CLIMATE CHANGE AND AGRICULTURE RESEARCH PAPER
An evaluation of urine patch simulation methods for nitrous oxide emission measurement

P. J. FORRESTAL[1]*, D. J. KROL[1], G. J. LANIGAN[1], M. M. R. JAHANGIR[2] AND K. G. RICHARDS[1]

[1] *Teagasc, Environment, Soils and Land-Use Department, Johnstown Castle, Co. Wexford, Ireland*
[2] *Department of Soil Science, Bangladesh Agricultural University, Mymensingh, Bangladesh*

SUMMARY

Global nitrous oxide (N_2O) inventory estimates for pasture systems are refined based on measurements of N_2O 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 $0 \cdot 156 \ m^2$ collar, with a naturally expanding effective area (NEEA) method, using a $0 \cdot 462 \ m^2$ collar under high (HL) and low (LL) N_2O loss conditions. The method chosen affects urine nitrogen (N) loading to the soil. Under HL the UWA method induced a N_2O-N loss of $280 \cdot 6$ mg/patch, significantly less than the $434 \cdot 8$ 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 (N_2O) and animal production accounts for an estimated 1·5 million tonnes N_2O-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 N_2O-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 N_2O-N emissions from urine and (b) assessing urine N_2O-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 m^2 by Williams & Haynes (1994), to have a mean patch area of 0·353 m^2 (Saarijarvi & Virkajarvi 2009) and to expand naturally over time (Williams & Haynes 1994). Dairy cow urine patches (4 year mean 0·37 m^2) 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 N_2O loss potential associated with a urine deposition.

There is considerable variability in methods used to simulate urine patches for N_2O loss estimation

* To whom all correspondence should be addressed. Email: Patrick. Forrestal@teagasc.ie

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

Chamber collar size (m²)	Patch size (m²)	Mean N loading (kg N/ha)	Urine volume (l/chamber area)	Mean volume urine in chamber (l/m²)	Urine N content (g N/l)	Method	Author
0·1164	*0·1164*	865, 911	1	*8·6*	10·07, 10·6	Install collar, urine within	Clough et al. (2008)
0·0962	*0·0962*	1030	*1·0*	9·9	10·4	Urine poured into 0·0962 m² ring, install 0·283 m² PVC ring and sealing area between internal ring and external ring	Wachendorf et al. (2008)
0·1195	*0·1195*	930	*1·1*	9·3	10	Install collar, urine within	Taghizadeh-Toosi et al. (2011)
0·083	0·083	890–3920	*1·0, 2·0, 3·0*	*11·9–35·6*	7·5–11	Install collar, urine within	Sordi et al. (2014)
0·24	0·24	*425*	1·0	*4·2*	10·2	Install collar, urine within	Lessa et al. (2014)
0·0875	0·0875	608, 1000	2·5	28·6	14·6, 21·6	Install collar, urine within	Baral et al. (2014)
0·2	0·2	300, 500, 700, 1000	2	*10*	3, 5, 7, 10	Lysimeter installed, urine within	Selbie et al. (2015)
0·0491	*0·5*	592	*0·49*	10	5·92	Uniform urine plot, install collar	de Klein et al. (2003)
0·0491		1000					Luo et al. (2008)
0·0491	*0·5*	496–551	*0·49*	10	4·96–5·51	Uniform urine plot, install collar	van der Weerden et al. (2011)
0·16	2	498	*0·8*	5	6·7	Uniform urine plot, install collar	Boon et al. (2014)
0·0314	*0·36*	420	1·8	5	8·4	Uniform urine plot, install collar	Bell et al. (2015)
0·24	0·2	*842*	2	*8·3*	*10·1*	Patch smaller than collar formed	Anger et al. (2003)
0·303	0·1	*92–481*	*0·9–1·4*	*3·0–4·6*	3·1–10·4	Patch smaller than collar formed	Rochette et al. (2014)
0·462	0·462	229, 359	2	4·33	5·3, 8·3	Install collar, urine to central point allowed to infiltrate naturally	Current study
0·156	0·156	679, 1064	2	12·8	5·3, 8·3	Install collar, urine 'ponding' resulted in uniform application	Current study

Values in italics are calculated from information provided in papers.

(Table 1). The two most common methods are to uniformly apply urine to either (a) a defined area larger than the footprint of the N$_2$O 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 N$_2$O 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 N$_2$O 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' N$_2$O loss, which occurred at a moderately drained site in autumn (HL) and (ii) 'low' N$_2$O 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, 30% silt, 12% clay, 73 g organic matter/kg, 32 g total C/kg, 3·0 g total N/kg, pH 5·7 0–10 cm) in autumn 2013 at the Teagasc Johnstown Castle Research Centre, Co. Wexford, Ireland (52°18′N, 6°30′W, 72 m a.s.l). The LL

occurred on a free-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 N$_2$O 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 m^2 collars and (b) NEEA, which closely mimicked natural urination by applying 2 litres of urine to a central point within collars of 0·462 m^2 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/m^2 and the NEEA a non-uniform urine loading with a mean of 4·33 litres/m^2. 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 N$_2$O-N emission associated with urination voided by an animal, which represents the unit of interest. The control treatment to measure the soil background N$_2$O emission (control) used a 0·156 m^2 collar. Up-scaling N$_2$O 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 m^2 area was calculated by up-scaling emissions from 0·156 m^2. 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

treatment dedicated to N_2O sampling. Additionally, in blocks 1, 3 and 5 each experimental unit contained an additional individual simulated urine patch (HL) or three additional simulated urine patches (LL), which were used solely for soil sampling and mineral N assessment. The dimensions of these experimental units was 4×2.5 m. For the experimental units containing one urine patch the plot size was 1.5×2.5 m with the treatment located centrally in the plot.

Urine was collected from grazing lactating Holstein Friesian dairy cows less than a week prior to application, homogenized and refrigerated at 4 °C until application. Urine N content was measured using an Aquakem 600 discrete analyser (Thermo Fisher Scientific, Waltham, MA, USA) (Cabrera & Beare 1993). Urine N content at application was 8·3 and 5·3 g/l for the HL and LL experiments, respectively.

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 N_2O sampling procedure of Chadwick *et al*. (2014) was followed. Eight samples of ambient air were collected at each sampling. Their mean N_2O concentration was set as a surrogate for N_2O concentration at time zero. The assumption of a linear increase in headspace N_2O 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 N_2O 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 N_2O emissions were calculated based on the rate of N_2O 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 sub-sample 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 N_2O, soil nitrate (NO_3-N) and ammonium (NH_4-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 N_2O-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.

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 N$_2$O-N loss*

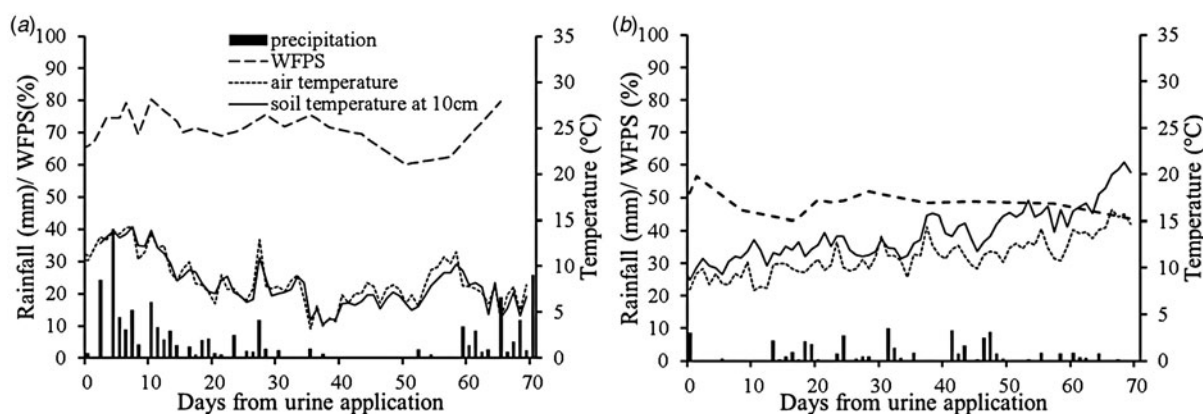| Urine patch simulation and measurement method	Patch/ collar area (m^2)	Urine volume (l/patch)	N load/patch (g N/patch)	Mean N loading (kg N/ha)	N$_2$O-N loss (mg/patch)	S.D. (mg N$_2$O-N/ patch)	Net emission relative to the NEEA (%)
HL-NEEA	0·462	2	16·6	359	434·8	156·5	100
HL-UWA	0·156	2	16·6	1064	280·6	65·8	64
HL-Control	0·156	0	–	0	5·5	1·8	–
LL-NEEA	0·462	2	10·6	229	35·1	10·2	100
LL-UWA	0·156	2	10·6	679	37·7	22·4	108
LL-Control	0·156	0	–	0	3·9	3·9	–
Pooled S.E. of the mean					31·3		
Degrees of freedom					20		

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

RESULTS AND DISCUSSION

Water-filled pore space is an important driver of N$_2$O-N loss (Smith *et al.* 1998). Conditions were not favourable for N$_2$O 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 N$_2$O-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 N$_2$O production related to temperature. Under these conditions, both urine treatments increased N$_2$O 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 N$_2$O-N loss in their experiment came from native soil N. It is likely that a significant portion of the urine-induced N$_2$O loss under HL in the current work also came from native soil N. A rapid emission peak exceeding 1100 µg N$_2$O-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 N$_2$O-N/patch/h for the UWA simulated patch (Fig. 3
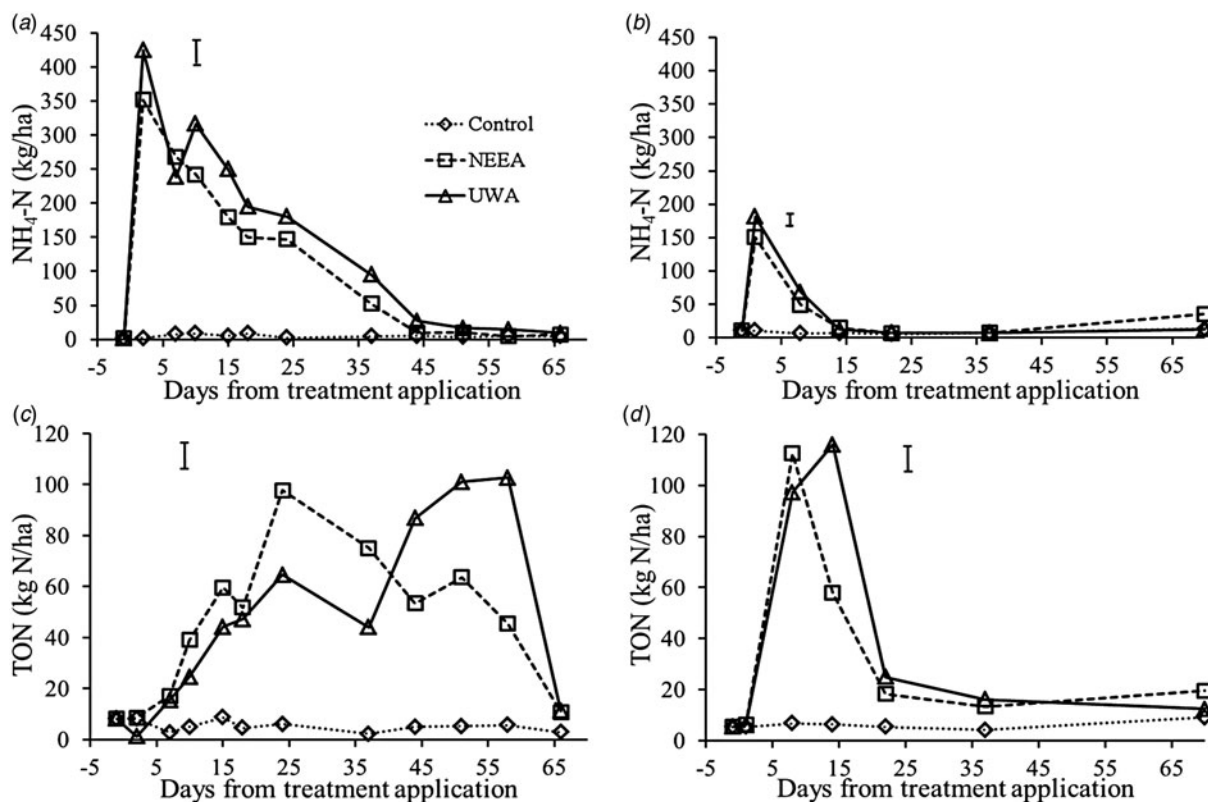
Fig. 2. Soil NH_4-N for (*a*) high loss and (*b*) low loss conditions, soil NO_2-N and NO_3-N (total oxidized N (TON)) for (*c*) high loss and (*d*) low loss conditions (0–10 cm) for naturally expanding effective area (NEEA) and uniform wetted area (UWA) methods. Error bars indicate the pooled s.e. of the mean.
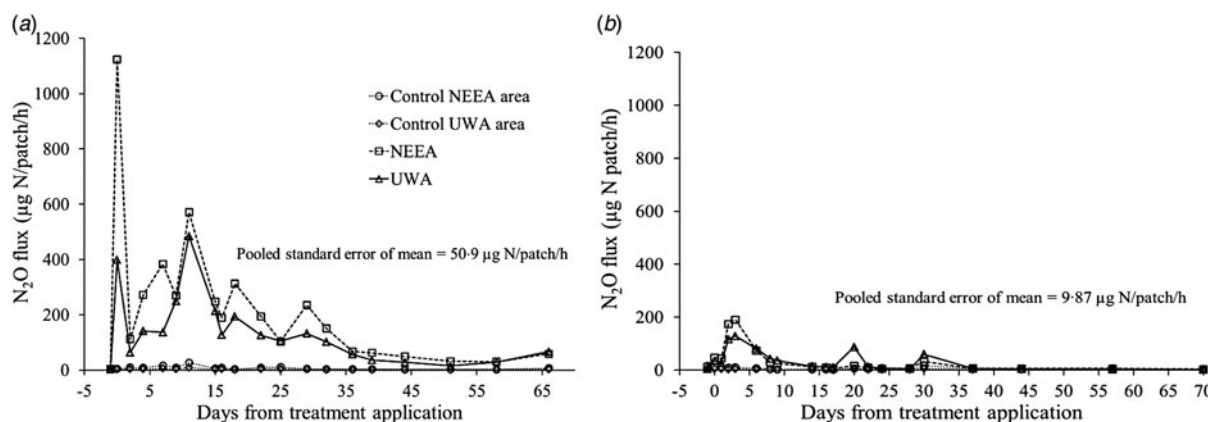

Fig. 3. Temporal flux of N_2O-N emission for (*a*) high loss and (*b*) low loss conditions in response to the uniform wetted area (UWA) and naturally expanding effective area (NEEA) urine patch simulation methods.

(*a*)). 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 as a result of the UWA method could contribute to an elevated NO$_3^-$-N pool, which persists for longer, favouring denitrification further from the time of urine application. Some evidence of such an effect is present in the LL data. A significant treatment × day of measurement interaction was detected ($P < 0\cdot001$) with two secondary peaks in emission on days 20 and 30 measured for the UWA method but not for the NEEA method (Fig. 3(*b*)). The direction of difference in N$_2$O loss between methods cannot be extrapolated from the present study to the diverse soil and environmental conditions in which researchers make N$_2$O loss estimates for urine patches. The present study simply highlights a need for greater attention to the method of urine patch simulation. 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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