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2 **Flooding-induced N<sub>2</sub>O emission bursts controlled by**  
3 **pH and nitrate in agricultural soils**

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20 *(DNRA), agriculture, microsensor, stabile isotope*

21

## 22 **Abstract**

23 Agricultural soils are a major source of the greenhouse gas nitrous oxide ( $N_2O$ ) to the  
24 atmosphere. Increasing frequency and severity of flooding as predicted for large intensively  
25 cropped areas may promote temporary denitrification and  $N_2O$  production but the effect of  
26 flooding events on  $N_2O$  emissions is poorly studied for agricultural systems. The overall  $N_2O$   
27 dynamics during flooding of an agricultural soil and the effect of pH and  $NO_3^-$  concentration  
28 has been investigated based on a combination of the use of microsensors, stable isotope  
29 techniques, KCl extractions and modelling. This study shows that non-steady state peak  $N_2O$   
30 emission events during flooding might potentially be at least in the order of reported annual  
31 mean  $N_2O$  emissions, which typically do not include flood induced  $N_2O$  emissions, and that  
32 more than one-third of the produced  $N_2O$  in the soil is not emitted but consumed within the  
33 soil. The magnitude of the emissions are, not surprisingly, positively correlated with the soil  
34  $NO_3^-$  concentration but also negatively correlated with liming (neutral pH). The redox  
35 potential of the soil is found to influence  $N_2O$  accumulation as the production and  
36 consumption of  $N_2O$  occurs in narrow redox windows where the redox range levels are  
37 negatively correlated with the pH. This study highlights the potential importance of  $N_2O$   
38 bursts associated with flooding and infers that annual  $N_2O$  emission estimates for tilled  
39 agricultural soils that are temporarily flooded will be underestimated. Furthermore, this study  
40 shows that subsurface  $N_2O$  reduction is a key process limiting  $N_2O$  emission and that a  
41 reduction in  $N_2O$  emissions is achievable if highly fertilized N-rich soils are limed.

## 42 **1. Introduction**

43 Future climate change will lead to changes in precipitation worldwide. A higher frequency of  
44 extreme rainfall events is predicted for temperate areas such as New Zealand and Northern  
45 Europe (IPCC, 2007; Min et al., 2011). This increases the risk of flooding for low-lying or  
46 poorly drained areas, which are the same areas receiving run off and ground water with  
47 potentially high nitrate ( $\text{NO}_3^-$ ) concentrations. As a consequence it can be expected that there  
48 will be an increase in nitrous oxide ( $\text{N}_2\text{O}$ ) production and emissions from these areas,  
49 particularly fertilized and nitrogen-rich agricultural fields (Knowles, 1982).

50 Nitrous oxide is a greenhouse gas with a global warming potential relative to  $\text{CO}_2$  of 298 on  
51 a 100 year time horizon assuming a lifetime of 114 years in the atmosphere (IPCC, 2007).  
52 Additionally,  $\text{N}_2\text{O}$  has a negative effect on stratospheric ozone as NO and other free radical  
53 species ( $\text{NO}_x$ ), generated from  $\text{N}_2\text{O}$ , deplete the ozone layer (Badr & Probert, 1993). The  
54 atmospheric concentration of  $\text{N}_2\text{O}$  has increased since pre-industrial times by 16% from 270  
55 ppb to 319 ppb in 2005 (IPCC, 2007) and it is currently considered the dominant  
56 anthropogenic ozone depleting substance emitted (Ravishanakara et al., 2009). Soils are the  
57 main source of both anthropogenically and naturally produced  $\text{N}_2\text{O}$  and changes in land use  
58 have been the primary driver for the observed increase in tropospheric  $\text{N}_2\text{O}$  concentration  
59 (IPCC, 2007). Today, agricultural fields account for 42% of the total anthropogenic  
60 contribution of  $\text{N}_2\text{O}$  to the atmosphere and  $\text{N}_2\text{O}$  is the single most important greenhouse gas  
61 when looking at agricultural soils (IPCC, 2007).

62 In oxygen ( $\text{O}_2$ ) limited environments production of  $\text{N}_2\text{O}$  in soil occurs as microbial  
63 processes utilize nitrogenous compounds as electron acceptors (Knowles, 1982). During  
64 denitrification  $\text{N}_2\text{O}$  is an obligate intermediary product in the reduction of  $\text{NO}_3^-$  to  $\text{N}_2$ , a

65 process performed by heterotrophic microorganisms. It is also a by-product during  
66 dissimilatory  $\text{NO}_3^-$  reduction (DNRA) to ammonium ( $\text{NH}_4^+$ ) as  $\text{NO}_3^-$  is reduced to  $\text{NH}_4^+$  via  
67 nitrite ( $\text{NO}_2^-$ ) by fermentative microorganisms (Tiedje et al., 1982). Denitrification rates  
68 increase with organic C and  $\text{NO}_3^-$  availability, soil water content, pH and temperature  
69 (Knowles, 1982; Šimek and Cooper, 2002). The  $\text{N}_2\text{O}:\text{N}_2$  ratio, describing the end product of  
70 denitrification, shifts in favour of  $\text{N}_2\text{O}$  as soil  $\text{NO}_3^-$  concentrations and acidity increase  
71 (Knowles, 1982; Weier et al., 1993). Not all  $\text{N}_2\text{O}$  produced in a soil will be emitted as it can  
72 be consumed during denitrification to  $\text{N}_2$  a process controlled by the presence of  $\text{N}_2\text{O}$   
73 reductase (NOS) (Knowles, 1982). Highly anoxic conditions, caused by high soil water  
74 content and high availability of easily degradable organic matter, favour the consumption of  
75  $\text{N}_2\text{O}$  (Wrage et al., 2001) as NOS is strongly inhibited by the presence of  $\text{O}_2$  (Knowles, 1982).  
76 Thus the balance between  $\text{N}_2\text{O}$  consumption and production rates controls  $\text{N}_2\text{O}$  emissions as  
77 well as the transport properties of  $\text{N}_2\text{O}$  in the soil (Clough et al., 2005). The primary mode of  
78 transport for  $\text{N}_2\text{O}$  in the soil is diffusion, which is controlled by concentration gradients  
79 according to Fick's law of diffusion.

80 The environmental factors for production of  $\text{N}_2\text{O}$  are optimal when fertilized fields are  
81 flooded. Non-steady state draining experiments have established the relationship between  
82 water-filled pore space and  $\text{N}_2\text{O}$  emissions (Castellano et al., 2010), however, to the authors  
83 knowledge, no studies on agricultural soils and only a few studies on natural soils have  
84 examined the effect of soil flooding on  $\text{N}_2\text{O}$  dynamics: the temporal and spatial trends of  
85 subsurface  $\text{N}_2\text{O}$  concentrations and net surface emissions. Jørgensen and Elberling (2012)  
86 found a distinct pulse pattern in  $\text{N}_2\text{O}$  concentrations and emissions when flooding an un-  
87 managed wetland peat soil. An increase in  $\text{N}_2\text{O}$  concentrations was observed within the first  
88 24 hrs followed by a rapid decline in concentration, until the  $\text{N}_2\text{O}$  concentration was below

89 detection after 40 hrs. It was concluded that for these wetland peat soils the increase in N<sub>2</sub>O  
90 production would not affect the annual N<sub>2</sub>O emission budget, even if flooding event  
91 frequency increases in the future (Jørgensen and Elberling, 2012). This may not be the case  
92 for agricultural fields, where tillage events can increase the availability of NO<sub>3</sub><sup>-</sup>-N (Eriksen  
93 and Jensen, 2001; Silgram and Shepherd, 1999) and thereby the potential for N<sub>2</sub>O production  
94 via denitrification.

95 The aim of this study was to investigate the overall N<sub>2</sub>O dynamics during a flooding event  
96 of a New Zealand agricultural soil as affected by soil pH and NO<sub>3</sub><sup>-</sup> concentration. Specific  
97 aims of the study were to determine the balance between produced, consumed and emitted  
98 N<sub>2</sub>O from the soil and to determine the depth- and time-specific production and consumption  
99 of N<sub>2</sub>O. Two methods were used in combination to achieve the aims: depth-specific profiling  
100 of the soil N<sub>2</sub>O concentration and the redox potential using microsensors as well as 2 M KCl  
101 extractions of 3 soil layers per soil core after <sup>15</sup>N labelled NO<sub>3</sub><sup>-</sup> addition. The study was  
102 designed based on the hypothesis that it is possible to mitigate N<sub>2</sub>O emissions by changes in  
103 agricultural practises (with a focus on changes in soil pH and N-input) and that annual N<sub>2</sub>O  
104 inventories made to date have potentially been underestimated because the impact of flooding  
105 has not been included in annual budgets.

## 106 **2. Materials and methods**

107 A Templeton silt loam soil (Udic Ustochrept) was collected from a field, with a management  
108 history of perennial pasture, from the top layer (0-10 cm deep) during cultivation for pasture  
109 renovation, Lincoln, Canterbury (43° 38.720S; 172° 26.753E Lat/Lon). The Canterbury  
110 region is temperate with mean annual precipitation of 600-700 mm and a daily mean air  
111 temperature range of 1-10°C in the coldest months and 12-22°C in the warmest (Cappelen and  
112 Jensen, 2001). The Templeton soil and similar inceptisols represents app. 25% of the  
113 Canterbury Plains (Molloy, 1988). Inceptisols in temperate areas are soils with high inputs of  
114 fertilizer N (Potter et al., 2010) with crop types typically consisting of cereals such as barley  
115 and wheat (Leff et al., 2004).

116 The sampled soil was air-dried, sieved (< 2 mm) and kept dry and cold (4°C). The soil pH  
117 was determined (10 g air-dried soil:25 mL water). Half of the soil was treated with 2.08 g  
118 Ca(OH)<sub>2</sub> kg<sup>-1</sup> dry soil (quicklime) in powder form to increase the pH by one unit. Lime  
119 treatment and the resulting pH increase were made consistently with previous experiments  
120 (Clough et al., 2003). Inorganic-N and dissolved organic carbon (DOC) were determined for  
121 both the un-treated and the limed soil. Inorganic-N was determined in a 2 M KCl extraction (4  
122 g soil:70 mL KCl, shaken on an end-over-end shaker for 60 min and filtered through  
123 Whatman 42 filter paper. Filtered samples were analysed using an Alpkem FS3000 twin  
124 channel flow injection analyser (FIA) with Alpkem Winflow 4.03 software). The DOC was  
125 analysed by a DI water extraction (1:10 soil:water ratio), shaken on an end-over-end shaker  
126 for 30 min followed by centrifuging at 3500 rev/min for 20 min and filtered through a  
127 Whatman 42 filter paper into a 30 mL sample vial (Ghani et al., 2003). The DOC was  
128 determined based on the difference between the total organic carbon (TOC) and the inorganic

129 carbon (IC) analysed using a Shimadzu Total Organic Carbon Analyser (TOC-5000A) fitted  
130 with a Shimadzu ASI-5000A autosampler.

### 131 *2.1 Core preparations*

132 Soil was packed into either stainless steel metal cores ( $D = 7.4$  cm) for microsensor  
133 measurements or PVC plastic cores ( $D = 7.5$  cm) for KCl extractions (see below). Soil core  
134 bases were covered with a 1 mm nylon mesh and packed to a depth of 3.5 cm. The soil was  
135 packed in layers to ensure an even bulk density of  $1 \text{ g cm}^{-3}$  throughout the profile. Four  
136 treatments were made: control (soil with no additions, **TC**), limed soil (soil plus lime, **TL**),  
137 soil with N added (soil plus nitrate- $^{15}\text{N}$ , **TN**), and soil with N and lime added (soil plus  
138 nitrate- $^{15}\text{N}$  and lime, **TLN**). For treatment TN and TLN a known volume of  $^{15}\text{N}$  enriched (50  
139 atom%)  $\text{KNO}_3$  solution (0.0154 M) was sprayed onto a designated mass of dry soil prior to  
140 packing the soil cores, supplying  $100 \mu\text{g NO}_3\text{-N g}^{-1}$  soil. Since  $\text{NO}_3^-$  is evenly distributed in  
141 the surface of a cultivated soil, the  $^{15}\text{N-NO}_3^-$  was applied to the entire depth of soil in the  
142 packed soil core. Soil cores were packed and adjusted with  $\text{KNO}_3$  immediately before  
143 flooding. The soil cores were then flooded from below immediately prior to commencement  
144 of microsensor measurements, to mimic the rise of a high groundwater table, by placing them  
145 in a water-filled box. This method of soil flooding also minimised soil drainage during  
146 wetting. In total 108 cores were made, of which 12 were used for microsensor measurements  
147 and 96 for KCl extractions. KCl extractions were performed on three replicates at eight time  
148 steps for each treatment. The timing of the KCl extraction was distributed throughout the  
149 pulse of  $\text{N}_2\text{O}$  production (see supporting information (SI) (Fig. S1)). Due to measurement  
150 constraints of the microsensor, replication was done in time by sequentially measuring one  
151 soil core from one treatment at any given time. For each treatment a total of 3 replicates were  
152 measured at  $t_1$ ,  $t_2$  and  $t_3$ . In practice, two replicates of each treatment (at  $t_1$  and  $t_2$ ) were



153 measured after each other. All  $t_1$  and  $t_2$  measurements for all four treatments were finished  
154 within 50 days. All  $t_3$  measurements were subsequently measured after this period (see SI Fig.  
155 S2 for exact specifications of the timing of  $t_1$ ,  $t_2$  and  $t_3$  for all treatments). Each soil core was  
156 followed until no more  $N_2O$  evolved (up till 7 days) before the next soil core was measured,  
157 hence only one soil core for microsensor measurements was flooded at a time. Soil remained  
158 sieved but unpacked at  $4^\circ C$  and unamended with  $^{15}N$ , with these procedures performed prior  
159 to microsensor measurements starting.

## 160 *2.2 Microsensors*

161 A standard  $N_2O$  microsensor ( $N_2O$ -100, Unisense, Science Park, DK-8000 Aarhus,  
162 Denmark), a redox microsensor (RD-100, Unisense) and a redox reference electrode (REF-  
163 RM, Unisense) were used to measure the  $N_2O$  concentration and the redox potential from the  
164 soil:water interface, down through the soil profile at  $500 \mu m$  steps (71 points per profile), with  
165 movement controlled by a motorized micromanipulator. To ensure a complete mixing of the  
166 water phase above the soil, an ambient air flow was generated over the water surface to avoid  
167  $N_2O$  accumulation in the water overlying the soil core. To ensure the soil water remained at a  
168 constant level at all times, water was added several times daily to offset any evaporation,  
169 maintaining a 1 cm water depth above the soil surface. The output current for the  $N_2O$   
170 microsensor was measured using a Microsensor Multimeter while the redox signal was  
171 measured using a pH/mV-METER. The  $N_2O$  microsensor was calibrated with a five-point  
172 calibration using a saturated  $N_2O$  solution to make standard solutions increasing by  $100 \mu M$   
173 each step. Repeated calibration, after profiling, revealed that instrument drift was  
174 insignificant. Each soil core profile was measured every two hours for 7 days or until the  $N_2O$   
175 concentration was below the detection limit ( $< 0.1 \mu M$ ) at all depths. The room temperature  
176 during the microsensor measurements was in the range of  $21-23^\circ C$ . Based on the microsensor

177 measurements, contour maps of the subsurface N<sub>2</sub>O concentration and the redox potential  
178 were constructed using kriging interpolation (Surfer Version 9.785, Golden Software Inc.,  
179 Colorado, USA).

### 180 *2.2.1 Flux determination*

181 The observed flux of N<sub>2</sub>O from the flooded soil core was determined as the diffusive gas  
182 exchange across the diffusive boundary layer (DBL) according to Elberling and Damgaard  
183 (2001). The DBL is a thin film of water at the soil:water interface (often < 2 mm) where the  
184 only form of transport is molecular diffusion (Gundersen and Jørgensen, 1990). The linear  
185 concentration gradient over the DBL was used to determine the N<sub>2</sub>O flux across the DBL  
186 using Fick's law (Clough et al., 2005). The diffusion coefficient for N<sub>2</sub>O in water at 20°C was  
187 taken to be  $2.2295 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$  (Ramsing and Gundersen, 2009). Verification of the flux was  
188 made based on dark, closed chambers (INNOVA 1313, LumaSense, Inc., Ballerup,  
189 Denmark), where the observed flux was compared to chamber measurements performed  
190 immediately after the microsensor measurements (see SI Fig. S3).

### 191 *2.2.2 Modelling N<sub>2</sub>O production and consumption*

192 Assuming the N<sub>2</sub>O concentration profile represents a pseudo-steady state, the SensorTrace  
193 PRO 3.0 programme (Unisense A/S, Science Park, DK-8000 Aarhus, Denmark) was used to  
194 model the depth-specific N<sub>2</sub>O production and consumption zones. The programme is based on  
195 the model PROFIL by Berg et al. (1998). Here the effective gas diffusion coefficient through  
196 the soil is described as a function of the water-filled pore space, the soil porosity and the  
197 associated gas diffusion coefficient through air (Berg et al., 1998).

### 198 *2.3 KCl extractions*

199 Based on the preliminary results of the maximum N<sub>2</sub>O concentrations in the soil core,  
200 measured using the microsensors, 8 time steps for the KCl extractions were chosen for each  
201 treatment distributed over time: One extraction was made at time zero (and subsequently used  
202 as a reference for the initial conditions), three were made between time zero and maximum  
203 N<sub>2</sub>O concentration where a further extraction was made, and then two further extractions  
204 taken after the maximum N<sub>2</sub>O concentration and the final extraction was taken when N<sub>2</sub>O  
205 was no longer measurable (for details, see SI Fig. S1).

206 Prior to KCl extraction, the flooded cores were removed to drain for app. 10 minutes. A  
207 subsample of the soil, 10 g, from the top (0-1.2 cm) of the core was mixed with 60 mL 2 M  
208 KCl in a 107 mL glass bottle capped with an aluminium screw-top lined with a rubber septum  
209 leaving a headspace of 43 mL and vigorously shaken (30 seconds). Gas samples (8 mL) were  
210 then collected from the headspace, using a gas-tight syringe fitted with a stop-cock to prevent  
211 under-pressurisation of the sample, and stored in a pre-evacuated 6 mL Exetainer®. For all  
212 <sup>15</sup>N-NO<sub>3</sub><sup>-</sup> treated samples an additional 16 mL gas sample was collected in a similar manner  
213 and placed in a pre-evacuated 12 mL Exetainer®. The soil/KCl solution was then shaken for 1  
214 hour on an end-over-end shaker, left to settle for 5 minutes and filtered through Whatman 42  
215 filter paper into a 30 mL sample vial and frozen (-20°C) until analysis. The KCl extraction  
216 was repeated with a soil sample from the middle of the soil core (1.2-2.3 cm) and from the  
217 bottom (2.3-3.5 cm). The remainder of the soil at each depth was weighed and dried to  
218 determine the gravimetric water content.

219 Gas samples in the 6 mL Exetainers were analysed for methane (CH<sub>4</sub>) and N<sub>2</sub>O using a SRI  
220 8610C gas chromatograph (SRI, Ca. USA) linked to a Gilson 222XL autosampler. CH<sub>4</sub> was  
221 determined using a flame ionisation detector (FID) while N<sub>2</sub>O was determined with an  
222 electron capture detector (ECD) calibrated with certified gas standards (BOC gases) that

223 covered the range of sample concentrations (Mosier and Mack, 1980). The gas samples in the  
224 12 mL Exetainers were analysed for  $^{15}\text{N-N}_2\text{O}$  and  $^{15}\text{N-N}_2$  using a continuous flow isotope  
225 ratio mass spectrometer (IRMS) on a Sercon 20-20 IRMS with  $^{15}\text{N}$  calculations performed  
226 according to the equations of Mulvaney and Boast (1986) and Stevens et al. (1993). When  
227 calculating the release of  $^{15}\text{N}$  labelled gas entrapped in the soil, corrections were made to  
228 allow for headspace gas dissolved in the KCl solution using the appropriate Bunsen  
229 coefficient (Moraghan and Buresh, 1977). Derived  $^{15}\text{N}$  data permitted the transformation and  
230 redistribution of the original  $^{15}\text{NO}_3^-$  to be observed across inorganic-N and gaseous N species  
231 at the times KCl extractions were performed.

232 Soil KCl extracts were thawed at room temperature and analysed for  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N  
233 concentrations using an Alpkem FS3000 twin channel flow injection analyser (FIA) with  
234 Alpkem Winflow 4.03 software. Following the method of Brooks et al. (1989) sub-samples of  
235 the KCl extracts were diffused and analysed to determine their  $\text{NH}_4^+$ - $^{15}\text{N}$  and  $\text{NO}_3^-$ - $^{15}\text{N}$   
236 enrichments.

#### 237 ***2.4 Statistical analyses***

238 Statistical analyses of Pearson product-moment correlation, F-test and one-way ANOVA  
239 analyses were performed using the SPSS statistical package (v. 19.0), with a significance  
240 level of at least 95 % (\*P < 0.05). For KCl extractions three replicates were analysed (n = 3)  
241 while for microsensor  $\text{N}_2\text{O}$  determinations two replicates were analysed (n= 2),  $t_1$  and  $t_2$ .

## 242 **3. Results**

243 The initial values of the soil pH,  $\text{NO}_3^-$  concentration and DOC concentration as well as the  
244 effect of liming on the parameters can be seen in Table 1.

### 245 *3.1 N<sub>2</sub>O dynamics*

246 For all microsensor measurements the repeated measurements at  $t_1$  and  $t_2$  can be seen as  
247 replicates because of the low variation (see SI Fig. S4) whereas  $t_3$  differs markedly. For all  
248 treatments, the maximum  $\text{N}_2\text{O}$  concentrations were lower and peaked in half the time  
249 compared with  $t_1$  and  $t_2$ , and at much lower levels. In the following, results will be based on  $t_1$   
250 and  $t_2$ , (see SI Fig. S4 for comments on  $t_3$ ).

251 Regardless of treatment the  $\text{N}_2\text{O}$  concentrations initially increased and then decreased over  
252 time but with differences in the timing of the maximum concentration (26-34 hrs) and the  
253 maximum magnitude of the  $\text{N}_2\text{O}$  concentration (Fig. 1 and SI Fig. S5). There was a  
254 significant correlation between the observed  $\text{N}_2\text{O}$  diffusion flux and the maximum  $\text{N}_2\text{O}$   
255 concentration in the soil ( $r = 0.94$ ,  $n = 256$ ,  $***P < 0.0001$ ) (Fig. 1a). Both parameters had a  
256 skewed bell-shaped pattern with a steep increase followed by a steep decrease after which  
257 they levelled out app. 72 hrs after flooding. The highest  $\text{N}_2\text{O}$  concentrations occurred in  
258 treatment TN, followed by TL, TC and TLN. The maximum  $\text{N}_2\text{O}$  diffusion flux was  $2.5 \cdot 10^{-11}$ ,  
259  $3.8 \cdot 10^{-11}$ ,  $5.7 \cdot 10^{-11}$  and  $2.9 \cdot 10^{-11}$  mol  $\text{N}_2\text{O}$   $\text{cm}^{-2}$   $\text{s}^{-1}$  for treatments TC, TL, TN and TLN,  
260 respectively.  $\text{N}_2\text{O}$  fluxes obtained using microsensor measurements are in agreement with  
261 levels obtained by INNOVA chambers (SI Fig. S3). During the first 96 hrs of flooding, the  
262 mean ( $\pm$  stdev,  $n = 2$ ) integrated  $\text{N}_2\text{O}$  fluxes for treatments TC, TL, TN and TLN were  $29 \pm$   
263  $4.3$ ,  $41 \pm 0.9$ ,  $54 \pm 5.2$  and  $39 \pm 8.9$  mmol  $\text{N}_2\text{O}$   $\text{m}^{-2}$  respectively.

264 After the initial short lag phase of app. 5 hrs the N<sub>2</sub>O concentrations increased primarily in  
265 the zone 1-2 cm below the soil surface (Fig. 1b). Concentrations of N<sub>2</sub>O below 2 cm depth  
266 decreased faster than in shallower depths and reached zero within 24-50 hrs after flooding  
267 (Fig. 1b and SI Fig. S5). After the maximum N<sub>2</sub>O concentrations in the soil profile occurred,  
268 they then decreased with time in all depths still with the highest concentrations in the middle  
269 zone of the soil profile, until the N<sub>2</sub>O concentrations were zero at all depths.

270 The decrease in NO<sub>3</sub><sup>-</sup> concentrations correlated negatively with increasing N<sub>2</sub>O  
271 concentrations (Fig. 1d) within the first 24-36 hrs (three time steps) for all four treatments.  
272 Subsequently, the rate of NO<sub>3</sub><sup>-</sup> decrease reached a minimum over the last three time steps. For  
273 treatment TLN, the rate difference in NO<sub>3</sub><sup>-</sup> decrease was small between the first and the last  
274 three time steps and the NO<sub>3</sub><sup>-</sup> concentration did not reach zero during the experiment. Despite  
275 this, the N<sub>2</sub>O concentrations were below detection for treatment TLN after 96 hrs.

276 As a consequence of flooding, the soil redox potential decreased over time with the rate of  
277 decrease increasing with increasing soil depth (Fig. 1c and SI Fig. S5). This depth driven  
278 change in redox potential happened primarily after the first 16 hrs of flooding. The primary  
279 redox range, where N<sub>2</sub>O accumulates in the soil, for each of the treatments can be seen in  
280 Table 1. High N<sub>2</sub>O concentrations were found in the same range for treatments TC, TN and  
281 TLN while the redox range for treatment TL was 100 mV lower. The N<sub>2</sub>O concentrations and  
282 the redox potentials were significantly (\*P < 0.05) correlated (Table 1), as the N<sub>2</sub>O  
283 concentrations portray a bell-shaped curve over time with a decreasing redox potential where  
284 a 'build-up' and a 'build-down' phase are divided around the occurrence of the maximum  
285 N<sub>2</sub>O concentration (see SI Fig. S6).

### 286 ***3.2 N<sub>2</sub>O production and consumption***

287 The depth-specific consumption and production rates over time were modelled using the  
288 SensorTrace PRO 3.0 programme for each treatment, while assuming that the porosity and the  
289 effective diffusion coefficient were uniform throughout the packed soil core. The diffusion  
290 flux was simultaneously determined for each profile. In Fig. 2, 6 time intervals of the depth-  
291 specific activity are shown for treatments TC and TN. The corresponding figure for TL and  
292 TLN can be seen in SI (Fig. S7). The production (positive activity rates) of N<sub>2</sub>O commenced  
293 after 10 hrs, primarily right below the DBL. The activity was several orders of magnitude  
294 larger for treatment TN compared to TC. After 20 hrs of flooding, N<sub>2</sub>O was produced in the  
295 near-surface zone (2 cm) of the soil while consumption of N<sub>2</sub>O (negative activity rates)  
296 increased below this depth. Thirty hrs after flooding, high production and maximum  
297 consumption rates were found, with consumption in the top (< 0.5 cm) and below 1.5 cm and  
298 production in a 1 cm zone from 0.5 to 1.5 cm below the surface. This pattern remained  
299 throughout the experiment, with rates of production and consumption decreasing with time.  
300 The model was successfully validated as a significant correlation, with a slope of 0.9, was  
301 found between the observed flux and the modelled flux ( $r = 0.77$ ,  $n = 754$ ,  $***P < 0.0001$ ).

302 Based on the time-integrated modelled diffusion flux and the time and depth-integrated N<sub>2</sub>O  
303 production, more than one-third of N<sub>2</sub>O produced in the soil was consumed within the soil  
304 and not released. Consumption accounted for  $41 \pm 6.9$ ,  $34 \pm 3.7$ ,  $51 \pm 0.4$  and  $48 \pm 10.9\%$  of  
305 N<sub>2</sub>O produced for treatments TC, TL, TN and TLN, respectively.

### 306 **3.3 <sup>15</sup>N recovery**

307 Percentage recovery of <sup>15</sup>N as NO<sub>3</sub><sup>-</sup>, N<sub>2</sub>O, N<sub>2</sub> and NH<sub>4</sub><sup>+</sup> over time for treatments TN and TLN  
308 can be seen in Fig. 3. The atom% <sup>15</sup>N enrichment for N<sub>2</sub>O, NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> as well as <sup>15</sup>X<sub>N</sub> for  
309 N<sub>2</sub> (the mole fraction of <sup>15</sup>N in the N pool from which the N<sub>2</sub> was derived) are shown in SI

310 (Fig. S8). At time 0 all added  $^{15}\text{N}$  was present as  $\text{NO}_3^-$ . Over time recovery of  $^{15}\text{N}$  in the  $\text{NO}_3^-$ -  
311  $^{15}\text{N}$  labelled pool is reduced while the other components increase.  $^{15}\text{N}$  recovered as  $\text{N}_2\text{O}$   
312 increased for the first 6 time steps (38 and 48 hrs after flooding for treatments TN and TLN  
313 respectively) after which it decreased to zero at 96 and 144 hrs after flooding.  $^{15}\text{N}$  recovered  
314 as  $\text{NH}_4^+$  increased steadily over the entire flooding period and ended up constituting 1.0 and  
315 0.6% of the  $^{15}\text{N}$  initially added for treatments TN and TLN, respectively.  $^{15}\text{N}$  recovered as  $\text{N}_2$   
316 also increased over time. For treatment TN the increase was slow for the first 38 hrs,  
317 subsequently it increased rapidly to  $115 \pm 12\%$   $^{15}\text{N}$  recovered at 72 hrs and levels out at  $108 \pm$   
318  $13\%$  after 96 hrs within the same range of the standard deviation. For treatment TLN the  $^{15}\text{N}$   
319 recovery of  $\text{N}_2$  was close to zero within the first 72 hrs. After 144 hrs of flooding  $^{15}\text{N-N}_2$   
320 constituted 26% of the initially added  $^{15}\text{N}$  label. The recovered  $^{15}\text{N}_2$  is displayed without  
321 standard deviations as less replicates are available as some fluxes were too low to be detected  
322 with only one replicate available in some cases. This is primarily the case for treatment TLN,  
323 and results should only be seen as best estimates.



## 324 **4. Discussion**

325 The soil profile N<sub>2</sub>O concentrations measured using the microsensor are in the same range as  
326 the methodologically comparable study by Jørgensen and Elberling (2012), as the maximum  
327 N<sub>2</sub>O concentration for TC is a factor 1.5 higher and the maximum N<sub>2</sub>O diffusion flux is a  
328 factor of 4 higher than their measurements on a flooded temperate peat soil. However, the  
329 duration of the high N<sub>2</sub>O emissions is longer when compared to other studies, resulting in an  
330 accumulated N<sub>2</sub>O release (29-54 mmol N<sub>2</sub>O m<sup>-2</sup> for the four treatments) that is at least a factor  
331 of 10 higher: For the study by Jørgensen and Elberling (2012), the duration was 40 hrs,  
332 resulting in an accumulated N<sub>2</sub>O release of 0.06 mmol N<sub>2</sub>O m<sup>-2</sup>, while an experimentally  
333 flooded natural tropical wetland soil resulted in peak emissions of 2.92 and 3.7 mmol N<sub>2</sub>O m<sup>-2</sup>  
334 for a 2.3 and 3 day peak duration (Liengaard et al., 2013).

335 There is a lack of studies examining peak N<sub>2</sub>O emissions during flooding events from  
336 temperate agricultural fields. Choudhary et al. (2001) found the annual N<sub>2</sub>O emission for a  
337 conventionally grown maize field in New Zealand to be 8.5-12.2 mmol N<sub>2</sub>O m<sup>-2</sup> yr<sup>-1</sup> while  
338 Roelandt et al. (2005), summarizing the data from 30 studies recording the annual N<sub>2</sub>O  
339 emissions from croplands and grasslands in North America and Europe, found emissions  
340 varied from 0.7 to 20.7 mmol N<sub>2</sub>O m<sup>-2</sup> yr<sup>-1</sup>. These reported values are of the same order or  
341 smaller than the peak emissions found in this study. Thus, the non-steady state emissions,  
342 over just a single four day flooding period, reported here, can potentially contribute more N<sub>2</sub>O  
343 to the atmosphere than the annual emissions of N<sub>2</sub>O on croplands and grasslands. This  
344 emphasizes the importance of incorporating flooding events in studies of annual N<sub>2</sub>O  
345 emissions and the need for further in-situ measurement of N<sub>2</sub>O fluxes during flood events.

### 346 ***4.1 Treatment effects***

347 During flooding redox conditions and N<sub>2</sub>O concentrations were markedly affected. The pH of  
348 the soil is also likely to have increased in all treatments following flooding due to reduction  
349 processes such as Fe and Mn oxide reduction (Yu and Patrick, 2003) and denitrification  
350 (Zárate-Valdez et al., 2006). Thus pH effects on N<sub>2</sub>O emission are therefore a consequence of  
351 both the direct effect of liming and the indirect effects of redox processes.

352 The redox potential in the soil was a time- and depth-specific parameter during flooding  
353 (Fig. 1c). The fact that the reduction was faster in the bottom part of the soil relative to the top  
354 is in line with the fact that reduction in the top will be counter-balanced by diffusion of  
355 atmospheric O<sub>2</sub> into the soil core. A lower redox potential at a higher pH is in agreement with  
356 the negative correlation between the two parameters described by Yu & Patrick (2003). The  
357 range in values of these parameters is likewise in agreement with their findings.

358 During flooding, the development of the subsurface N<sub>2</sub>O concentration over time portrays  
359 the same bell-shaped profile and the same depth-specific distribution, independent of  
360 treatment, also described by Liengaard et al. (2013). Higher NO<sub>3</sub><sup>-</sup> concentrations resulted in  
361 higher N<sub>2</sub>O concentrations and emissions, except in treatment TLN. The reduced N<sub>2</sub>O  
362 production due to higher soil pH was significant when comparing TN and TLN, as expected  
363 (Šimek & Cooper 2002), whereas this effect was not seen between TC and TL.

364 The unambiguous depth-specific distribution of the N<sub>2</sub>O concentration can be explained by  
365 the correlation between N<sub>2</sub>O concentration and redox potential based on the distinct redox  
366 ranges for N<sub>2</sub>O accumulation and reduction. The low N<sub>2</sub>O concentration in the top soil can be  
367 explained by the combination of redox potentials being too high for N<sub>2</sub>O accumulation and by  
368 the diffusion of N<sub>2</sub>O to the atmosphere. The level of reduction in the middle part of the soil  
369 displayed the optimum redox potential where the denitrification process is promoted, but not

370 to an extent where  $N_2O$  was rapidly reduced to  $N_2$ . The low  $N_2O$  concentrations found in the  
371 bottom part of the soil was either due to the fact that the low redox potentials favoured  
372 complete denitrification, not allowing  $N_2O$  to accumulate, or that the denitrification process  
373 was completely inhibited, allowing  $NO_3^-$  to be preserved (as seen for treatment TLN), where  
374 any measured  $N_2O$  was a result of diffusion of  $N_2O$  produced in the middle zone. Subsurface  
375 accumulation of  $N_2O$  and resulting emissions are therefore time-dependent as the redox  
376 potential of flooded soils will continuously decrease.

377 The depth-specific distribution of the  $N_2O$  concentrations and the finding that distinct zones  
378 of production and consumption of  $N_2O$  occur underlines the fact that spatial and temporal  
379 changes in denitrification rates are not a sequential process, but rather a consequence of micro  
380 zones of specific environmental conditions affecting the  $N_2O$  dynamics. More than one-third  
381 of the produced  $N_2O$  in the soil was consumed within the soil, with the highest ratios for  
382 treatments TN and TLN with high  $NO_3^-$  concentrations. The high  $N_2O$  production rate  
383 observed for treatment TN was counterbalanced by high consumption rates. The consumption  
384 fraction of  $N_2O$  is low when compared to the study by Liengaard et al. (2013) where about  
385 two-thirds of the produced  $N_2O$  was consumed within the soil. The balance between  
386 produced, consumed and emitted  $N_2O$  is therefore not a universal value, but a soil and  
387 environment dependent value.

388 The net effect of flooding, liming and addition of N was reduced  $N_2O$  emissions, as  
389 treatment TLN had the lowest emission during the time of study. The high  $NO_3^-$  concentration  
390 of treatment TLN and the lower maximum  $N_2O$  concentration indicate that the denitrification  
391 process was limited even at the  $NO_3^-$  reducing step. The lack of  $NO_3^-$  reduction explains the  
392 higher redox potential, as the soil was not exhausted of easily available electron acceptors.  
393 The limitation was not caused by the addition of  $^{15}N$  as the incomplete reduction of  $^{15}N-NO_3^-$

394 was of the same magnitude as the total  $\text{NO}_3^-$  reduction over time, dismissing isotope  
395 fractionation as the cause. Additionally, Pan et al. (2012) did not find  $\text{NO}_3^-$  reduction to be  
396 sensitive to pH variations within the pH range of the present study. Contrary to the other  
397 treatments, no  $\text{CH}_4$  production was seen for TLN (see SI Fig. S9) despite the fact that a higher  
398 pH should increase the production (Le Mer and Roger, 2001). Thus, the net effect of the  
399 treatment on the gas producing processes remains uncertain.

400 Small concentrations of  $\text{CH}_4$  were produced during flooding (see SI Fig. S9). Compared to  
401  $\text{N}_2\text{O}$ ,  $\text{CH}_4$  is not a significant greenhouse gas to consider during short term floodings as the  
402 redox potential in the soil is not fully reduced to levels ( $< -100$  mV) where  $\text{CH}_4$  is the primary  
403 end product of mineralisation over  $\text{CO}_2$  (Yu and Patrick, 2003).

#### 404 ***4.2 Pathways of N transformation***

405 The primary production mechanism of  $\text{N}_2\text{O}$  in the anoxic soil environment is denitrification.  
406 The intermediate products of denitrification were detected following the reduction of  $^{15}\text{N}$   
407 labelled  $\text{NO}_3^-$  (Fig. 3). The  $^{15}\text{N}$  not accounted for 14-26 hrs after flooding is expected to be  
408 found as  $\text{NO}_2^-$  or  $\text{NO}$ . The recovery of  $^{15}\text{N}$  was quantitative in treatment TN with effectively  
409 100% of  $^{15}\text{N}$  recovered as  $\text{N}_2$  after 72 hrs. However, this was not the case for treatment TLN  
410 where after 144 hrs only 26% of the  $^{15}\text{N}$  was recovered as  $\text{N}_2$  with total  $^{15}\text{N}$  recovery of  $54 \pm$   
411 5%. Reasons for the lower recovery in TLN may be due to volatilization of  $\text{NH}_4^+$  to  $\text{NH}_3$   
412 enhanced by the higher pH (Sommer and Hutchings, 2001), as the  $\text{NH}_4^+$  concentration was  
413 lower in treatment TLN compared to TN (see SI Fig. S9) or that the residual  $^{15}\text{N}$  was  
414 incorporated in other non-measured N pools. Alternatively, a major difference between the  
415 TN and TLN treatments at the end of the 144 hours was the higher  $\text{NO}_3^-$  concentration in the  
416 TLN treatment. Thus if any  $\text{NO}_3^-$  was lost as the soil cores were drained prior to performing

417 the KCl extracts then this may have had a greater impact on the  $^{15}\text{N}$  balance in the TLN  
418 treatment. Another pathway may contribute to  $\text{N}_2\text{O}$  production. Only a small fraction of the  
419 applied  $^{15}\text{N}$  was recovered as  $\text{NH}_4^+$  for both treatments (Fig. 3). Although small, the increase  
420 over time indicates that  $\text{NH}_4^+$  has been produced from DNRA of the applied  $^{15}\text{N}\text{-NO}_3^-$  (Buresh  
421 and Patrick, 1978). The  $\text{NH}_4^+$  produced from the applied  $^{15}\text{N}\text{-NO}_3^-$  was minimal when  
422 compared to the total  $\text{NH}_4^+$  concentration during the flooding event (see SI Fig. S9), but the  
423 process could potentially be important if flooding continued for prolonged periods. It also  
424 cannot be dismissed that the increase in  $^{15}\text{N}\text{-NH}_4^+$  was caused by a release of assimilatory  
425 reduced  $^{15}\text{N}\text{-NO}_3^-$  during extraction, even though the extraction setup should not destroy  
426 microbial cells.

#### 427 ***4.3 $\text{N}_2\text{O}$ emissions and agricultural management practice under future climate conditions***

428 Based on these results, a marked reduction in  $\text{N}_2\text{O}$  production and emission during soil  
429 flooding could be achieved if soils were limed prior to tillage. As the reduction is achieved  
430 with only an increase in pH of 1.3 units it emphasises the importance of liming often, to keep  
431 the pH constant. Low lying areas are of highest risk of flooding and are also likely to receive  
432 additional inputs of N from the surrounding elevated areas. To reduce the risk of flood-  
433 induced  $\text{N}_2\text{O}$  emissions, N-application in low lying areas should be minimized and where  
434 possible these areas should be drained. If flooded, drainage should be avoided, as it is at the  
435 boundary between aerobic and anaerobic conditions when  $\text{N}_2\text{O}$  accumulation is seen.  
436 Consequently, there is a need for additional experiments to include more soil types and land  
437 uses before implications are scaled to larger areas.

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442 **6. References**

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533 **Supporting information**

534 Figure S1: Distribution of KCl extractions over time.

535 Figure S2: Timing of repeated measurements.

536 Figure S3: Validation of microsensors measurements by chamber measurements.

537 Figure S4: Maximum N<sub>2</sub>O concentration over time for t<sub>1</sub>, t<sub>2</sub> and t<sub>3</sub>.

538 Figure S5: N<sub>2</sub>O concentration, diffusion flux, redox potential and NO<sub>3</sub><sup>-</sup> concentration  
539 development over time.

540 Figure S6: Relationship between N<sub>2</sub>O concentration and redox potential.

541 Figure S7: Depth-specific activity of N<sub>2</sub>O production and consumption.

542 Figure S8: Atom% <sup>15</sup>N enrichment.

543 Figure S9: Concentration of NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, CH<sub>4</sub> and the total sum of N over time.

544 **Table 1.** Overview of soil pH, NO<sub>3</sub><sup>-</sup> and DOC concentration as well as the absolute and the  
 545 primary redox range and the redox range for the ‘build-up’ and ‘build-down’ phase of N<sub>2</sub>O  
 546 for all four treatments (n =3).

	Control (TC)	Limed (TL)	N added (TN)	N added & limed (TLN)
<i>pH</i>	6.1 ± 0.1	7.4 ± 0.1		
<i>NO<sub>3</sub><sup>-</sup> (µgN g soil<sup>-1</sup>)</i>	97.8 ± 27.6	108.9 ± 49.9	197.9 ± 73.3	183.3 ± 31.8
<i>DOC (µg g soil<sup>-1</sup>)</i>	129.4 ± 3.9	160.9 ± 2.7		
<i>Primary redox range (mV)</i>	300-450	200-350	250-450	300-400
<i>N<sub>2</sub>O build-up redox range (mV)</i>	414-339	335-242	418-312	384-331
<i>N<sub>2</sub>O build-down redox range (mV)</i>	366-289	298-194	342-232	361-282

547

548 **Figure 1.** Development of mean maximum N<sub>2</sub>O concentration and observed diffusion flux  
549 (a), time- and depth-specific contour plot of the N<sub>2</sub>O concentration over time for treatment TC  
550 (b), time- and depth-specific contour plot of the redox potential over time for treatment TC (c)  
551 and the sum of the NO<sub>3</sub><sup>-</sup> concentration in the soil core over time (d). For contour plots of  
552 treatment TL, TN and TLN see SI (Fig. S5; 5A, 5B and 5C respectively).

553

554 **Figure 2.** Modelled activity of N<sub>2</sub>O production (positive values) and consumption (negative  
555 values) over 6 time intervals (10, 20, 30, 50, 70 and 90 hrs after flooding) for treatment TC  
556 (solid line) and TN (dashed line). Values < 0.0007 mol cm<sup>-3</sup> s<sup>-1</sup> are not shown.

557

558 **Figure 3.** <sup>15</sup>N recovered as NO<sub>3</sub><sup>-</sup>, N<sub>2</sub>O, N<sub>2</sub> and NH<sub>4</sub><sup>+</sup> (secondary axis) over time for treatment  
559 TN (a) and TLN (b). All values are normalized against the recovered <sup>15</sup>N-NO<sub>3</sub><sup>-</sup> at time 0 (n =  
560 3).