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

AUTHORS FORRESTAL, P., KROL, D., LANIGAN, G., JAHANGIR, M., & RICHARDS, K.

NOTICE: This is the author's version of a work that was accepted for publication in *Journal of Agricultural Science* © Cambridge University Press 2016 the Version of Record can be accessed at doi: http://dx.doi.org/10.1017/S0021859616000939

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

Summary

Global N_2O-N inventory estimates for pasture systems are refined based on measurements of N_2O-N loss from simulated urine patches using a variety of methods but frequently using 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 L urine the UWA method was compared, using a 0.156 m^2 collar, with a naturally expanding effective area (NEEA) method, using a 0.462 m^2 collar under high (HL) and low (LL) N_2O loss conditions. The method chosen affects urine N loading to the soil. Under HL the UWA method induced a N_2O-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) use of a urine volume and N content similar to that of the animal of interest.
b) allow natural infiltration of the chosen urine volume to permit tapering toward the edges.
c) measure from the zone of influence in addition to the wetted area i.e. the patch effective area.

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

Introduction

Nitrogen (N) inputs to agricultural soils contribute to the production of the greenhouse gas, nitrous oxide (N_2O) and animal production accounts for an estimated 1.5 Tg N_2O-N/yr (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 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*. (2014) 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*. 2010). 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*. 2014). It follows that effective area of the patch 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 (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_2O 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. First, 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 33% of the N_2O measurement collar area thus ensuring the zone of influence was accounted for. .

The objective of this work was to summarise patch simulation approaches in the literature and to evaluate the hypothesis that the N_2O 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 to 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_2O loss, which occurred at a moderately drained site in autumn (HL) and ii) "low" N_2O 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, 7.3% organic matter, 3.2% total C, 0.30% total N, 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). The LL occurred on a free-draining Cambisol (58% sand, 28% silt, 14% clay, 7.9% organic matter, 3% total C, 0.32% total N, pH 5.8 0-10 cm) in spring 2014 at the Teagasc Moorepark Research Centre, Co. Cork, Ireland (52°09'N; 8° 14'W). Both sites were in long-term grassland dominated by perennial ryegrass (*Lolium perenne* L.). No organic manures or fertilisers were applied and animals were excluded for a period of at least six 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_2O measurement collars were inserted to 7 to 10 cm depth at least four days prior to treatment application. Soil volumetric moisture (0-10 cm) was measured using a Theta probe soil moisture sensor (Delta-T, Cambridge, U.K.) in the area surrounding the simulated urine patches. Soil bulk density (0-10 cm) was measured to calculate water filled pore space (WFPS) per 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 L of urine within 0.156 m^2 collars and b) NEEA which closely mimicked a natural urination by applying 2 L urine to a central point within collars of 0.462 m^2 and allowing urine migrate outward as it would naturally. Although the simulated patches originated from the same simulated urination (2 L) the UWA method resulted in a uniform volume loading of 12.8 L/m^2 and the NEEA a non-uniform urine loading with a mean of 4.33 L/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_2O-N emission associated with a urination voided by an animal which represents the unit of interest. The control treatment to measure the soil background N_2O emission (control) used a 0.156 m^2 collar. Up-scaling N_2O emissions from a chamber scale to area scales is a common practice for presenting results, in a like manner the background emission for a 0.462 m^2 area was calculated by up-scaling emissions from 0.156

99 m^2. 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 N_2O 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 discreet 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 N_2O sampling procedure of Chadwick *et al*.
121 (2014) was followed. Eight samples of ambient air were collected at each sampling. Their
122 mean N_2O concentration was set as a surrogate for N_2O concentration at time zero. The
123 assumption of a linear increase in headspace N_2O accumulation (Chadwick *et al*., 2014)

during the 40 minute 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 minute enclosure period. Of the sub-set of chambers which had a flux, 87% were linear according to the criteria of Chadwick *et al*. (2014). At the end of the 60 minute enclosure period the mean nitrous oxide concentration inside chambers in the linear group was 3.5 ppm (standard deviation 3.96 ppm). For the quadratic group it was 2.62 ppm (standard deviation 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*. (2016), 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 and 12 am 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 the HL and LL experiments, respectively. In each experiment sampling was conducted on 20 occasions with the highest sampling intensity following treatment application (Fig. 3).

Soil sampling and analysis

Soil samples (0-10 cm) were collected 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 (Fig. 2). Samples were fresh sieved using a four mm sieve, sub-sample gravimetric moisture content and mineral N content was

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

Data presentation and statistical analysis

The flux data is presented per simulated urine patch as has previously been done by Rochette *et al*. (2014). The effect of treatment and time after urine application on the dependent variables of N_2O, soil NO_3-N and 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 pooled standard error of the mean is presented in Figures. 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. This 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

WFPS is an important driver of N_2O-N loss (Smith *et al*., 1998). Conditions were not favourable for N_2O 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 patch simulation approach had no effect. In contrast, under the HL conditions precipitation occurred almost daily following urine application (Fig. 1a) and WFPS exceeded 65% for at least 40 days following urine application. Additionally, soil temperature at patch simulation, a time when N_2O-N losses are frequently greatest (Williams *et al*. 1999; Maljanen *et al*. 2007; Krol *et al*. 2015), was also three to five degrees Celsius higher. Smith *et al*. (1998) reported an exponential increase in N_2O production related to temperature. Under these conditions both urine treatments increased N_2O 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 µg N_2O-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 µg
182 N_2O-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

NEEA method was approximately 3 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 mean zone of grass response of 0.37 m^2 reported by Moir *et al*. (2010). It was also larger than any of the collars used in 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_2O 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_2O 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 x day of measurement interaction was detected ($P<0.001$) with two secondary peaks in emission on days 20 and 30 measured for the UWA method but not for the NEEA method (Fig. 3b). The direction of difference in N_2O loss between methods cannot be extrapolated from this study to the diverse soil and environmental conditions in which researchers make N_2O loss estimates for urine patches. This manuscript 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_2O 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 L for dairy cattle (Selbie *et al*. 2014).

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*. 2014) or the NEEA.

References

Anger, M., Hoffmann, C. & Kühbauch, W. (2003). Nitrous oxide emissions from artificial urine patches applied to different N-fertilized swards and estimated annual N_2O emissions for differently fertilized pastures in an upland location in Germany. *Soil Use and Management*, 19(2): 104-111.

Bell. M.J., Rees, R.M., Cloy, J.M., Topp, C.F.E., Bagnall, A. & Chadwick, D.R. (2016). Nitrous oxide emissions from cattle excreta applied to a Scottish grassland: Effects of soil and climatic conditions and a nitrification inhibitor. *Science of the Total Environment*, 508: 343-353.

Blackmer, A.M., Robbins, S.G. & Bremner, J.M. (1982). Diurnal variability in rate of emission of nitrous-oxide from soils. *Soil Science Society of America Journal*, 46(5): 937-942.

Boon, A., Robinson, J.S., Chadwick, D.R. & Cardenas, L.M. (2014). Effect of cattle urine addition on the surface emissions and subsurface concentrations of greenhouse gases in a UK peat grassland. *Agriculture Ecosystems and Environment*, 186: 23-32.

244 Cabrera, M.L. & Beare, M.H. (1993). Alkaline persulfate oxidation for determining total
245 nitrogen in microbial biomass extracts. *Soil Science Society of American Journal*, 57
246 (4), 1007–1012.

247 Chadwick, D.R., Cardenas, L., Misselbrook, T.H., Smith, K.A., Rees, R.M., Watson, C.J.,
248 Mcgeough, K.L., Williams, J.R., Cloy, J.M., Thorman, R.E. & Dhanoa, M.S. (2014).
249 Optimizing chamber methods for measuring nitrous oxide emissions from plot-based
250 agricultural experiments. *European Journal of Soil Science*, 65(2): 295-307.

251 Clough, T.J., Ray, J.L., Buckthought, L.E., Calder, J., Baird, D., O'Callaghan, M., Sherlock,
252 R.R. & Condron, L.M. (2009). The mitigation potential of hippuric acid on N_2O
253 emissions from urine patches: An in situ determination of its effect. *Soil Biology and*
254 *Biochemistry*, 41(10): 2222-2229.

255 Clough, T.J., Ray, J.L., Sherlock, R.R., Condron, L.M., O'Callaghan, M. & Buckthought,
256 L.E. (2008). Nitrous oxide-novel mitigation methodologies. Retrieved 17.09.2015,
257 from www.mpi.govt.nz/document-vault/6928

258 de Klein, C.A.M., Barton, L., Sherlock, R.R., Li, Z. & Littlejohn, R.P. (2003). Estimating a
259 nitrous oxide emission factor for animal urine from some New Zealand pastoral soils.
260 *Australian Journal of Soil Research*, 41(3): 381.

261 de Klein, C. A. M. & Harvey M. (2012). Nitrous oxide chamber methodology guidelines.
262 Retrieved 17.09.2015, from https://mpi.govt.nz/document-vault/3687

263 Fischer, K., Burchill, W., Lanigan, G.J., Kaupenjohann, M., Chambers, B., Richards, K.G.
264 and Forrestal, P.J. Ammonia emissions from cattle dung, urine and urine with
265 dicyandiamide. Soil Use and Management, 32: 83-91.

266 Hillel, D. (2004). Introduction to experimental soil physics. Elsevier Academic Press,
267 Amsterdam, 112 pp.

Jahangir, M.M.R., Khalil, M.I., Johnston, P., Cardenas, L.M., Hatch, D.J., Butler, M., Barrett, M., O'flaherty, V. & Richards, K.G. (2012). Denitrification potential in subsoils: A mechanism to reduce nitrate leaching to groundwater. *Agriculture Ecosystems and Environment*, 147: 13-23.

Kelly, K.B., Phillips, F.A. & Baigent, R. (2008). Impact of dicyandiamide application on nitrous oxide emissions from urine patches in northern Victoria, Australia. *Australian Journal of Experimental Agriculture*, 48(2): 156.

Krol, D.J., Forrestal, P.J., Lanigan, G.J., & Richards, K.G. (2015). In situ N_2O emissions are not mitigated by hippuric and benzoic acids under denitrifying conditions. *Science of the Total Environment*, 511: 352-358.

Krol, D.J., Carolan, R., Minet, E., McGeough, K.L., Watson, C.J., Forrestal, P.J., Lanigan, G.J. and Richards, K.G. (2016). Improving and disaggregating N_2O emission factors for ruminant excreta on temperate pasture soils. *Science of the Total Environment*. 568: 327-338.Lessa, A.C.R., Madari, B.E., Paredes, D.S., Boddey, R.M., Urquiaga, S., Jantalia, C.P. & Alves, B.J.R. (2014). Bovine urine and dung deposited on Brazilian savannah pastures contribute differently to direct and indirect soil nitrous oxide emissions. *Agriculture Ecosystems and Environment,* 190: 104-111.

Luo, J., Lindsey, S.B. & Ledgard, S.F. (2008). Nitrous oxide emissions from animal urine application on a New Zealand pasture. *Biology and Fertility of Soils*, 44(3): 463-470.

Luo, J., Tillman, R.W., White, R.E. & Ball, P.R. (1998). Variation in denitrification activity with soil depth under pasture. *Soil Biology and Biochemistry*, 30(7): 897-903.

Maljanen, M., Martikkala, M., Koponen, H.T., Virkajarvi, P. & Martikainen, P.J. (2007). Fluxes of nitrous oxide and nitric oxide from experimental excreta patches in boreal agricultural soil. *Soil Biology and Biochemistry*, 39(4): 914-920.

292 Minet, E.P., Jahangir, M.M.R., Krol, D.J., Rochford, N., Fenton, O., Rooney, D., Lanigan,
293 G., Forrestal, P.J., Breslin, C. & Richards, K.G. (2016). Amendment of cattle slurry
294 with the nitrification inhibitor dicyandiamide during storage: A new effective and
295 practical N_2O mitigation measure for land spreading. Agriculture, Ecosystems and
296 Environment, 215: 68-78.

297 Moir, J.L., Cameron, K.C., Di, H.J. & Fertsak, U. (2010). The spatial coverage of dairy cattle
298 urine patches in an intensively grazed pasture system. *The Journal of Agricultural*
299 *Science*, 149(04): 473-485.

300 Oenema, O., Wrage, N., Velthof, G.L., Groenigen, J.W., Dolfing, J. & Kuikman, P.J. (2005).
301 Trends in global nitrous oxide emissions from animal production systems. *Nutrient*
302 *Cycling in Agroecosystems*, 72(1): 51-65.

303 Rochette, P., Chantigny, M.H., Ziadi, N., Angers, D.A., Bélanger, G., Charbonneau, E.,
304 Pellerin, D., Liang, C. & Bertrand, N. (2014). Soil nitrous oxide emissions after
305 deposition of dairy cow excreta in Eastern Canada. *Journal of Environmental Quality*,
306 43:829–841.

307 Saarijarvi, K. & P. Virkajarvi. (2009). Nitrogen dynamics of cattle dung and urine patches on
308 intensively managed boreal pasture. *Journal of Agricultural Science*. 147:479–491.

309 Selbie, D.R., Buckthought, L.E. & Shepherd, M.A. (2014). The challenge of the urine patch
310 for managing nitrogen in grazed pasture systems. *Advances in Agronomy*, vol. 129:
311 229-292.

312 Smith, K.A., Thomson, P.E., Clayton, H., McTaggart, I.P. and Conen, F. (1998). Effects of
313 temperature, water content and nitrogen fertilisation on emissions of nitrous oxide by
314 soils. *Atmospheric Environment*, 32(19): 3301-3309.

315 Sordi, A., Dieckow, J., Bayer, C., Alburquerque, M.A., Piva, J.T., Zanatta, J.A., Tomazi, M.,
316 da Rosa, C.M. & de Moraes, A. (2014). Nitrous oxide emission factors for urine and

dung patches in a subtropical Brazilian pastureland. *Agriculture, Ecosystems and Environment*, 190: 94-103.

Taghizadeh-Toosi, A., Clough, T.J., Condron, L.M., Sherlock, R.R., Anderson, C.R. & Craigie, R.A. (2011). Biochar incorporation into pasture soil suppresses in situ nitrous oxide emissions from ruminant urine patches. *Journal of Environmental Quality*, 40(2): 468-476.

van der Weerden, T.J., Luo, J., de Klein, C.A.M., Hoogendoorn, C.J., Littlejohn, R.P. & Rys, G.J. (2011). Disaggregating nitrous oxide emission factors for ruminant urine and dung deposited onto pastoral soils. *Agriculture, Ecosystems and Environment*, 141(3-4): 426-436.

Wachendorf, C., Lampe, C., Taube, F. & Dittert, K. (2008). Nitrous oxide emissions and dynamics of soil nitrogen under N-15-labeled cow urine and dung patches on a sandy grassland soil. *Journal of Plant Nutrition and Soil Science*, 171: 171-180.

Williams, D.L., Ineson, P. & Coward, P.A. (1999). Temporal variations in nitrous oxide fluxes from urine-affected grassland. *Soil Biology and Biochemistry*, 31(5): 779-788.

Williams, P.H. & Haynes, R.J. (1994). Comparison of initial wetting pattern, nutrient concentrations in soil solution and the fate of N-15-labeled urine in sheep and cattle urine patch areas of pasture soil. *Plant and Soil*, 162: 49-59.

List of Tables

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

Table 2. Effect of the uniform wetted area (UWA) and naturally expanding effective area (NEEA) urine patch simulation methods on N_2O-N loss.

List of Figures

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

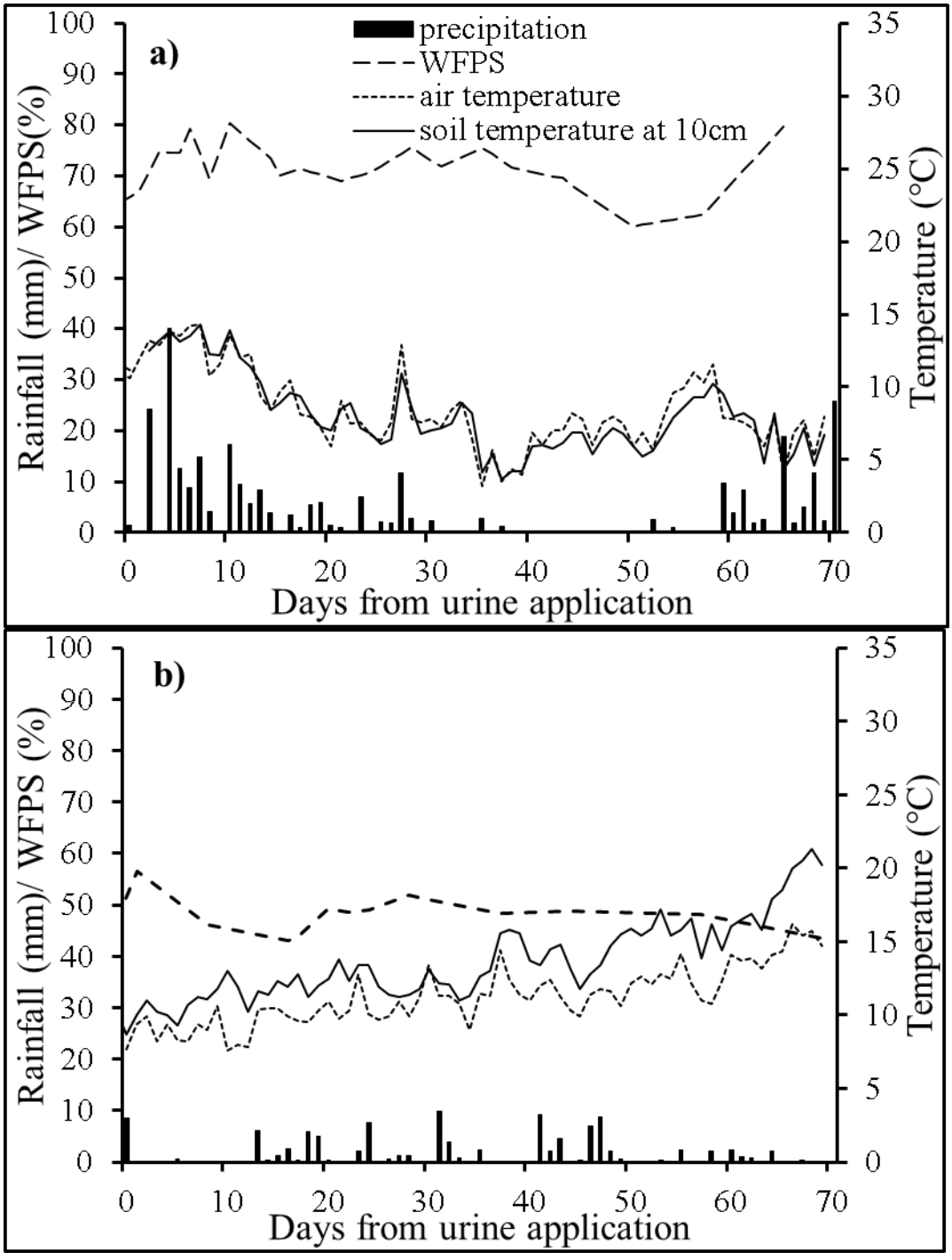
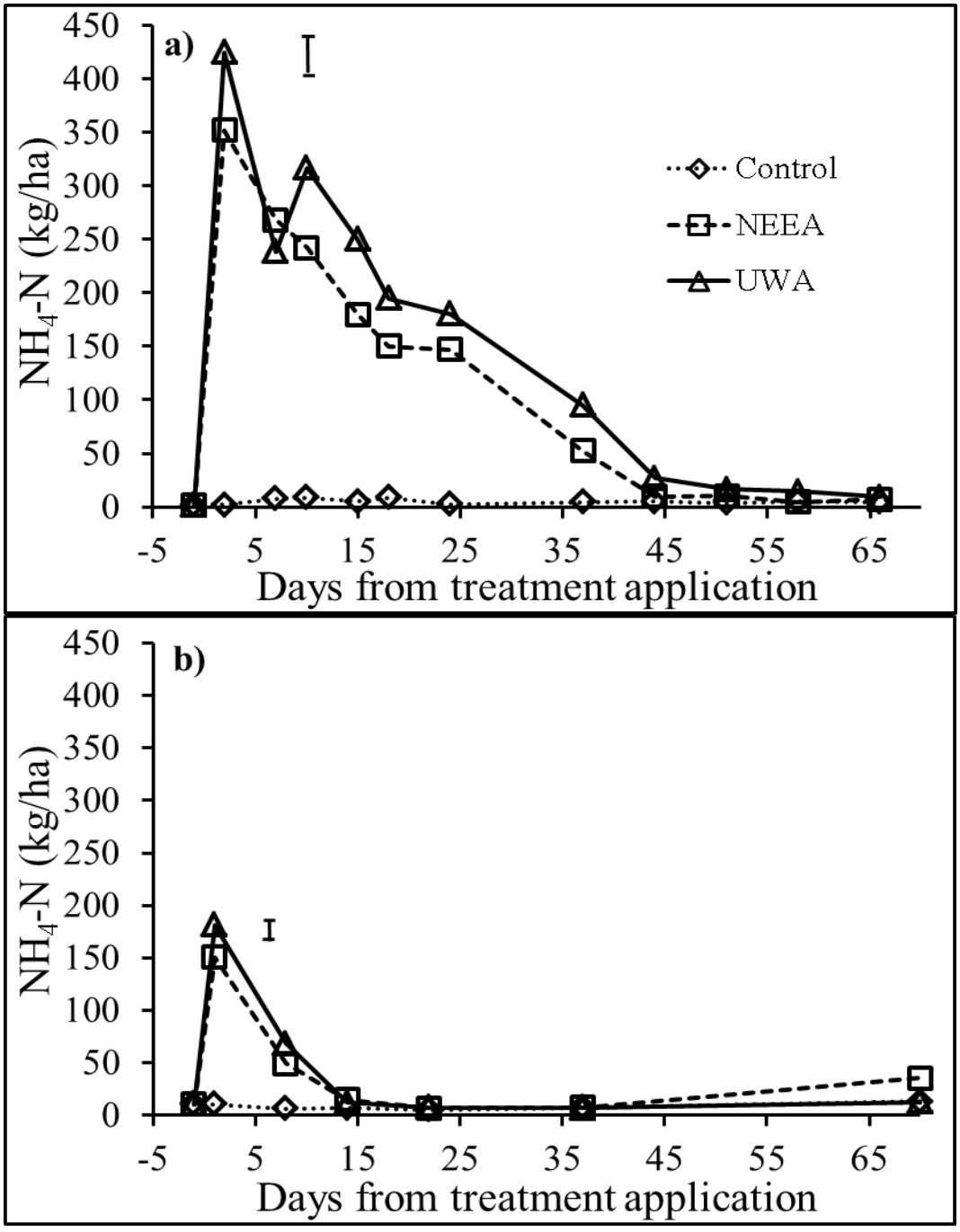
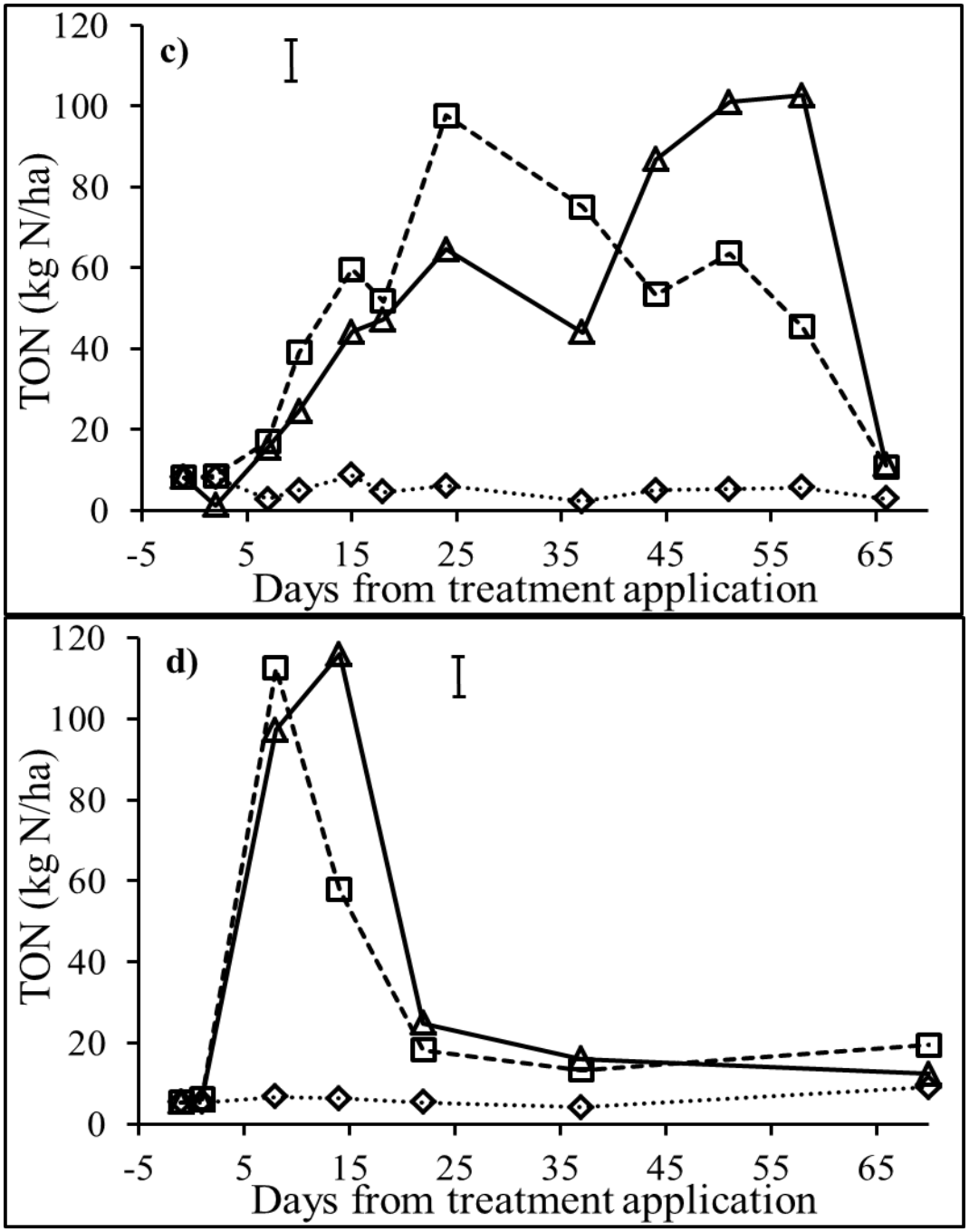


Fig. 2. Soil NH_4-N for a) high loss and b) low loss conditions, soil NO_2-N and NO_3-N [total oxidisable N (TON)] for c) high loss and d) low loss conditions (0-10 cm) for naturally expanding effective area (NEEA) and uniform wetted area (UWA) methods. Error bars indicate the pooled standard error of the mean.


a)
b)
NH_4-N (kg/ha)
Days from treatment application
Control
NEEA
UWA
450
400
350
300
250
200
150
100
50
0
-5
5
15
25
35
45
55
65

373

374



375

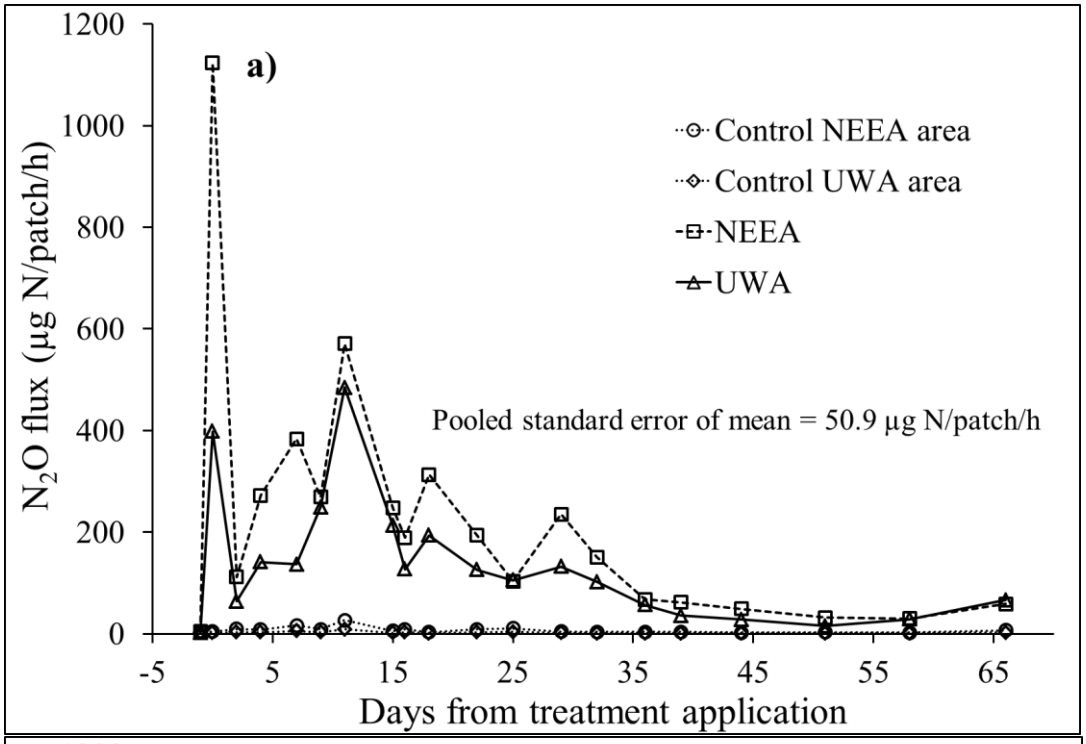
376

377

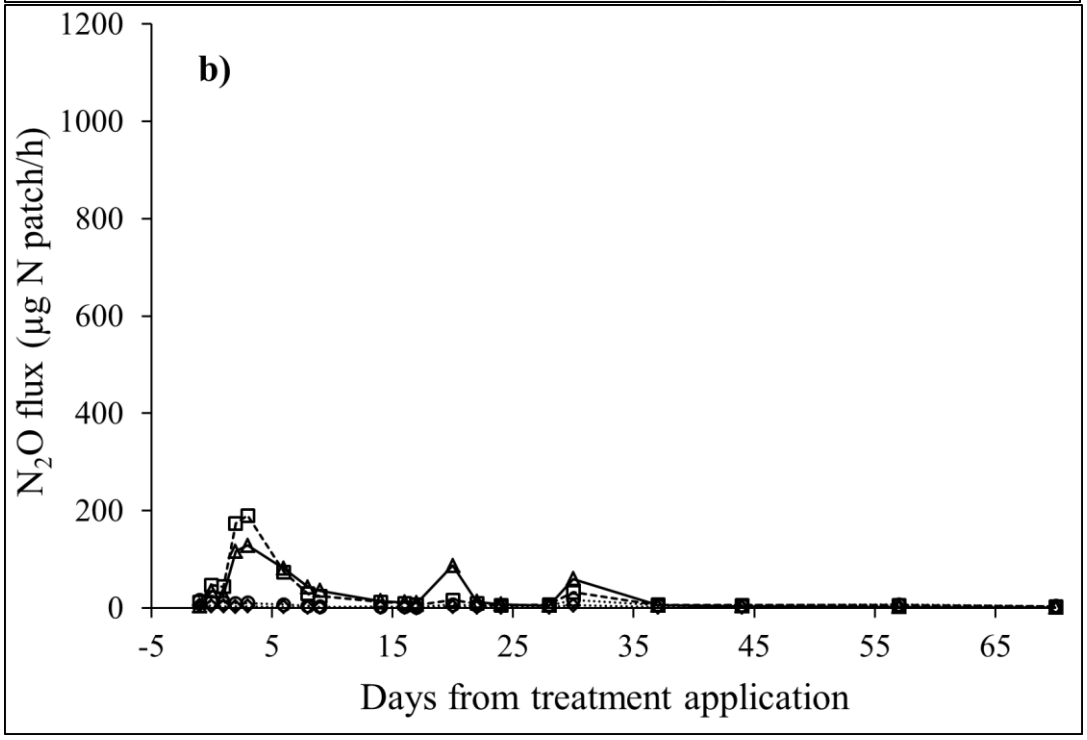
378 Fig. 3. Temporal flux of N_2O-N emission for a) high loss and b) low loss conditions in

379 response to the uniform wetted area (UWA) and naturally expanding effective area (NEEA)

380 urine patch simulation methods.



381



382

383

384

385

386

Table 1. A selection of studies using urine patch simulation for nitrous oxide loss 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	
0.1164	*0.1164*	865, 911	1	*8.6*	10.07, 10.6	Install collar, urine within
0.0962	*0.0962*	1030	*1.0*	*9.9*	10.4	Urine poured into 0.0962 m^2 ring, install 0.283 m^2 pvc ring and sealing area between internal ring and external ring
0.1195	*0.1195*	930	*1.1*	*9.3*	10	Install collar, urine within,
0.083	0.083	890 – 3920	*1.0, 2.0, 3.0*	*11.9-35.6*	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	*10*	3, 5, 7, 10	Lysimeter installed, urine within
0.0491	0.5	592	*0.49*	10	5.92	Uniform urine plot, install collar
0.0491		1000				
0.0491	*0.5*	496-551	*0.49*	10	4.96-5.51	Uniform urine plot, install collar
0.16	2	498	*0.8*	5	6.7	Uniform urine plot, install collar
0.0314	*0.36*	420	1.8	5	8.4	Uniform urine plot, install collar
0.24	0.2	*842*	2	*8.3*	*10.1*	Patch smaller than collar formed
0.303	0.1	*92 - 481*	0.9 – 1.4	*3.0 – 4.6*	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

Values in italic are calculated from information provided in papers.

Table 2. Effect of the uniform wetted area (UWA) and naturally expanding effective area (NEEA) urine patch simulation methods under high loss (HL) and low loss (LL) conditions on N_2O-N loss.

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_2O-N loss mg/ patch	Standard deviation mg N_2O-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		

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