



TITLE An evaluation of urine patch simulation methods for nitrous oxide emission measurement

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1 Title: An evaluation of urine patch simulation methods for nitrous oxide emission
2 measurement

3

4 **Summary**

5 Global N₂O-N inventory estimates for pasture systems are refined based on measurements of
6 N₂O-N loss from simulated urine patches using a variety of methods but frequently using a
7 uniform wetted area (UWA), often smaller than a bovine urine patch. However, natural
8 patches follow non-uniform infiltration patterns expanding naturally from a point of deposit
9 with a non-wetted zone of influence. Using 2 L urine the UWA method was compared, using
10 a 0.156 m² collar, with a naturally expanding effective area (NEEA) method, using a 0.462
11 m² collar under high (HL) and low (LL) N₂O loss conditions. The method chosen affects
12 urine N loading to the soil. Under HL the UWA method induced a N₂O-N loss of 280.6
13 mg/patch, significantly less than the 434.8 mg/patch loss for the NEEA method, for the same
14 simulated urination. Under LL there was no method effect. Efforts should be made to employ
15 patch simulation methods which mimic natural deposits and can be achieved, at least in part,
16 by:

- 17 a) use of a urine volume and N content similar to that of the animal of interest.
- 18 b) allow natural infiltration of the chosen urine volume to permit tapering toward the
19 edges.
- 20 c) measure from the zone of influence in addition to the wetted area i.e. the patch
21 effective area.

22

23 **Keywords: nitrous oxide, urine, patch, denitrification, pasture range paddock**

24

25 **Introduction**

26 Nitrogen (N) inputs to agricultural soils contribute to the production of the greenhouse gas,
27 nitrous oxide (N₂O) and animal production accounts for an estimated 1.5 Tg N₂O-N/yr
28 (Oenema, *et al.* 2005). In pasture systems, urination by grazing animals causes a mosaic of
29 discrete patches of highly concentrated N loading to soil. Approximately 41% of N₂O-N
30 emissions from animal production are attributable to urine and dung deposition by grazing
31 animals (Oenema, *et al.* 2005). An increasing number of studies have focused on a)
32 quantifying N₂O-N emissions from urine, and b) assessing urine N₂O-N emission mitigation
33 strategies in pasture systems. These studies typically use simulated urine patches (Table 1).
34 Natural urine patches are intrinsically heterogeneous in their within-patch N loading and size.
35 Selbie *et al.* (2014) summarized the drivers of this variability as urine volume, wind, slope,
36 antecedent soil moisture and soil physical properties. Cattle urine patches were observed to
37 range from 0.16 to 0.49 m² by Williams & Haynes (1994), to have a mean patch area of 0.353
38 m² (Saarijarvi & Virkajarvi, 2009) and to expand naturally over time (Williams & Haynes,
39 1994). Dairy cow urine patches (4 year mean 0.37 m²) have also been measured using the
40 zone of grass response as a proxy for the urine wetting front (Moir *et al.* 2010). Saarijarvi &
41 Virkajarvi (2009) reported that the non-wetted zone of influence extended up to 150 mm
42 from the wetted patch edge. The total area is termed the “effective area” of a urine patch
43 (Selbie *et al.* 2014). It follows that effective area of the patch be expected to delineate the
44 zone of increased N₂O loss potential associated with a urine deposition.

45 There is considerable variability in methods used to simulate urine patches for N₂O loss
46 estimation (Table 1). The two most common methods are to uniformly apply urine to either a)
47 a defined area larger than the footprint of the N₂O measurement collar and subsequently
48 install the collar or b) install the collar prior to application to constrain urine (Table 1). These
49 methods, though practical, do not perfectly simulate a naturally occurring urine patch for a

50 number of reasons. First, they create a uniformly wetted area. Secondly, when constrained by
51 a collar, urine infiltration along the horizontal plain in the surface soil, the most active zone
52 of denitrification (Luo *et al.* 1998), is restricted. Thirdly, the constraint interferes with the
53 pattern of urine interaction with soil. Fourthly, there are discrepancies between the average
54 footprints of naturally deposited urine patches and the collars used to simulate them (Table
55 1). In recent work, Rochette *et al.* (2014) took an alternative approach by simulating a urine
56 patch with a wetted area which was 33% of the N₂O measurement collar area thus ensuring
57 the zone of influence was accounted for. .

58 The objective of this work was to summarise patch simulation approaches in the literature
59 and to evaluate the hypothesis that the N₂O loss induced by a simulated dairy cow urination
60 would be affected by patch simulation and measurement approach. The typical “uniform
61 wetted area” (UWA) method which artificially limits horizontal movement of urine, is
62 compared to a “natural expanding effective area” (NEEA) method using a collar large enough
63 to allow natural infiltration of urine.

64 **Materials and methods**

65 *Site description, experimental design, and treatments*

66 Field experiments were conducted under two conditions i) “high” N₂O loss, which occurred
67 at a moderately drained site in autumn (HL) and ii) “low” N₂O loss, which occurred at a
68 freely draining site in spring (LL). This approach permitted comparison of the methods under
69 contrasting loss conditions and was not designed to explore specific site or seasonal
70 differences which are heavily influenced by specific soil and environmental factors following
71 treatment application. The HL occurred on a moderately draining Cambisol (58% sand, 30%
72 silt, 12% clay, 7.3% organic matter, 3.2% total C, 0.30% total N, pH 5.7 0-10 cm) in autumn
73 2013 at the Teagasc Johnstown Castle Research Centre, Co. Wexford, Ireland (52°18'N; 6°

74 30'W). The LL occurred on a free-draining Cambisol (58% sand, 28% silt, 14% clay, 7.9%
75 organic matter, 3% total C, 0.32% total N, pH 5.8 0-10 cm) in spring 2014 at the Teagasc
76 Moorepark Research Centre, Co. Cork, Ireland (52°09'N; 8° 14'W). Both sites were in long-
77 term grassland dominated by perennial ryegrass (*Lolium perenne* L.). No organic manures or
78 fertilisers were applied and animals were excluded for a period of at least six months in
79 advance of the experiments. Grass was cut to approximately 5 cm before the experiments and
80 allowed to regrow to approximately 8 cm. Stainless steel N₂O measurement collars were
81 inserted to 7 to 10 cm depth at least four days prior to treatment application. Soil volumetric
82 moisture (0-10 cm) was measured using a Theta probe soil moisture sensor (Delta-T,
83 Cambridge, U.K.) in the area surrounding the simulated urine patches. Soil bulk density (0-10
84 cm) was measured to calculate water filled pore space (WFPS) per Maljanen *et al.* (2007).
85 Precipitation, air and soil temperature (0-10 cm) were measured at a nearby (<500 m)
86 meteorological station.

87 The treatments were a) UWA, a patch simulated by uniformly applying 2 L of urine within
88 0.156 m² collars and b) NEEA which closely mimicked a natural urination by applying 2 L
89 urine to a central point within collars of 0.462 m² and allowing urine migrate outward as it
90 would naturally. Although the simulated patches originated from the same simulated
91 urination (2 L) the UWA method resulted in a uniform volume loading of 12.8 L/m² and the
92 NEEA a non-uniform urine loading with a mean of 4.33 L/m². The urine N loading differed
93 on an area basis but not on a simulated urination basis or on a patch basis. This is an
94 important point because it is the N₂O-N emission associated with a urination voided by an
95 animal which represents the unit of interest. The control treatment to measure the soil
96 background N₂O emission (control) used a 0.156 m² collar. Up-scaling N₂O emissions from a
97 chamber scale to area scales is a common practice for presenting results, in a like manner the
98 background emission for a 0.462 m² area was calculated by up-scaling emissions from 0.156

99 m². Treatments were applied on the morning of 14 October 2013 and 8 April 2014 for the HL
100 and LL experiments, respectively. The experimental design was a randomised block design,
101 with three treatments (UWA, NEEA and untreated control) present in each of the five
102 replicate blocks. The experimental unit was the plot, which in all blocks contained one
103 simulated urine patch per urine treatment dedicated to N₂O sampling. Additionally, in blocks
104 1, 3 and 5 each experimental unit contained an additional individual simulated urine patch
105 (HL) or three additional simulated urine patches (LL) which were used solely for soil
106 sampling and mineral N assessment. The dimension of these experimental units was 4 m by
107 2.5 m. For the experimental units containing one urine patch the plot size was 1.5 m by 2.5 m
108 with the treatment located centrally in the plot.

109 Urine was collected from grazing lactating Holstein Friesian dairy cows less than a week
110 prior to application, homogenised and refrigerated at 4°C until application. The urine N
111 content was measured using an Aquakem 600 discrete analyser (Cabrera and Beare, 1993).
112 The urine N content at application was 8.3 g/L and 5.3 g/L for the HL and LL experiments,
113 respectively.

114 *Nitrous oxide sampling and analysis*

115 Unvented stainless steel covers (10 cm in height) were used to form a headspace. Chamber to
116 collar sealing was via a neoprene gasket, compressed by a 6 kg weight. A 10 mL gas sample
117 was taken through a rubber septum after 40 minutes (Becton Dickinson, UK) using a 10 mL
118 polypropylene syringe (BD Plastipak, Becton Dickinson, UK) fitted with a hypodermic
119 needle (BD Microlance 3, Becton Dickinson, UK) and was injected into pre-evacuated 7 mL
120 screw-cap septum glass vials (Labco, UK). The N₂O sampling procedure of Chadwick *et al.*
121 (2014) was followed. Eight samples of ambient air were collected at each sampling. Their
122 mean N₂O concentration was set as a surrogate for N₂O concentration at time zero. The
123 assumption of a linear increase in headspace N₂O accumulation (Chadwick *et al.*, 2014)

124 during the 40 minute enclosure period was verified on each sampling occasion by collecting
125 five headspace samples per chamber from a random sub-set of urine treated chambers during
126 a 60 minute enclosure period. Of the sub-set of chambers which had a flux, 87% were linear
127 according to the criteria of Chadwick *et al.* (2014). At the end of the 60 minute enclosure
128 period the mean nitrous oxide concentration inside chambers in the linear group was 3.5 ppm
129 (standard deviation 3.96 ppm). For the quadratic group it was 2.62 ppm (standard deviation
130 1.93 ppm). The quadratic group was not dominated by any particular urine treatment. The
131 methodology of Chadwick *et al.* (2014) has been used in the generation of emission factors
132 e.g. Bell *et al.* (2016), Krol *et al.* (2016) and treatment inter-comparison e.g. Minet *et al.*
133 (2016). Nitrous oxide concentrations were determined using a gas chromatograph (GC)
134 (Varian CP 3800 GC, Varian, USA). Hourly N₂O emissions were calculated based on the rate
135 of N₂O concentration change during the enclosure period. Flux calculations accounted for air
136 temperature, atmospheric pressure, and the ratio of surface area to chamber volume.
137 Sampling took place between 10 and 12 am and was used to calculate daily emissions (de
138 Klein *et al.*, 2003). Cumulative emissions were calculated by integrating the daily fluxes and
139 linear interpolation between measurement points (de Klein & Harvey, 2012) over 66 and 70
140 days in the HL and LL experiments, respectively. In each experiment sampling was
141 conducted on 20 occasions with the highest sampling intensity following treatment
142 application (Fig. 3).

143 *Soil sampling and analysis*

144 Soil samples (0-10 cm) were collected were collected by sampling at 15 cm intervals across a
145 horizontal cross-section of each patch to obtain a composite sample. In total there were 12
146 soil samplings in the HL and 7 in the LL experiment (Fig. 2). Samples were fresh sieved
147 using a four mm sieve, sub-sample gravimetric moisture content and mineral N content was

148 measured. Samples were extracted with 2M KCl and mineral N in the extract was determined
149 using an Aquakem 600 discrete analyser.

150 *Data presentation and statistical analysis*

151 The flux data is presented per simulated urine patch as has previously been done by Rochette
152 *et al.* (2014). The effect of treatment and time after urine application on the dependent
153 variables of N₂O, soil NO₃-N and NH₄-N were evaluated using the REPEATED statement of
154 the PROC MIXED procedure of SAS 9.3 (© 2002-2010, SAS Institute Inc., Cary, NC, USA).
155 The factors in the model were treatment, time of sampling and block with time of sampling as
156 the repeated factor. The pooled standard error of the mean is presented in Figures. The
157 treatment effect on the cumulative mass of N₂O-N loss during the measurement period was
158 tested using the PROC GLMMIX procedure of SAS. This analysis included treatment, loss
159 condition i.e. HL or LL and their interaction as fixed effects and block as a random effect.

160

161 **Results and discussion**

162 WFPS is an important driver of N₂O-N loss (Smith *et al.*, 1998). Conditions were not
163 favourable for N₂O loss under LL due to lower WFPS levels (45-55%). Under LL the urine
164 treatments were not significantly different from the control (Table 2). Consequently, it is not
165 surprising that patch simulation approach had no effect. In contrast, under the HL conditions
166 precipitation occurred almost daily following urine application (Fig. 1a) and WFPS exceeded
167 65% for at least 40 days following urine application. Additionally, soil temperature at patch
168 simulation, a time when N₂O-N losses are frequently greatest (Williams *et al.* 1999; Maljanen
169 *et al.* 2007; Krol *et al.* 2015), was also three to five degrees Celsius higher. Smith *et al.*
170 (1998) reported an exponential increase in N₂O production related to temperature. Under
171 these conditions both urine treatments increased N₂O loss significantly compared with the

172 control ($P<0.001$). The NEEA which closely mimics a natural urine deposit induced a
173 significantly greater loss compared with the UWA method ($P<0.01$). The UWA patch had a
174 net relative emission of 64% compared to the NEEA method (Table 2). An important factor
175 explaining the lower loss by the UWA method is thought to be the differential urine-soil
176 interactions between methods. Wachendorf *et al.* (2008) reported that 75% of the urine
177 induced N_2O -N loss in their experiment came from native soil N. It is likely that a significant
178 portion of the urine-induced N_2O loss under HL also came from native soil N. A rapid
179 emission peak exceeding $1100 \mu\text{g N}_2\text{O-N/patch/h}$ was induced from the NEEA simulated
180 patch on the day of application. This peak in emission, occurring at a time when soil TON
181 levels were low (Fig. 2c) and was almost three times larger than the initial peak of $398 \mu\text{g}$
182 $\text{N}_2\text{O-N/patch/h}$ for the UWA simulated patch (Fig. 3a). In the NEEA method the urine can
183 interact with a greater volume of surface soil as it migrates outwards from the point of
184 application within the collar and tapers off naturally towards the edges. In the case of these
185 experiments the NEEA area was approximately three times larger than the UWA. We suggest
186 that these tapering (Williams & Haynes, 1994) and edge effects could be important because
187 interfaces or edges are often the most active zones of ecosystems. Another factor likely to
188 affect the urine-soil interaction is a degree of transient ponding observed at application in the
189 UWA method. The hydraulic head (Hillel, 2004) created by the artificial urine ponding which
190 occurred in the UWA treatment may have promoted deeper infiltration. Deeper infiltration
191 could reduce N_2O production because the nitrification rate in the upper soil layer could be at
192 least an order of magnitude higher than in the lower soil layers (Luo *et al.* 1988). It is also
193 conceivable that ammonia volatilisation loss, an important N loss pathway from urine patches
194 (Fischer *et al.*, 2016), could be differentially affected by the patch simulation approach.

195 The NEEA method allowed measurement of the naturally occurring patch effective area for
196 the specific soil environmental conditions of this experiment. The 0.462 m^2 collar used in the

197 NEEA method was approximately 3 times larger than the 0.156 m² collar used in the UWA
198 method. It was larger than the mean wetted area of 0.353 m² reported for a 2.37 kg urination
199 by Saarijarvi & Virkajarvi (2009) and mean zone of grass response of 0.37 m² reported by
200 Moir *et al.* (2010). It was also larger than any of the collars used in previous work listed in
201 Table 1. Anger *et al.* (2003) accounted for the patch zone of influence to a degree by
202 simulating a 0.2 m² patch in a 0.24 m² N₂O measurement collar (Table 1) and Rochette *et al.*
203 (2014) specifically designed their experiment to account for it by simulating 0.1 m² patches
204 in 0.303 m² N₂O measurement collars.

205 The method which most closely mimics natural conditions is expected to deliver the most
206 credible quantitative estimate of loss. In the case of these experiments the NEEA mimicked
207 natural conditions much more closely than the UWA method. Although higher loss was
208 recorded for the NEEA method under HL, this may not always be the outcome, for instance
209 no effect was observed under LL. Under different conditions a concentrated zone of N
210 loading as a result of the UWA method could contribute to an elevated NO₃⁻-N pool which
211 persists for longer favouring denitrification further from the time of urine application. Some
212 evidence of such an effect is present in the LL data. A significant treatment x day of
213 measurement interaction was detected (P<0.001) with two secondary peaks in emission on
214 days 20 and 30 measured for the UWA method but not for the NEEA method (Fig. 3b). The
215 direction of difference in N₂O loss between methods cannot be extrapolated from this study
216 to the diverse soil and environmental conditions in which researchers make N₂O loss
217 estimates for urine patches. This manuscript simply highlights a need for greater attention to
218 the method of urine patch simulation. The nature of urine patches raises practical questions of
219 how to best simulate patches for N₂O emission measurements. It is suggested that a
220 representative patch can be achieved, at least in part by the following:

- 221 a) use of a defined urine volume and N content similar to that of the animal of interest
222 e.g. close to 2.1 L for dairy cattle (Selbie *et al.* 2014).
- 223 b) allow natural infiltration of the chosen defined volume of urine for the soil of choice
224 to permit tapering toward the edges as observed in natural patches by Williams &
225 Haynes (1994).
- 226 c) measure from the zone of influence (Saarijarvi & Virkajarvi, 2009) in addition to the
227 wetted area i.e. the patch effective area (Selbie *et al.* 2014) or the NEEA.

228

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343 experiment for a) high loss and b) low loss conditions.

344 Fig. 2. Soil $\text{NH}_4\text{-N}$ for a) high loss and b) low loss conditions, soil $\text{NO}_2\text{-N}$ and $\text{NO}_3\text{-N}$ [total
345 oxidised N (TON)] for c) high loss and d) low loss conditions (0-10 cm) for naturally
346 expanding effective area (NEEA) and uniform wetted area (UWA) methods.

347 Fig. 3. Temporal flux of $\text{N}_2\text{O-N}$ emission for a) high loss and b) low loss conditions in
348 response to the uniform wetted area (UWA) and naturally expanding effective area (NEEA)
349 urine patch simulation methods.

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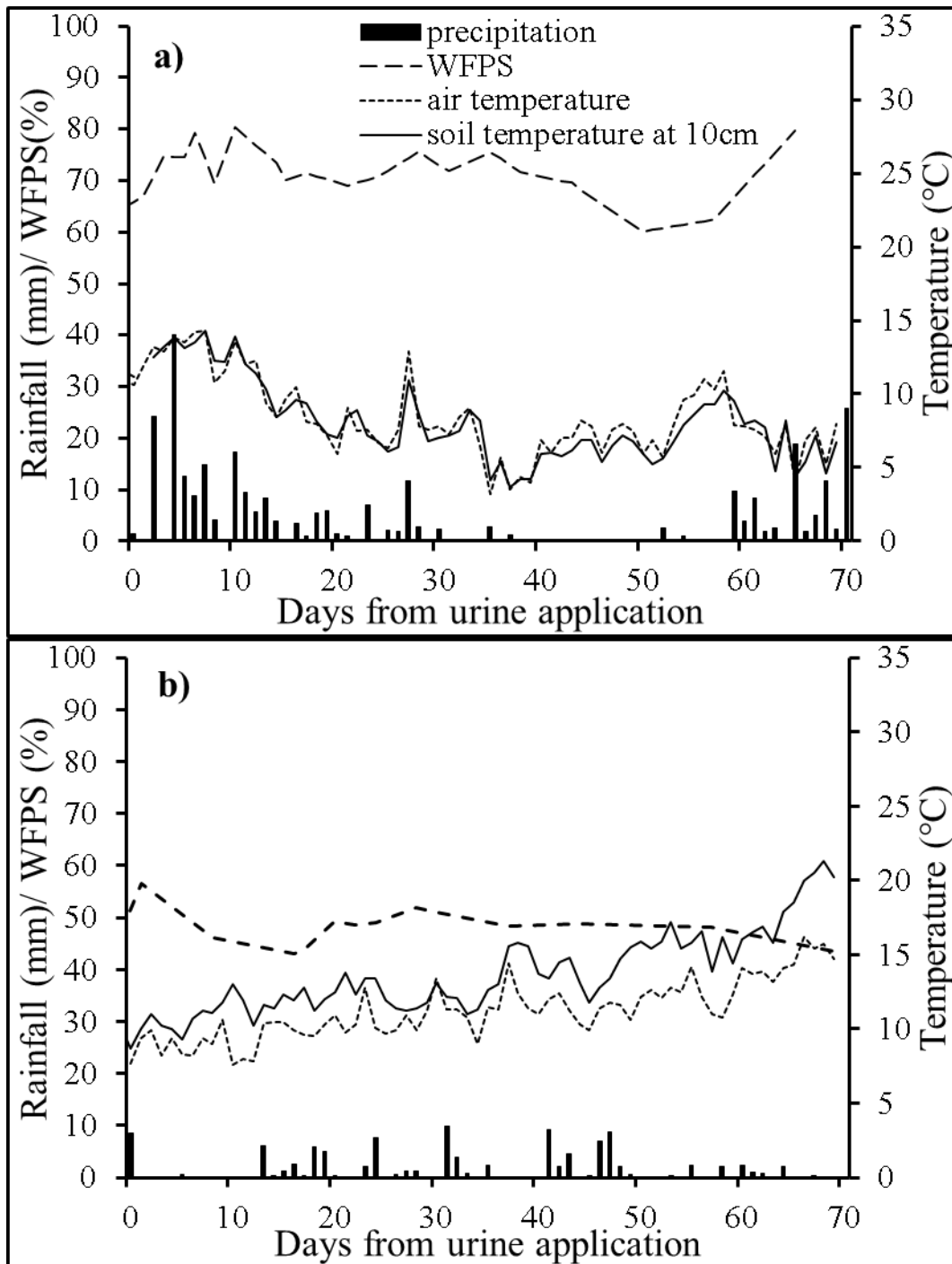
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364 Fig. 1. Precipitation, water filled pore space (WFPS), soil and air temperature during the
 365 experiment for a) high loss and b) low loss conditions.

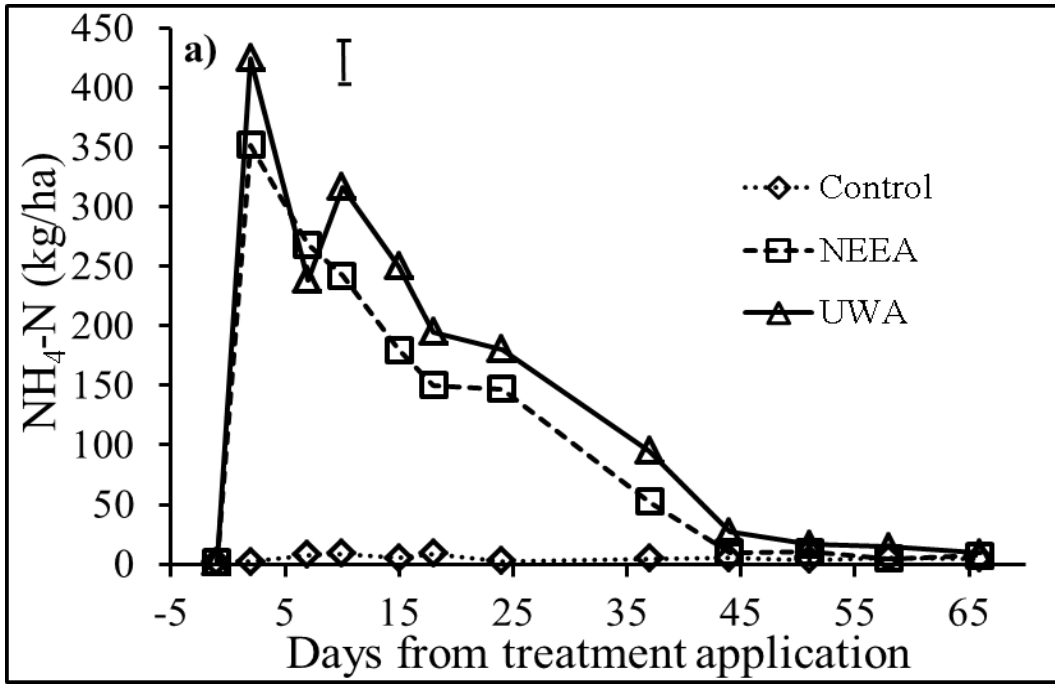


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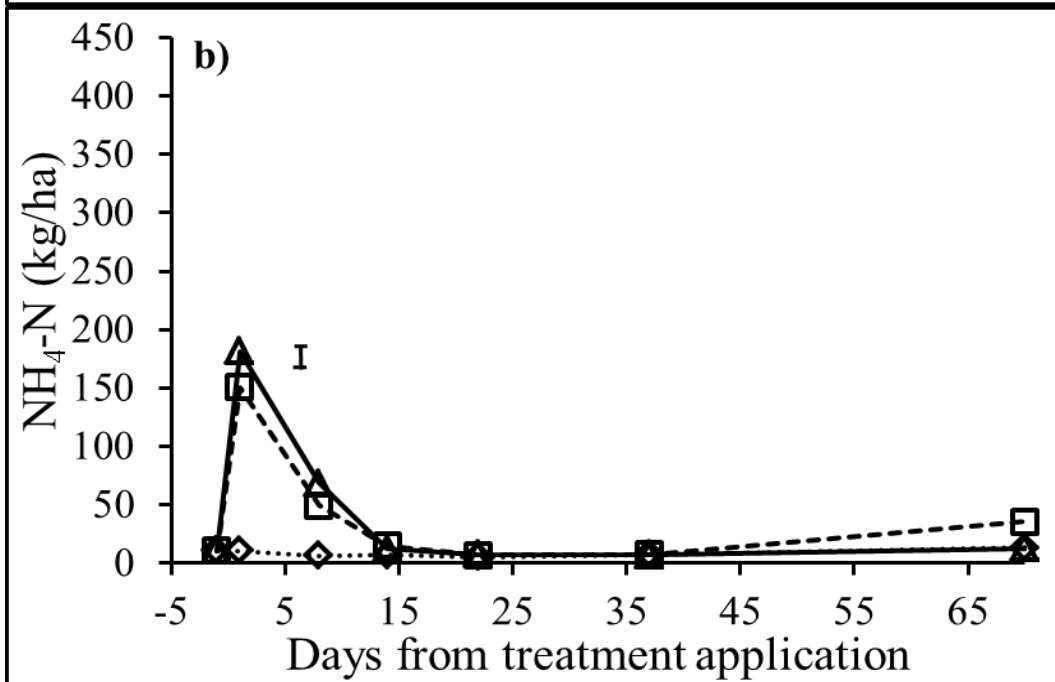
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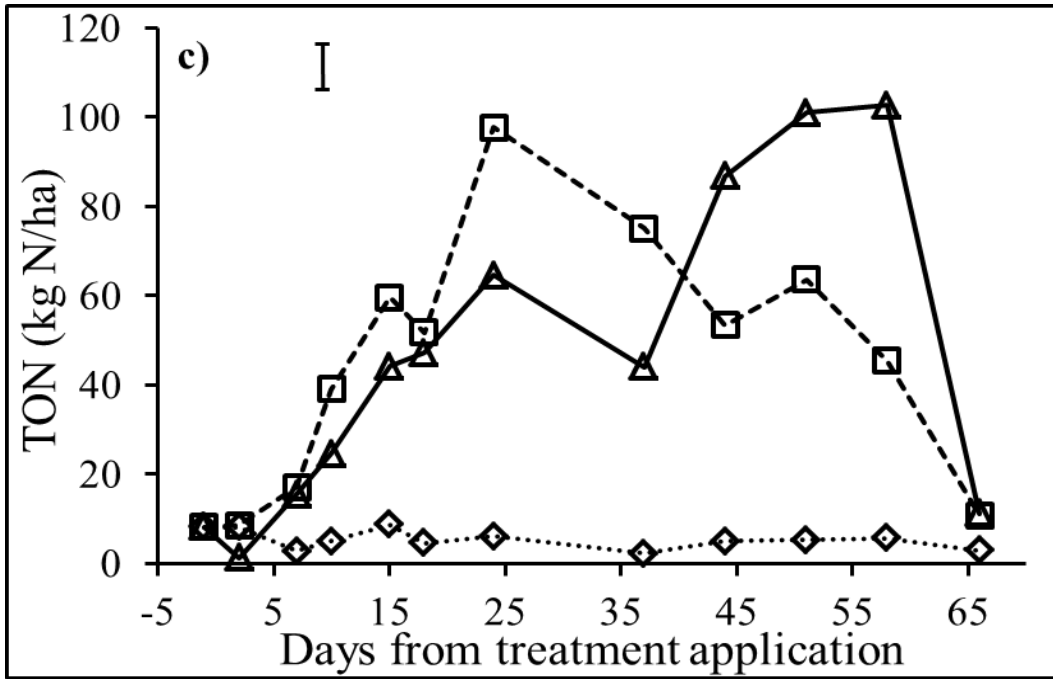
369 Fig. 2. Soil $\text{NH}_4\text{-N}$ for a) high loss and b) low loss conditions, soil $\text{NO}_2\text{-N}$ and $\text{NO}_3\text{-N}$ [total
 370 oxidisable N (TON)] for c) high loss and d) low loss conditions (0-10 cm) for naturally
 371 expanding effective area (NEEA) and uniform wetted area (UWA) methods. Error bars
 372 indicate the pooled standard error of the mean.



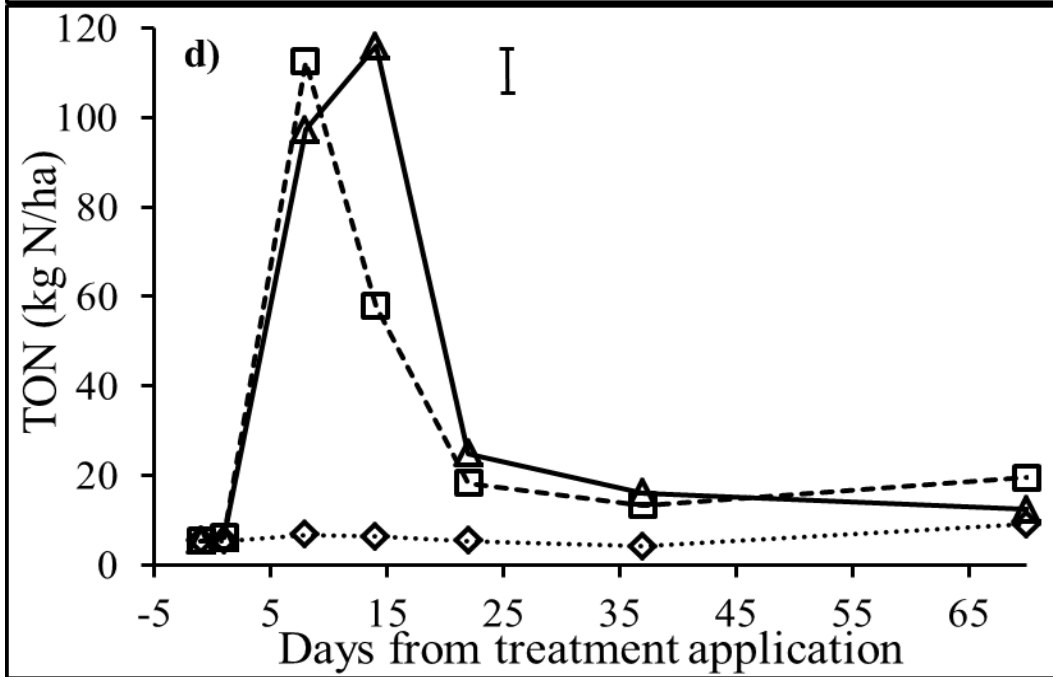
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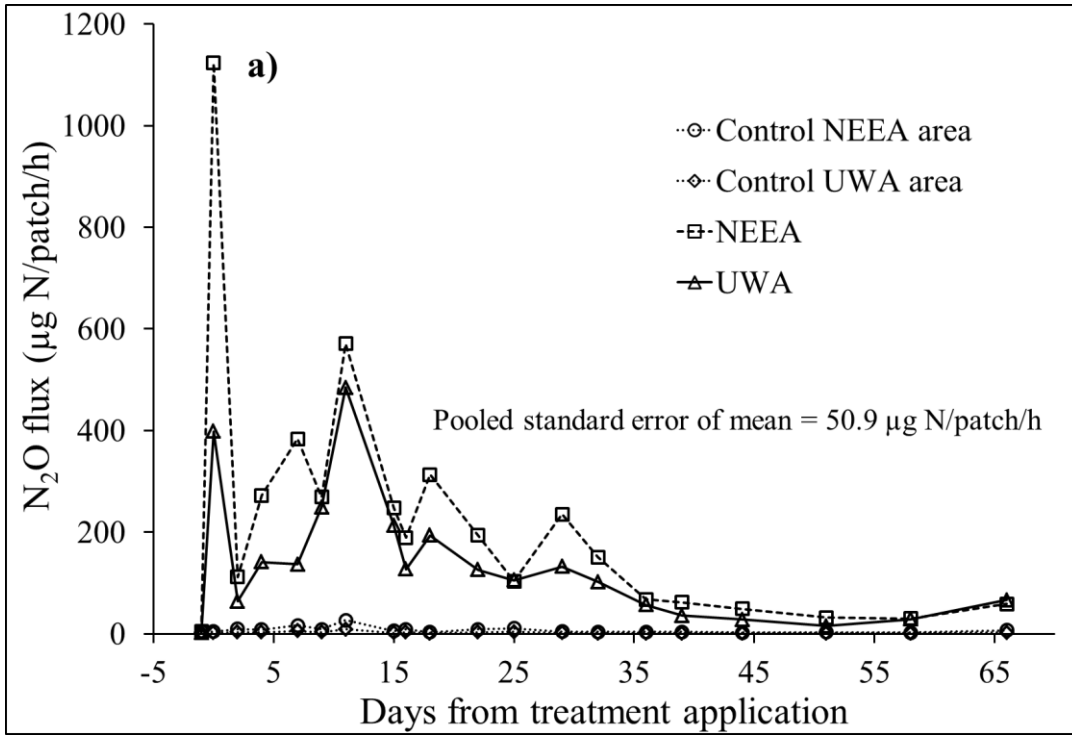
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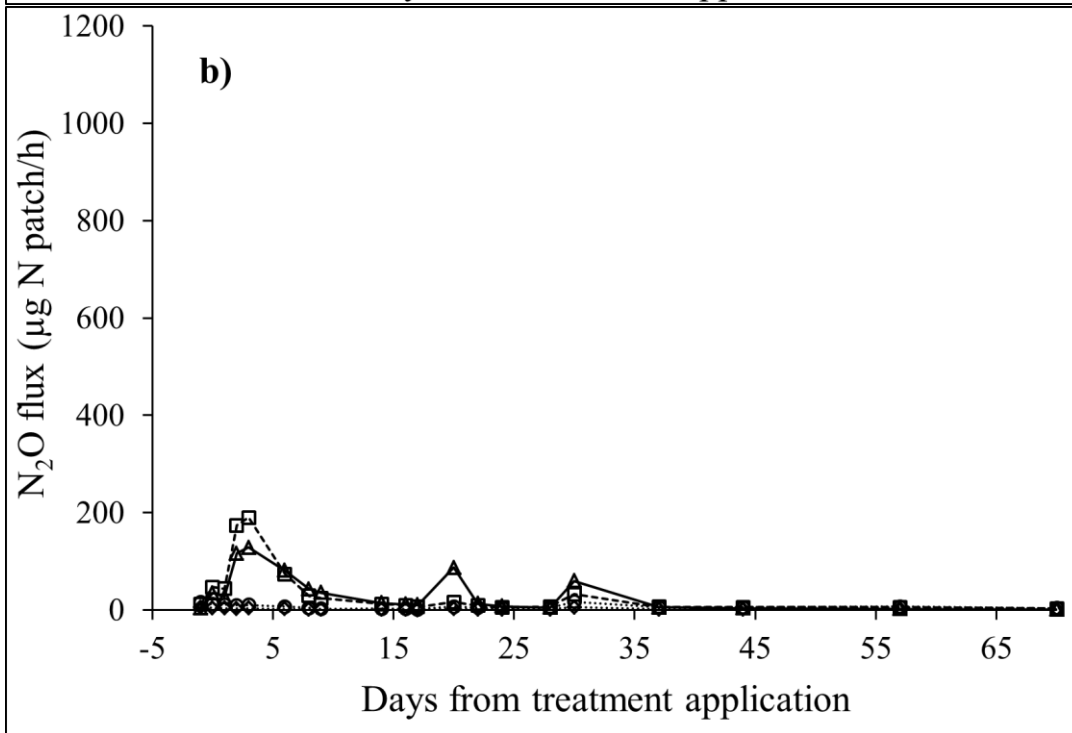
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378 Fig. 3. Temporal flux of N_2O-N emission for a) high loss and b) low loss conditions in
 379 response to the uniform wetted area (UWA) and naturally expanding effective area (NEEA)
 380 urine patch simulation methods.



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387 Table 1. A selection of studies using urine patch simulation for nitrous oxide loss
 388 measurement.

Chamber collar size	Patch size	Mean N loading kg N/ha.	Urine volume	Mean volume urine in chamber	Urine N content	Method
m ²	m ²	kg N/ha	L per chamber area	L m ²	g N/L	
<i>0.1164</i>	<i>0.1164</i>	865, 911	1	8.6	10.07, 10.6	Install collar, urine within Urine poured into 0.0962 m ² ring, install 0.283 m ² pvc ring and sealing area between internal ring and external ring
<i>0.0962</i>	<i>0.0962</i>	1030	<i>1.0</i>	9.9	10.4	
<i>0.1195</i>	<i>0.1195</i>	930	<i>1.1</i>	9.3	10	Install collar, urine within,
0.083	0.083	890 – 3920	<i>1.0, 2.0, 3.0</i>	<i>11.9-35.6</i>	7.5 – 11	Install collar, urine within,
0.24	0.24	425	1.0	4.2	10.2	Install collar, urine within,
0.0875	0.0875	608, 1000	2.5	28.6	14.6, 21.6	Install collar, urine within,
0.2	0.2	300, 500, 700, 1000	2	<i>10</i>	3, 5, 7, 10	Lysimeter installed, urine within
<i>0.0491</i>	0.5	592	<i>0.49</i>	10	5.92	Uniform urine plot, install collar
<i>0.0491</i>		1000				
<i>0.0491</i>	0.5	496-551	<i>0.49</i>	10	4.96-5.51	Uniform urine plot, install collar
<i>0.16</i>	2	498	<i>0.8</i>	5	6.7	Uniform urine plot, install collar
<i>0.0314</i>	<i>0.36</i>	420	1.8	5	8.4	Uniform urine plot, install collar
<i>0.24</i>	0.2	842	2	8.3	<i>10.1</i>	Patch smaller than collar formed
<i>0.303</i>	0.1	92 - 481	0.9 – 1.4	<i>3.0 – 4.6</i>	3.1 – 10.4	Patch smaller than collar formed
0.462	0.462	229, 359	2	4.33	5.3, 8.3	Install collar, urine to central point allowed to infiltrate naturally
0.156	0.156	679, 1064	2	12.8	5.3, 8.3	Install collar, urine “ponding” resulted in uniform application

389 Values in italic are calculated from information provided in papers.

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396 Table 2. Effect of the uniform wetted area (UWA) and naturally expanding effective area
 397 (NEEA) urine patch simulation methods under high loss (HL) and low loss (LL) conditions
 398 on N₂O-N loss.
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Urine patch simulation & measurement method	Patch/collar area m ²	Urine volume L/patch	N load/patch g N/patch	Mean N loading kg N/ha	N ₂ O-N loss mg/patch	Standard deviation mg N ₂ O-N/patch	Net emission relative to the NEEA %
HL-NEEA	0.462	2	16.6	359	434.8 a*	156.5	100
HL-UWA	0.156	2	16.6	1064	280.6 b	65.8	64
HL-Control	0.156	0	-	0	5.5 c	1.8	-
LL-NEEA	0.462	2	10.6	229	35.1 c	10.2	100
LL-UWA	0.156	2	10.6	679	37.7 c	22.4	108
LL-Control	0.156	0	-	0	3.9 c	3.9	-
Pooled standard error of the mean					31.3		
Degrees of freedom					20		

400 * Means with different letters at $P \leq 0.05$.

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