CLIMATE CHANGE AND AGRICULTURE RESEARCH PAPER

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

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SUMMARY

Global nitrous oxide (N₂O) inventory estimates for pasture systems are refined based on measurements of N₂O loss from simulated urine patches. A variety of methods are used for patch simulation but they frequently use a uniform wetted area (UWA), often smaller than a bovine urine patch. However, natural patches follow non-uniform infiltration patterns expanding naturally from a point of deposit with a non-wetted zone of influence. Using 2 litres of urine the UWA method was compared, using a 0·156 m² collar, with a naturally expanding effective area (NEEA) method, using a 0·462 m² collar under high (HL) and low (LL) N₂O loss conditions. The method chosen affects urine nitrogen (N) loading to the soil. Under HL the UWA method induced a N₂O-N loss of 280·6 mg/patch, significantly less than the 434·8 mg/patch loss for the NEEA method, for the same simulated urination. Under LL there was no method effect. Efforts should be made to employ patch simulation methods, which mimic natural deposits and can be achieved, at least in part, by: (a) Using a urine volume and N content similar to that of the animal of interest. (b) Allowing natural infiltration of the chosen urine volume to permit tapering towards the edges. (c) Measuring from the zone of influence in addition to the wetted area, i.e. the patch effective area.

INTRODUCTION

Nitrogen (N) inputs to agricultural soils contribute to production of the greenhouse gas nitrous oxide (N₂O) and animal production accounts for an estimated 1·5 million tonnes N₂O-N/year (Oenema et al. 2005). In pasture systems, urination by grazing animals causes a mosaic of discrete patches of highly concentrated N loading to soil. Approximately 0·41 of N₂O-N emissions from animal production are attributable to urine and dung deposition by grazing animals (Oenema et al. 2005). An increasing number of studies have focused on (a) quantifying N₂O-N emissions from urine and (b) assessing urine N₂O-N emission mitigation strategies in pasture systems. These studies typically use simulated urine patches (Table 1). Natural urine patches are intrinsically heterogeneous in their within-patch N loading and size. Selbie et al. (2015) summarized the drivers of this variability as urine volume, wind, slope, antecedent soil moisture and soil physical properties. Cattle urine patches were observed to range from 0·16 to 0·49 m² by Williams & Haynes (1994), to have a mean patch area of 0·353 m² (Saarijarvi & Virkajarvi 2009) and to expand naturally over time (Williams & Haynes 1994). Dairy cow urine patches (4 year mean 0·37 m²) have also been measured using the zone of grass response as a proxy for the urine wetting front (Moir et al. 2011). Saarijarvi & Virkajarvi (2009) reported that the non-wetted zone of influence extended up to 150 mm from the wetted patch edge. The total area is termed the ‘effective area’ of a urine patch (Selbie et al. 2015). It follows that effective area of the patch would be expected to delineate the zone of increased N₂O loss potential associated with a urine deposition.

There is considerable variability in methods used to simulate urine patches for N₂O loss estimation.
Table 1. A selection of studies using urine patch simulation for nitrous oxide loss measurement

<table>
<thead>
<tr>
<th>Chamber collar size (m²)</th>
<th>Patch size (m²)</th>
<th>Mean N loading (kg N/ha)</th>
<th>Urine volume (l/chamber area)</th>
<th>Mean volume urine in chamber (l/m²)</th>
<th>Urine N content (g N/l)</th>
<th>Method</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1164</td>
<td>0.1164</td>
<td>865, 911</td>
<td>1</td>
<td>8·6</td>
<td>10·07, 10·6</td>
<td>Install collar, urine within</td>
<td>Clough et al. (2008)</td>
</tr>
<tr>
<td>0.0962</td>
<td>0.0962</td>
<td>1030</td>
<td>1·0</td>
<td>9·9</td>
<td>10·4</td>
<td>Urine poured into 0·0962 m² ring, install 0·283 m² PVC ring and sealing area between internal ring and external ring</td>
<td>Wachendorf et al. (2008)</td>
</tr>
<tr>
<td>0.1195</td>
<td>0.1195</td>
<td>930</td>
<td>1·1</td>
<td>9·3</td>
<td>10</td>
<td>Install collar, urine within</td>
<td>Taghizadeh-Toosi et al. (2011)</td>
</tr>
<tr>
<td>0·083</td>
<td>0·083</td>
<td>890–3920</td>
<td>1·0, 2·0, 3·0</td>
<td>11·9–35·6</td>
<td>7·5–11</td>
<td>Install collar, urine within</td>
<td>Sordi et al. (2014)</td>
</tr>
<tr>
<td>0·24</td>
<td>0·24</td>
<td>425</td>
<td>1·0</td>
<td>4·2</td>
<td>10·2</td>
<td>Install collar, urine within</td>
<td>Lessa et al. (2014)</td>
</tr>
<tr>
<td>0·0875</td>
<td>0·0875</td>
<td>608, 1000</td>
<td>2·5</td>
<td>28·6</td>
<td>14·6, 21·6</td>
<td>Install collar, urine within</td>
<td>Baral et al. (2014)</td>
</tr>
<tr>
<td>0·2</td>
<td>0·2</td>
<td>300, 500, 700, 1000</td>
<td>2</td>
<td>10</td>
<td>3, 5, 7, 10</td>
<td>Lysimeter installed, urine within</td>
<td>Selbie et al. (2015)</td>
</tr>
<tr>
<td>0·0491</td>
<td>0·5</td>
<td>592</td>
<td>0·49</td>
<td>10</td>
<td>5·92</td>
<td>Uniform urine plot, install collar</td>
<td>de Klein et al. (2003)</td>
</tr>
<tr>
<td>0·0491</td>
<td>1000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Luo et al. (2008)</td>
</tr>
<tr>
<td>0·0491</td>
<td>0·5</td>
<td>496–551</td>
<td>0·49</td>
<td>10</td>
<td>4·96–5·51</td>
<td>Uniform urine plot, install collar</td>
<td>van der Weerden et al. (2011)</td>
</tr>
<tr>
<td>0·16</td>
<td>2</td>
<td>498</td>
<td>0·8</td>
<td>5</td>
<td>6·7</td>
<td>Uniform urine plot, install collar</td>
<td>Boon et al. (2014)</td>
</tr>
<tr>
<td>0·0314</td>
<td>0·36</td>
<td>420</td>
<td>1·8</td>
<td>5</td>
<td>8·4</td>
<td>Uniform urine plot, install collar</td>
<td>Bell et al. (2015)</td>
</tr>
<tr>
<td>0·24</td>
<td>0·2</td>
<td>842</td>
<td>2</td>
<td>8·3</td>
<td>10·1</td>
<td>Patch smaller than collar formed</td>
<td>Anger et al. (2003)</td>
</tr>
<tr>
<td>0·303</td>
<td>0·1</td>
<td>92–481</td>
<td>0·9–1·4</td>
<td>3·0–4·6</td>
<td>3·1–10·4</td>
<td>Patch smaller than collar formed</td>
<td>Rochette et al. (2014)</td>
</tr>
<tr>
<td>0·462</td>
<td>0·462</td>
<td>229, 359</td>
<td>2</td>
<td>4·33</td>
<td>5·3, 8·3</td>
<td>Install collar, urine to central point allowed to infiltrate naturally</td>
<td>Current study</td>
</tr>
<tr>
<td>0·156</td>
<td>0·156</td>
<td>679, 1064</td>
<td>2</td>
<td>12·8</td>
<td>5·3, 8·3</td>
<td>Install collar, urine ‘ponding’ resulted in uniform application</td>
<td>Current study</td>
</tr>
</tbody>
</table>

Values in italics are calculated from information provided in papers.
The two most common methods are to uniformly apply urine to either (a) a defined area larger than the footprint of the N₂O measurement collar and subsequently install the collar or (b) install the collar prior to application to constrain urine (Table 1). These methods, though practical, do not perfectly simulate a naturally occurring urine patch for a number of reasons. Firstly, they create a uniformly wetted area. Secondly, when constrained by a collar, urine infiltration along the horizontal plain in the surface soil, the most active zone of denitrification (Luo et al. 1998), is restricted. Thirdly, the constraint interferes with the pattern of urine interaction with soil. Fourthly, there are discrepancies between the average footprints of naturally deposited urine patches and the collars used to simulate them (Table 1). In recent work, Rochette et al. (2014) took an alternative approach by simulating a urine patch with a wetted area, which was 0·33 of the N₂O measurement collar area, thus ensuring the zone of influence was accounted for.

The objective of the current work was to summarize patch simulation approaches in the literature and to evaluate the hypothesis that N₂O loss induced by a simulated dairy cow urination would be affected by patch simulation and measurement approach. The typical ‘uniform wetted area’ (UWA) method, which artificially limits horizontal movement of urine, is compared with a ‘natural expanding effective area’ (NEEA) method using a collar large enough to allow natural infiltration of urine.

MATERIALS AND METHODS

Site description, experimental design and treatments

Field experiments were conducted under two conditions: (i) ‘high’ N₂O loss, which occurred at a moderately drained site in autumn (HL) and (ii) ‘low’ N₂O loss, which occurred at a freely draining site in spring (LL). This approach permitted comparison of the methods under contrasting loss conditions and was not designed to explore specific site or seasonal differences, which are heavily influenced by specific soil and environmental factors following treatment application. The HL occurred on a moderately draining Cambisol (58% sand, 28% silt, 14% clay, 79 g organic matter/kg, 30 g total C/kg, 3·2 g total N/kg, pH 5·8 0–10 cm) in spring 2014 at the Teagasc Moorepark Research Centre, Co. Cork, Ireland (52°09′N, 8°14′W, 35 m a.s.l.). Both sites were in long-term grassland dominated by perennial ryegrass (Lolium perenne L.). No organic manures or fertilizers were applied and animals were excluded for a period of at least 6 months in advance of the experiments. Grass was cut to approximately 5 cm before the experiments and allowed to regrow to approximately 8 cm. Stainless steel N₂O measurement collars were inserted to 7–10 cm depth at least 4 days prior to treatment application. Soil volumetric moisture (0–10 cm) was measured using a Theta probe soil moisture sensor (Delta-T, Cambridge, UK) in the area surrounding the simulated urine patches. Soil bulk density (0–10 cm) was measured to calculate water-filled pore space (WFPS) following the method of Maljanen et al. (2007). Precipitation, air and soil temperature (0–10 cm) were measured at a nearby (<500 m) meteorological station.

The treatments were: (a) UWA, a patch simulated by uniformly applying 2 litres of urine within 0·156 m² collars and (b) NEEA, which closely mimicked natural urination by applying 2 litres of urine to a central point within collars of 0·462 m² and allowing urine to migrate outward as it would naturally. Although the simulated patches originated from the same simulated urination (2 litres) the UWA method resulted in a uniform volume loading of 12·8 litres/m² and the NEEA a non-uniform urine loading with a mean of 4·33 litres/m². The urine N loading differed on an area basis but not on a simulated urination basis or on a patch basis. This is an important point because it is the N₂O-N emission associated with urination voided by an animal, which represents the unit of interest. The control treatment to measure the soil background N₂O emission (control) used a 0·156 m² collar. Up-scaling N₂O emissions from a chamber scale to area scales is a common practice for presenting results, in a similar manner the background emission for a 0·462 m² area was calculated by up-scaling emissions from 0·156 m². Treatments were applied on the morning of 14 October 2013 and 8 April 2014 for the HL and LL experiments, respectively. The experimental design was a randomized block design, with three treatments (UWA, NEEA and untreated control) present in each of the five replicate blocks. The experimental unit was the plot, which in all blocks contained one simulated urine patch per urine...
Nitrous oxide sampling and analysis

Unvented stainless steel covers (10 cm high) were used to form a headspace. Chamber to collar sealing was via a neoprene gasket, compressed by a 6 kg weight. A 10 ml gas sample was taken through a rubber septum after 40 min (Becton Dickinson, Oxford, UK) using a 10 ml polypropylene syringe (BD Plastipak, Becton Dickinson, Oxford, UK) fitted with a hypodermic needle (BD Microlance 3, Becton Dickinson, Oxford, UK) and was injected into pre-evacuated 7 ml screw-cap septum glass vials (Labco, High Wycombe, UK). The N₂O sampling procedure of Chadwick et al. (2014) was followed. Eight samples of ambient air were collected at each sampling. Their mean N₂O concentration was set as a surrogate for N₂O concentration at time zero. The assumption of a linear increase in headspace N₂O accumulation (Chadwick et al. 2014) during the 40-min enclosure period was verified on each sampling occasion by collecting five headspace samples per chamber from a random sub-set of urine treated chambers during a 60-min enclosure period. Of the sub-set of chambers, which had a flux, 0.87 were linear according to the criteria of Chadwick et al. (2014).

At the end of the 60-min enclosure period, the mean N₂O concentration inside chambers in the linear group was 3.5 ppm (s.d. 3.96 ppm). For the quadratic group it was 2.62 ppm (s.d. 1.93 ppm). The quadratic group was not dominated by any particular urine treatment. The methodology of Chadwick et al. (2014) has been used in the generation of emission factors (e.g. Bell et al. (2015); Krol et al. (2016)) and treatment inter-comparison (e.g. Minet et al. (2016)). Nitrous oxide concentrations were determined using a gas chromatograph (GC) (Varian CP 3800 GC, Varian, USA). Hourly N₂O emissions were calculated based on the rate of N₂O concentration change during the enclosure period. Flux calculations accounted for air temperature, atmospheric pressure and the ratio of surface area to chamber volume. Sampling took place between 10:00 and 12:00 h and was used to calculate daily emissions (de Klein et al. 2003).

Cumulative emissions were calculated by integrating the daily fluxes and linear interpolation between measurement points (de Klein & Harvey 2012) over 66 and 70 days in HL and LL experiments, respectively. In each experiment, sampling was conducted on 20 occasions with the highest sampling intensity following treatment application.

Soil sampling and analysis

Soil samples (0–10 cm) were collected by sampling at 15 cm intervals across a horizontal cross-section of each patch to obtain a composite sample. In total, there were 12 soil samplings in the HL and 7 in the LL experiment with the highest sampling intensity following treatment application. Samples were fresh-sieved using a 4 mm sieve, and subsample gravimetric moisture content and mineral N content was measured. Samples were extracted with 2 M potassium chloride (KCl) and mineral N in the extract was determined using an Aquakem 600 discrete analyser.

Data presentation and statistical analysis

The flux data are presented per simulated urine patch, as previously done by Rochette et al. (2014). The effect of treatment and time after urine application on the dependent variables of N₂O, soil nitrate (NO₃-N) and ammonium (NH₄-N) were evaluated using the REPEATED statement of the PROC MIXED procedure of SAS 9.3 (© 2002–2010, SAS Institute Inc., Cary, NC, USA). The factors in the model were treatment, time of sampling and block with time of sampling as the repeated factor. The treatment effect on the cumulative mass of N₂O-N loss during the measurement period was tested using the PROC GLMMIX procedure of SAS. The analysis included treatment, loss condition, i.e. HL or LL and their interaction as fixed effects and block as a random effect.
RESULTS AND DISCUSSION

Water-filled pore space is an important driver of N₂O-N loss (Smith et al. 1998). Conditions were not favourable for N₂O loss under LL due to lower WFPS levels (45–55%). Under LL the urine treatments were not significantly different from the control (Table 2). Consequently, it is not surprising that the patch simulation approach had no effect. In contrast, under HL conditions precipitation occurred almost daily following urine application (Fig. 1(a)) and WFPS exceeded 65% for at least 40 days following urine application. Additionally, soil temperature at patch simulation, a time when N₂O-N losses are frequently greatest (Williams et al. 1999; Maljanen et al. 2007; Krol et al. 2015), was also 3–5 °C higher. Smith et al. (1998) reported an exponential increase in N₂O production related to temperature. Under these conditions, both urine treatments increased N₂O loss significantly compared with the control (P < 0.001).

The NEEA, which closely mimics a natural urine deposit, induced a significantly greater loss compared with the UWA method (P < 0.01). The UWA patch had a net relative emission of 64% compared with the NEEA method (Table 2). An important factor explaining the lower loss by the UWA method is thought to be the differential urine-soil interactions between methods. Wachendorf et al. (2008) reported that 75% of the urine induced N₂O-N loss in their experiment came from native soil N. It is likely that a significant portion of the urine-induced N₂O loss under HL in the current work also came from native soil N. A rapid emission peak exceeding 1100 µg N₂O-N/patch/h was induced from the NEEA simulated patch on the day of application. The peak in emission occurred at a time when soil total oxidized nitrogen (TON) levels were low (Fig. 2(c)) and was almost three times larger than the initial peak of 398 µg N₂O-N/patch/h for the UWA simulated patch (Fig. 3).

### Table 2. Effect of the uniform wetted area (UWA) and naturally expanding effective area (NEEA) urine patch simulation methods under high (HL) and low loss (LL) conditions on N₂O-N loss

<table>
<thead>
<tr>
<th>Urine patch simulation and measurement method</th>
<th>Patch/collar area (m²)</th>
<th>Urine volume (l/patch)</th>
<th>N load/patch (g N/patch)</th>
<th>Mean N loading (kg N/ha)</th>
<th>N₂O-N loss (mg/patch)</th>
<th>s.d. (mg N₂O-N/patch)</th>
<th>Net emission relative to the NEEA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL-NEEA</td>
<td>0.462</td>
<td>2</td>
<td>16.6</td>
<td>339</td>
<td>434.8</td>
<td>156.5</td>
<td>100</td>
</tr>
<tr>
<td>HL-UWA</td>
<td>0.156</td>
<td>2</td>
<td>16.6</td>
<td>1064</td>
<td>280.6</td>
<td>65.8</td>
<td>64</td>
</tr>
<tr>
<td>HL-Control</td>
<td>0.156</td>
<td>0</td>
<td>–</td>
<td>0</td>
<td>5.5</td>
<td>1.8</td>
<td>–</td>
</tr>
<tr>
<td>LL-NEEA</td>
<td>0.462</td>
<td>2</td>
<td>10.6</td>
<td>229</td>
<td>35.1</td>
<td>10.2</td>
<td>100</td>
</tr>
<tr>
<td>LL-UWA</td>
<td>0.156</td>
<td>2</td>
<td>10.6</td>
<td>679</td>
<td>37.7</td>
<td>22.4</td>
<td>108</td>
</tr>
<tr>
<td>LL-Control</td>
<td>0.156</td>
<td>0</td>
<td>–</td>
<td>0</td>
<td>3.9</td>
<td>3.9</td>
<td>–</td>
</tr>
</tbody>
</table>

Pooled S.E. of the mean 31.3
Degrees of freedom 20

![Fig. 1.](image-url) Precipitation, water filled pore space (WFPS), soil and air temperature during the experiment for (a) high loss and (b) low loss conditions.
In the NEEA method, the urine can interact with a greater volume of surface soil as it migrates outwards from the point of application within the collar and tapers off naturally towards the edges. In the current experiments, the NEEA area was approximately three times larger than the UWA. It is suggested that these tapering (Williams & Haynes 1994) and edge effects could be important because interfaces or edges are often the most active zones of ecosystems. Another factor likely to affect the urine–soil interaction is a degree of transient ponding observed at application in the UWA method. The hydraulic head (Hillel 2004) created by the artificial urine ponding which occurred in the UWA treatment may have promoted...
deeper infiltration. Deeper infiltration could reduce N$_2$O production because the nitrification rate in the upper soil layer could be at least an order of magnitude higher than in the lower soil layers (Luo et al. 1998). It is also conceivable that ammonia volatilization loss, an important N loss pathway from urine patches (Fischer et al. 2016), could be differentially affected by the patch simulation approach.

The NEEA method allowed measurement of the naturally occurring patch effective area for the specific soil environmental conditions of the current experiment. The 0.462 m$^2$ collar used in the NEEA method was approximately three times larger than the 0.156 m$^2$ collar used in the UWA method. It was larger than the mean wetted area of 0.353 m$^2$ reported for a 2.37 kg urination by Saarijarvi & Virkajarvi (2009) and the mean zone of grass response of 0.37 m$^2$ reported by Moir et al. (2011). It was also larger than any of the collars used in the previous work, listed in Table 1. Anger et al. (2003) accounted for the patch zone of influence to a degree by simulating a 0.2 m$^2$ patch in a 0.24 m$^2$ N$_2$O measurement collar (Table 1) and Rochette et al. (2014) specifically designed their experiment to account for it by simulating 0.1 m$^2$ patches in 0.303 m$^2$ N$_2$O measurement collars.

The method which most closely mimics natural conditions is expected to deliver the most credible quantitative estimate of loss. In the case of these experiments, the NEEA mimicked natural conditions much more closely than the UWA method. Although higher loss was recorded for the NEEA method under HL, this may not always be the outcome, for instance no effect was observed under LL. Under different conditions a concentrated zone of N loading can be achieved, at least in part by the patch simulation approach.

The nature of urine patches raises practical questions of how to best simulate patches for N$_2$O emission measurements. It is suggested that a representative patch can be achieved, at least in part by the following:

(a) Use of a defined urine volume and N content similar to that of the animal of interest, e.g. close to 2.1 litres for dairy cattle (Selbie et al. 2015).

(b) Allow natural infiltration of the chosen defined volume of urine for the soil of choice to permit tapering toward the edges as observed in natural patches by Williams & Haynes (1994).

(c) Measure from the zone of influence (Saarijarvi & Virkajarvi 2009) in addition to the wetted area, i.e. the patch effective area (Selbie et al. 2015) or the NEEA.

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REFERENCES


