the ploughed fields in  
507 2012 (SF) and 2014 (NF) were 44% and 21% lower than from the unploughed fields.

508

509 Mineralisation rates are also favoured by the physical turnover of soil and break up of  
510 aggregates during ploughing by exposing new surfaces to the more oxygen rich atmosphere  
511 and by ploughing in the grass turf. Depending on the C/N ratio of the plant material,  
512 incorporation can either lead to immobilisation or mineralisation (Davis et al., 2001). At  
513 Easter Bush, the C/N ratios in the top 10 cm of the soil did not change significantly over the 6  
514 week period, 1 week before to 5 weeks after tillage (Table 2). We observed a 10 and 5 fold  
515 increase in top soil (0 -10 cm)  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentrations in the first 5 weeks after  
516 ploughing in 2012 from the ploughed SF, but also a 7 and 5 fold increase in  $\text{NH}_4^+$  and  $\text{NO}_3^-$

517 concentrations from the unploughed NF. This implies that the raised concentrations are a  
518 result of several factors; weather and ploughing on the SF and climate and excreta and urine  
519 from the sheep grazed NF. The reason for the much larger  $\text{NH}_4^+$  concentrations before  
520 ploughing in 2014 compared to 2012 are not obvious. In 2014 ploughing resulted in a  
521 significant decrease of  $\text{NH}_4^+$  and increase of  $\text{NO}_3^-$  concentrations, presumably caused by  
522 nitrification (Table 2). With hindsight, total C and N and  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentrations  
523 should have been measured for the entire plough depth (30 cm). The mixing of the soil layers  
524 and incorporation of the turf to the deeper layers will have created hotspots of  
525 mineralisation/immobilisation, which we could not account for by the 0-10 cm soil analysis.

526

## 527 **Conclusions**

528 Ploughing significantly increased fluxes of  $\text{N}_2\text{O}$ , reduced ecosystem respiration rate, and had  
529 a variable effect on  $\text{CH}_4$  fluxes. The effect on  $\text{N}_2\text{O}$  is small, but distinguishable from the  
530 effects of N fertilisation, soil temperature and soil moisture. Tillage-induced  $\text{N}_2\text{O}$  fluxes were  
531 close to expectations based on the IPCC default methodology. By far the dominant effect on  
532 the GHG balance was the temporary reduction in GPP.

533

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647

648

649

650 **Figure Captions**

651 Figure 1: Satellite image (Google Earth; imagery date July 2012) showing the outline of the  
652 South field (SF) and the North field (NF), and the locations of the three eddy-covariance  
653 systems used during the two ploughing events of 2012 and 2014. The long-term, fixed eddy-  
654 covariance system (“EC fenceline”) is located along the fence which separates the two fields.  
655 A temporary eddy-covariance system was deployed in the SF (“EC (May – August 2012)”)   
656 during the spring and summer of 2012 to monitor pre- and post-ploughing fluxes within the  
657 ploughed field. A different system (see materials and methods section for details) was  
658 deployed in the NF (“EC (May-August 2014)”) during the spring and summer of 2014.  
659 Overlain onto the satellite image are median values of  $x_{\max}$  (red line),  $x_{50}$  (green line) and  $x_{70}$   
660 (purple line) (distance in meters from the EC mast where peak, 50% and 70% of the  
661 measured fluxes originated, respectively) for spring and summer 2012 as in this instance  
662 fluxes from the same tower could come from either field and plotted per 10 deg wind  
663 direction bins. These footprint statistics were obtained with the analytical Kormann-Meixner  
664 footprint model for non-neutral stratification (Kormann and Meixner, 2001).

665  
666 Figure 2: Average daily air temperature (°C) (a, b) and daily rainfall (mm) (c, d) in 2012 (left,  
667 a & c) and 2014 (right, b & d). Ploughing was on the 1<sup>st</sup> May in 2012 and the 20<sup>th</sup> May in  
668 2014 indicated by the dashed vertical red line.

669  
670 Figure 3: a) Soil Temperature (°C) and b) Volumetric Soil Moisture (%) in 2012 (left panel)  
671 and 2014 (right panel) for North Field (NF) and South Field (SF), respectively.  
672 Measurements after ploughing in red and unploughed in blue. Fertilisation events indicated  
673 by blue horizontal line and ploughing by red horizontal line. To aid visualisation a smooth  
674 line was fitted through the data points.

675

676 Figure 4: Log fluxes of N<sub>2</sub>O (a), CH<sub>4</sub> (b) [ $\mu\text{g m}^{-2} \text{h}^{-1}$ ] and CO<sub>2</sub> (c) [ $\text{mg m}^{-2} \text{h}^{-1}$ ] in 2012 (left  
677 panel) and 2014 (right panel) for North Field (NF) and South Field (SF), respectively.  
678 Measurements after ploughing in red and unploughed in blue. Fertilisation events indicated  
679 by blue horizontal line and ploughing by red horizontal line. A simple exponential decay after  
680 fertilisation fitted as blue line through log N<sub>2</sub>O fluxes to indicate fertilisation induced  
681 predicted flux.

682

683 Figure 5: Soil CO<sub>2</sub> respiration rates on the day of ploughing. The bars represent average  
684 values from 4 measurement positions, the error bars are standard deviation. Time is the  
685 period in minutes after the plough passed the 4 plots on 5 repeated occasions.

686

687 Figure 6: Day time and night time fluxes of carbon dioxide (CO<sub>2</sub>) measured by an eddy-  
688 covariance system installed along the fence line separating the north field (NF) and the south  
689 field (SF); (a)-(b) 2012 fluxes and (c)-(d) 2014 fluxes.

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