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1 **Organic matter and nitrogen balance in rabbit fattening and gaseous** 2 **emissions during manure storage and simulated land application**

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4

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10

11 **Abstract**

12 Expansion in global rabbit populations and in the number of rabbits raised for consumption
13 necessitates assessment of the environmental impact and sustainability of rabbit production systems.
14 This study undertook two evaluations: utilization (animal efficiency) of organic matter (OM) and
15 nitrogen (N) produced from feed during rabbit fattening, and emission of ammonia (NH₃), carbon
16 dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O) from rabbit manure during its storage and
17 land application in laboratory-simulated conditions. Results demonstrated manure contained
18 approximately 30 and 65% of the OM and N in the daily feed intake, respectively. Additionally, rabbit
19 manure was shown to produce sizeable NH₃ and GHG emissions. Specifically, cumulative N losses
20 from storage and subsequent land application averaged 32.4 (NH₃) and 2.2% (N₂O) of excreted TN;
21 cumulative proportions of OM lost as CO₂ and CH₄ averaged 51.3 and 0.4%, respectively. Finally,
22 while manure incorporation into the soil effectively abated NH₃ emissions, it also showed its potential
23 to increase N₂O losses, a potent greenhouse gas (GHG). Future research should focus on identifying
24 appropriate emission mitigation measures. Accurate field-scale measurements are also needed to

25 make data available for the development of a reliable national emission inventory of the investigated
26 gases.

27

28 **Keywords**

29 Nutrient excretion, Ammonia, Greenhouse gases, Environmental impact.

30

31 **1. Introduction**

32 Raising rabbits is an active industry in several countries. The United Nations Food and Agriculture
33 Organization estimates that rabbit livestock production has grown an average of 2.6% in each of the
34 past 10 years; approximately 770 million rabbits are now counted each year worldwide (FAO-STAT,
35 2016). Currently, Asia accounts for about 83% of global rabbit production. Europe represents 14%
36 of the market, while Africa (2%) and the Americas (1%) follow distantly. Sixty-eight percent of the
37 107 million rabbits counted in Europe in 2014 were in Italy, which made it the top European producer
38 and second world producer of rabbit meat (262,500 t in 2013) (FAO-STAT, 2016). As is true for
39 other livestock sectors (e.g., cattle, pigs, poultry), about 80% of total rabbit production is concentrated
40 in the north of Italy. However, rabbit fattening farms in Italy are distributed differently; 50% are
41 located in the south, 30% in the central, and 20% in the north of the country (Filiou, 2015).

42 Intensification and specialization of livestock farming systems is widely recognized as a major cause
43 of environmental pollution (FAO, 2013) mainly due to the increased amount of manure produced by
44 animals per unit of utilized agricultural area. Two topics of major importance are surface and
45 groundwater nitrate contamination as a result of runoff and soil leaching, and atmospheric impacts
46 from ammonia (NH₃) and greenhouse gas (GHG) emissions, namely carbon dioxide (CO₂), methane
47 (CH₄), and nitrous oxide (N₂O) (Capri et al., 2009; Sanz-Cobena et al., 2017).

48 Ammonia and GHG production and release are influenced by the physical-chemical characteristics
49 of the manure (especially pH, dry matter - DM, organic matter - OM, total nitrogen – TN, and total
50 ammoniacal nitrogen - TAN), which in turn are determined primarily by feeding factors (such as feed

51 intake and diet digestibility, protein content and solubility, fiber content and degradability, presence
52 of antinutritional substances), and to a lesser extent by animal age, housing system, environmental
53 conditions, and production stage (e.g., Monteny et al., 2001; Ndegwa et al., 2008; Gerber et al., 2013;
54 Sheppard et al., 2013). However, information on the nature of manure production and N excretion
55 from rabbit production systems under specific conditions in Italy is scarce, especially from the
56 fattening period. Similarly, even though ammonia and GHG emissions may originate from any or all
57 stages of rabbit manure management (i.e., production, storage, and application), the relative share of
58 NH₃ and GHG emissions associated with the rabbit sector has yet to be precisely determined due to
59 a lack of data. Most available emission data refer to those released from buildings during rearing
60 (Estellés et al., 2010a; Estellés et al., 2010b; Calvet et al., 2011; Méda et al., 2014). Only a few data
61 on emissions from rabbit manure storage (Estellés et al., 2014) have been reported, whereas little or
62 none exists on emissions from application to land.

63 What makes investigation and quantification of these N processes important at this time is the
64 convergence of three facts. One, widespread expansion of the rabbit livestock farming and their
65 increasing numbers make it necessary to assess the environmental impact in relation to the production,
66 storage, and utilization of manure as a crop fertilizer. Two, information is available on NH₃ and GHG
67 emissions from rabbit manure managements in estimates produced by the Italian national inventory
68 from a combination of default emission factors and personal communications from researchers and
69 sector experts (Valli and Condòr, 2011; ISPRA, 2016). Three, rabbits have a particular digestive
70 strategy (coprophagy) that may affect hindgut fermentation and potentially, gaseous production from
71 manure.

72 To this end, this study investigated two related processes of rabbit production. The first aim evaluated
73 the efficiency of the use of OM and N during rabbit rearing and fattening under intensive conditions.
74 The second explored, under controlled laboratory conditions, the NH₃, CO₂, CH₄, and N₂O emissions
75 from rabbit manure during its storage and subsequent land application by analysing and comparing
76 two manure managements: 1) storage followed by application to the soil surface (S+SA), and 2)

77 storage followed by application to the soil surface with incorporation into the soil at six hours after
78 surface application (S+MI).

79 Scenario S+SA represents the traditional and most common rabbit manure application practice,
80 whereas S+MI represents a technique increasingly adopted by Italian farmers to reduce the
81 environmental impacts of NH₃ emissions. This relatively new practice aligns with current national
82 regulations (DL 152/2006) that recommend incorporating solid manure into the soil within 24 hours
83 of broadcasting.

84

85 **2. Materials and methods**

86 All procedures involving animals were conducted according to Italian law (DL 146/2001) governing
87 animal welfare in scientific experiments.

88

89 *2.1. Animals, their housing, and their diet*

90 The manure was collected from a rabbit farm (Carmagnola, Torino, Italy; 44°53' N, 7°41' E, at an
91 altitude of 240 m a.s.l.), in which 120 rabbits (weaned crossbred Grimaud × Hycole, 35 days of age,
92 935 g live weight - LW) were reared under semi-controlled environmental conditions (temperature
93 23±5 °C, light length >8 h) in individual California type cages (30×30×40 cm; 0.12 m² head⁻¹) for 55
94 days (90 d of age, 3275 g LW). The animals were fed a standard pelleted diet *ad libitum* (Table 1)
95 and had free access to clean drinking water.

96

97 *2.2. Preparation of samples for analyses*

98 During a four-day period (at 42 to 45 days of age; Perez et al., 1995), feed intake was measured and
99 recorded for each of 12 randomly selected rabbits (1:1 sex ratio) housed in individual metabolic cages
100 that separately collected faeces and urine (Perez et al., 1995). Feed intake was measured by weighing
101 daily the individual feed administration and the remnant feed. Losses of feed during the rearing period
102 were considered negligible, as the pellet form of the feedstuff enabled the rabbits to feed efficiently

103 and prevented feed falling from the manger to the wire-mesh floor of the cage. Faeces and urine
104 samples were also collected from the same individuals during the same period. Each sample was
105 placed in a two-layer plastic bag to prevent moisture loss, and immediately frozen at -20 °C. Each
106 sample was then thawed, mixed thoroughly, pooled, and then ground in a homogenizer (Tecator,
107 Herndon, VA). Representative sub-samples were then taken and weighed in an aluminium foil pan,
108 dried in a draft oven at 80 °C to a constant weight, and then stored for later chemical analysis. The
109 excreta (urine and faeces) produced by the same group of rabbits were also collected each day for six
110 days (48-53 days of age) and maintained at 4 °C for the gaseous emission trials. Each individual
111 rabbit raised in each cage represented one replicate.

112

113 *2.3. Feed and manure pH measurement and chemical analysis*

114 A Crison portable pH-meter (Crison Instruments, S.A., Alella, Spain) fitted with a spear-type,
115 automatic temperature compensation electrode was used for all pH measures. Proximate composition
116 analyses of the diet (Table 1) and faeces were performed on duplicate samples according to the
117 following AOAC (2006) methods: preparation of analytical sample (950.02), dry matter (DM)
118 content (934.01), OM (942.05), total nitrogen (TN) and crude protein (CP) content (984.13), total
119 ammoniacal nitrogen (TAN) content (941.04), ether extract (EE) content (2003.05), neutral detergent
120 fibre (NDF) content (2002.04), acid detergent fibre (ADF) and acid detergent lignin (ADL) content
121 (973.18). The acid-insoluble ash (AIA) content of the diet and faeces was determined using the Van
122 Keulen and Young (1977) method while gross energy (GE) was measured with an adiabatic
123 calorimeter bomb (IKA C7000, Staufen, Germany).

124

125 *2.4. Determination of Organic matter balance and digestibility*

126 The organic matter balance was calculated via the general equation to estimate OM excretion:

127
$$\text{OM excretion} = \text{OM intake} - \text{OM digestible}$$

128 where: OM intake is the amount of OM contained in feed consumed during the entire raising period
129 (kg), and OM digestible is the amount of OM (kg) calculated as follows:

$$130 \quad \text{OM digestible} = \text{Feed Intake (kg)} \times \text{Feed OM\%} \times \text{Feed OM apparent digestibility \%}$$

131 in which: the indirect digestibility method (Furuichi and Takahashi, 1981) using AIA as an inert
132 marker was employed to determine Feed OM apparent digestibility.

133

134 *2.5. Nitrogen balance and crude protein digestibility*

135 The nitrogen balance was calculated according to the final report of the European Commission
136 Directorate General XI (ERM/AB-DLO, 1999) following the general equation:

$$137 \quad \text{N excretion} = \text{N intake} - \text{N gain}$$

138 where: N excretion is the entire amount of N excreted by rabbits during raising (kg), N intake is the
139 amount of N contained in feed consumed during the same period (kg), and N gain is the amount of N
140 in rabbit live weight (LW) gain in the considered period (kg).

141 Additional measures were calculated as follows: N intake was determined by dividing feed CP by
142 6.25; N gain was determined by applying an N concentration for LW gain of 3.1% (Xiccato et al.,
143 2005) to the difference in initial and final animal weights; N efficiency equalled the ratio of N gain
144 to N diet; the Furuichi and Takahashi (1981) method produced the Feed CP apparent digestibility
145 measure.

146

147 *2.6. NH₃ and GHG emissions from storage and simulated land application of rabbit manure*

148 After the six-day collection period, the faeces and urine excreted from each selected rabbit were
149 accurately mixed. Then, 0.50 kg sub-samples of the 12 animal-specific manure mixtures were placed
150 in 1.5 dm³ vessels (Ø 0.113 m) and stored for 35 days in a climate controlled room at 20 ± 0.5 °C to
151 simulate farm storage before field spreading. The 20 °C temperature used in this study was chosen to
152 approximate the annual average air temperature of most rabbit production areas in Italy. In practice,
153 the duration of rabbit manure storage in Italy can vary and be for as long as 2–3 months. Bulk density

154 of the investigated animal-specific manure mixtures was measured with three replicates by using a
155 cubic box with an inner volume of 1 dm³. Following the recommendations of the EN 13080 (2002)
156 Standard, the box was completely filled with randomly collected manure sub-sample and carefully
157 levelled without compaction. The bulk density (ρ , in kg dm⁻³) was then determined after measuring
158 the total mass (kg) of manure introduced into the box. The average bulk density of the investigated
159 manure mixtures was found to be 0.60 kg dm⁻³, a value that agrees well with the weight to volume
160 ratio of 0.62 suggested by official guidelines (Regione Piemonte, 2007) for rabbit manure heaps
161 stored under typical farm conditions in Italy. Emissions of NH₃, CO₂, CH₄, and N₂O from each stored
162 manure sample (n=12) were measured by a dynamic chamber method using a gas trace analyser
163 (described in 2.7. section). Measurements were performed and recorded three times per week for the
164 first two weeks, twice weekly for the next two weeks, and once during the final week, for a total of
165 11 times during the 35-day experimental period.

166 At the beginning and end of the storage period, all investigated manure samples (n=12) were also
167 analysed for DM, OM, TN, TAN, fibre content (NDF, ADF, ADL), and pH using the procedures
168 previously described.

169

170 *2.7. Simulated land application trial*

171 After storage, replicate manure samples were collected and used in a simulated land application
172 experiment. Each experimental unit consisted of a cylindrical glass vessel (volume = 1.5 dm³; \varnothing
173 0.113 m) filled with 1 dm³ of loam soil (USDA, 1999). The loam soil used in the experiment had 48.4
174 g kg⁻¹ sand, 43.1 g kg⁻¹ silt, 8.5 g kg⁻¹ clay; pH = 7.93, total organic carbon = 8.79 g kg⁻¹, N = 0.18 g
175 kg⁻¹. The soil samples came from a farm field cultivated mainly with maize for grain in rotation with
176 wheat, and were representative of arable soil types of the western Po River plain (Northern Italy).
177 After collection, the soil was sieved through a 4 mm screen and stored in moist form, in the dark, at
178 4 °C prior to trial start. The bulk density (1160 g dm⁻³) of the undisturbed soil was achieved by shaking
179 the vessels until the required soil volume was reached.

180 A replicate sample of each individual rabbit manure mixture was homogeneously applied to the soil
181 surface of each vessel at a rate of 300 kg N ha⁻¹ (36.6 t ha⁻¹ of manure on a wet weight basis). The
182 choice to use 300 kg N ha⁻¹ considered manure inputs (270-320 kg N ha⁻¹) typically used in Italy for
183 maize, the most frequently cultivated in either livestock or non-livestock Italian farms. However, in
184 areas designed as Nitrates Vulnerable Zones (NVZs), or approximately 15% of Italian agricultural
185 land (Bassanino et al., 2007), European Union Nitrate Directive 91/676/EEC, aimed at protection of
186 waters from agriculturally-sourced nitrate pollution, mandates that manure N application rates not
187 exceed 170 kg ha⁻¹ year⁻¹. After application, manure mixtures were randomly selected either to remain
188 on the soil surface for the remainder of the investigation (SA) or to be manually incorporated (MI)
189 into the soil six hours after its application. In addition, non-fertilized soil was used as a control. The
190 number of replicates was 12 for SA, 12 for MI and 4 for the control. In the soil application trials, the
191 vessels were maintained in a climate controlled room at 20 ± 0.5 °C.

192 At the time of manure application, the gravimetric soil moisture content equalled 198 g kg⁻¹,
193 corresponding to 52.5% water-filled pore space (WFPS) based on the bulk density of the soil and
194 assuming particle density equalled to 2.65 g dm⁻³ (Zavattaro and Grignani, 2001). The application
195 experiment continued for 35 days, during which NH₃, CO₂, CH₄, and N₂O gas emission measurements
196 were taken at the following time intervals (days): immediately after manure application (t=0), at 0.25
197 (i.e., 6 h), and at day 1, 2, 3, 4, 7, 9, 11, 14, 16, 18, 21, 25, 28, 32, and 35 days after manure application.
198 The trial was suspended when two consecutive measurement sessions of the emission fluxes for all
199 investigated gases from the manure-amended soils SA and MI failed to be significantly different than
200 those recorded from the control (i.e., unfertilized soil). During the experimental period the soil
201 moisture content was kept constant (198 g kg⁻¹), gravimetrically adjusted for each individual jar,
202 always after a flux measurement session.

203 Gaseous emissions from both the storage and simulated land application trials were measured with
204 the dynamic chamber method using a gas trace analyser (1412 Photoacoustic Multi-gas Monitor,
205 Innova Air Tech Instruments) per the procedures described by Dinuccio et al. (2008; 2013a). Briefly,

206 prior to emission measurements, each vessel was sealed with a lid equipped with two ports—an air
207 inlet and outlet. The inlet was connected and sealed to a pump and flow meter. In all experiments the
208 air exchange was adjusted by the flow meter to guarantee a complete air exchange per minute,
209 according to the experimental set-up adopted by Balsari et al. (2008) to assess farmyard manure heap
210 NH₃ emissions using a large open dynamic chamber technique. Specifically, the airflow rate was set
211 to 0.04 m³ h⁻¹ for storage and to 0.03 m³ h⁻¹ for the land application trials. After lid placement, the
212 headspace was ventilated for 10 minutes with compressed air to create an airflow through the dynamic
213 chamber and to achieve steady conditions. Gas sampling was performed for the ensuing 10 minutes;
214 after which the lid was removed and the procedure repeated with the next vessel. The NH₃, CO₂, CH₄,
215 and N₂O readings (mg m⁻³) by the photoacoustic analyser were converted into fluxes that left each
216 vessel as follows:

$$217 \quad F = Q (C_{\text{out}} - C_{\text{in}}) / A$$

218 where: F (mg m⁻² h⁻¹) is the gas flux, Q is the airflow rate (m³ h⁻¹) dosed to the vessels, C_{in} (mg m⁻³)
219 is the air inlet gas concentration, C_{out} (mg m⁻³) is the vessel air outlet gas concentration, and A (m²)
220 is the emitting surface area of the vessel. In the land application trial, net NH₃, N₂O, CO₂, and CH₄
221 emission fluxes were calculated as the difference between emission fluxes recorded from the amended
222 soils and those from the control.

223 The cumulative emissions (g mg⁻²) of each gas recorded during the complete measurement period
224 were calculated (Pampuro et al., 2016) and expressed in CO₂-eq (IPCC, 2013) using conversion
225 factors of 1, 28, 265, and 2.65 for CO₂, CH₄, N₂O, and NH₃, respectively.

226 Finally, using the emission data recorded during the experiment and after accounting for OM and N
227 excretion, it was possible to estimate the emission factors (EFs) of NH₃, N₂O, CO₂, and CH₄ for the
228 full rabbit raising period using traditional rabbit manure management practices for storage and land
229 application. The investigated gas EFs were expressed as follows: 1) grams of N lost as NH₃ and N₂O
230 per kg of excreted TN (i.e., gNH₃-N kg⁻¹ excr. TN; gN₂O-N kg⁻¹ excr. TN); grams of OM lost as CO₂
231 and CH₄ per kg of excreted OM (i.e., gCO₂ kg⁻¹ excr. OM; gCH₄ kg⁻¹ excr. OM); grams per year per

232 livestock unit (LU) ($\text{g year}^{-1} \text{LU}^{-1}$). The latter calculation assumed six 55-day fattening cycles per
233 year.

234

235 *2.8. Statistical analyses*

236 Single emission data ($\text{g m}^{-2} \text{min}^{-1}$) of all investigated gases at each reading time were analysed after
237 testing their normal distribution (Shapiro-Wilk test) using the GLM ANOVA procedure (IBM SPSS
238 Statistics 22.0, SPSS Inc., Chicago, IL) with the following model:

$$239 \quad y = \mu + \alpha_i + \varepsilon_{ij}$$

240 where: μ = general mean; α_i = session effect; ε_{ij} = random error effect.

241 Their differences in mean values were tested by Tukey's test with a first class error value set at $\alpha=0.01$
242 to accept the differences as significant. Cumulative emission data (g m^{-2}) were analysed by the GLM
243 Repeated Measures procedure (IBM SPSS Statistics 22.0, SPSS Inc., Chicago, IL) with the following
244 model:

$$245 \quad y = \mu + \alpha_i + \tau_j + \varepsilon_{ij}$$

246 where: μ = general mean; α_i = session effect; τ_j = time effect; ε_{ij} = random error effect.

247 Their differences in mean values were tested by the multiple comparisons test in ANOVA with a first
248 class error value set at $\alpha=0.01$ to accept the differences as significant.

249 The effects of storage on manure characteristics were also tested by analysis of variance (ANOVA).

250

251 **3. Results and discussion**

252

253 *3.1. Manure composition: fresh versus stored*

254 The proximate composition of the freshly collected rabbit manure is reported in Table 2. Its average
255 DM content was 20.9%. Its OM content equalled 18.0%, whereas TN content averaged 33.7 g kg^{-1}
256 DM or $7.04 \text{ kg N Mg}^{-1}$ of fresh manure.

257 During storage, rabbit manure composition changed considerably. Specifically, the DM content of
258 stored manure significantly ($P<0.01$) increased by 32.4% relative to fresh manure, due to water
259 evaporation that typically occurs during this management phase (Dinuccio et al., 2008). In contrast,
260 during the 35-day storage period, the OM/DM ratio and TN content of manure decreased by 4.5%
261 and 11.9% ($P<0.01$), respectively, while the TAN content fell slightly. This resulted from the
262 concomitant occurrence of biological (e.g., organic matter degradation) and chemical (e.g.,
263 conversion of ammonium ions to ammonia and vice versa) processes, all of which are associated with
264 the formation and emissions of gaseous compounds from manure during storage (Dinuccio et al.,
265 2008; Petersen et al., 1998; Moral et al., 2012).

266 The lignin content of fibres in manure was also modified during the 35 days of storage, as evidenced
267 in its average decrease of 28.1%, which reflected the predominantly aerobic conditions in the stored
268 manure. Degradation of lignin is a complex process (Pérez et al., 2002) promoted by the action of
269 specific enzymes from different fungi and bacterial species. Although specialized microorganisms
270 can degrade lignin in either aerobic or anaerobic conditions (De Angelis et al., 2013), the majority of
271 anaerobes cannot. There are indications that the enzymatic reactions that promote lignin
272 depolymerisation decrease in oxygen-depleted conditions (Janusz et al., 2017). Furthermore,
273 Dinuccio et al. (2013b) suggested that in a strict anaerobic environment, lignin degradation may be
274 completely inhibited.

275

276 *3.2. Organic matter and nitrogen balances*

277 During the rabbit raising period, 144.4 g head⁻¹ (SEM 8.06) of fresh urine and faeces were produced
278 daily. The faecal balance analyses (Table 3) found it contained approximately 30% of the OM and
279 53% of the N consumed in daily feed. The N concentration in urine and faeces averaged 0.3% (SEM
280 0.03) and 1.3% (SEM 0.04) on fresh basis, respectively. Organic matter and N balances, as well as
281 the N partition value, in rabbit excreta are crucial inputs to estimation of the gaseous emissions linked
282 to rabbit manure management. Carbon dioxide and CH₄ are primarily formed by microbial

283 degradation of OM in faeces (Estellés et al., 2010b, 2014), while most of the N in the rabbit diet is
284 excreted as urea in urine, which is rapidly converted by the microbial enzyme urease to ammonium
285 (NH_4^+), which is a precursor in the pathway to NH_3 and N_2O emissions. Because urease is not usually
286 present in urine (Montes et al., 2013), its presence in manure was assumed to be associated with its
287 having come in contact with rabbit faeces.

288 Organic matter and N excreted by faeces and urine are closely related to feed OM and CP apparent
289 digestibility (Table 3), which reflect not only the conditions and diet used in the experimental, but
290 also those typically used at commercial rabbit farms. Hagen et al. (2015) tested different rabbit
291 fattening diets and reported an average OM apparent digestibility of 45% with a hay-only diet and
292 72% with a seed mix plus fresh parsley diet, while Samkol et al. (2006) reported that a water spinach
293 and broken rice diet yielded a higher OM digestibility value (81-83%). The CP apparent digestibility
294 of the diet used in this study fell within the range (76-80%) of values reported for typical rabbit
295 fattening diets (Xiccato et al., 2002; Samkol et al., 2006). Furthermore, comparable results were
296 reported by studies that assessed feed CP apparent digestibility in relation to animal age: 77-78% in
297 rabbits between 32 and 36 days of age (Gutierrez et al., 2003), 75% in rabbits between 49 and 52 days
298 of age (Pascual et al., 2007), and 76% in rabbits between nine and ten weeks old (Fernandez et al.,
299 1994). On the contrary, we observed higher levels of N excretion, and consequently lower N
300 efficiency, than generally reported in the literature (Maertens et al., 2005; Calvet et al., 2008), which
301 most likely relates to the higher rabbit slaughter weight (over 3 kg) preferred by the Italian market.
302 Available data suggest that N excretion rises with rabbit weight and age. In Spain, where rabbits are
303 fattened with a diet of 16% CP and slaughtered between 2.0 and 2.2 kg, Calvet et al. (2008) calculated
304 total excretions ranging from 40.1 to 42.4 g N kg^{-1} LW and a N efficiency of about 42%. Maertens et
305 al. (2005) reported excretions of 38 g N kg^{-1} LW for rabbits fed with 16.5% CP and slaughtered
306 between 2.0 and 2.8 kg.

307

308 *3.3. NH_3 and GHG emissions from storage of rabbit manure*

309 Fig. 1 shows average emission fluxes for all investigated gases recorded at each reading during the
310 storage trial. Ammonia and N₂O emissions were low when storage began, but increased rapidly over
311 time. Peak rates of 315.0 mg NH₃ m⁻² h⁻¹ and 1.8 mg N₂O m⁻² h⁻¹ were recorded on day 7, and then
312 fell rapidly to result in average values of 249.1 mg NH₃ m⁻² h⁻¹ and 0.7 mg N₂O m⁻² h⁻¹ at the end of
313 the storage period. In contrast, the highest CH₄ emissions occurred very early on (day 1: 132.2 mg m⁻²
314 h⁻¹), and then quickly decreased to 30.1 mg m⁻² h⁻¹ on day 4. At the end of the trial, the average CH₄
315 flux rate was less than 20.0 mg m⁻² h⁻¹. Average CO₂ flux rates ranged from 5594.9 on day 2 to 6680.4
316 mg m⁻² h⁻¹ on day 29, decreasing only slightly at the end of the storage period.

317 Overall, NH₃, N₂O, CH₄, and CO₂ emission fluxes during the experimental 35-day storage period
318 averaged 231.8, 0.9, 43.6, and 6296.9 mg m⁻² h⁻¹, respectively. In rabbit manure storage tests
319 conducted in the laboratory using dynamic chambers, Estellés et al. (2014) measured N₂O, CO₂, and
320 CH₄ fluxes at magnitudes similar to those we recorded. However, NH₃ emission results were
321 noticeably lower (average of 26.4 mg m⁻² h⁻¹) than those found in our study, which can be mainly
322 attributed to the approximately two-fold increase in manure N content exhibited in this experiment
323 (Table 2), compared to that (16.7-18.6 g N kg⁻¹ DM) found by Estellés et al. (2014). Total N content
324 of manure influences NH₃ emissions (Kulling et al., 2003) during storage because significant
325 biodegradation of organic nitrogen compounds into ammonium (Petersen and Sørensen, 2008)
326 generally occurs, such that NH₃ is generated and emitted from the manure surface. The higher NH₃
327 emission fluxes found in our study might also be explained by the differences in experimental
328 conditions. In particular, in our study the headspace air in the dynamic chambers was exchanged once
329 min⁻¹, which was approximately 14-fold the rate (0.07 times min⁻¹) used by Estellés et al. (2014).
330 According to Montes et al. (2009), increasing the rate of air exchange over a manured surface may
331 increase the mass transfer coefficient of NH₃, and thereby enhance the NH₃ volatilisation process. In
332 our case, we used an air flow rate based on the work of Balsari et al. (2007) that was representative
333 of the area.

334 Cumulative emissions of all investigated gases were affected ($P < 0.01$) by sampling date (Table 4).
335 Total nitrogen losses measured as NH_3 , represented 22.5% of the initial N content, and approached
336 the range maximum (1.8-23.5%) quoted in literature available for solid manure (Hou et al., 2015)
337 storage periods of between two and twelve months. In our study emissions were measured over a
338 shorter (35 days) period, which is likely to have resulted in an underestimation of cumulative
339 emissions.

340 Total N losses measured as N_2O emissions (Table 4) accounted for 0.10% of initial N content, which
341 fell well within guidelines set by the National GHG inventories Inter-governmental Panel on Climate
342 Change (IPCC, 2006), which has suggested an emission factor for the general category of solid
343 storage systems of 0.50% or $0.005 \text{ kg N-N}_2\text{O kg}^{-1} \text{ TN}$ in the manure. It is worth noting on this broad
344 guideline, is that stored solid manure N_2O emissions can range from <1.0 to 4.3% of total N content
345 (Chadwick et al., 2011), depending on manure type and characteristics (e.g., C/N ratio, DM content),
346 duration of storage, and climatic conditions.

347 Over the 35 days of storage, 45.0% of the initial OM content was lost as CO_2 emissions. Carbon
348 dioxide emissions are generally the largest pathway for C loss in manure, equating to OM content
349 percentages of 40.0 in cattle, 34.8 in dairy cow, 48.0 in pig, and 42.3% in poultry of farmyard manure
350 (FYM) heaps (Pardo et al., 2015). Overall, CH_4 losses accounted for 0.25% of the initial OM content
351 observed in this study, which aligns with manure-specific average OM content (Pardo et al., 2015)
352 percentages of 0.1% (poultry) and 3.2% (cattle).

353

354 *3.4. NH_3 and GHG emissions from simulated land application of rabbit manure*

355 Cumulative gas emissions recorded during the simulated land application experiment demonstrated
356 they were affected ($P < 0.01$) by both treatment and sampling date (Table 5). Ammonia volatilization
357 rates peaked ($591.8 \text{ mg NH}_3 \text{ m}^{-2} \text{ h}^{-1}$) immediately after manure application, and then rapidly dropped
358 with time (Fig. 2). As expected, MI significantly reduced NH_3 emissions at rates in line with the basic
359 principle that NH_3 emissions decrease with decreased exposure of the manure to air (Sommer et al.,

2003). Specifically, immediately after soil incorporation (day 0.25), MI averaged an NH₃ emission flux of 25.2 mg m⁻² h⁻¹, or approximately one-sixth of the flux recorded for SA (153.1 mg m⁻² h⁻¹). The significantly (P<0.01) lower NH₃ volatilization from MI relative to SA continued up to day 4; thereafter, the NH₃ emission flux differences were no longer significant (P>0.05). Furthermore, after 14 days, NH₃ emission from manure-amended soils resulted as negligible, regardless of soil treatment. As for total NH₃ losses calculated in SA (Table 5), they corresponded to 15.96 and 91.07% of applied N and TAN, respectively. By comparison, a literature review of NH₃ emission from land spread with manure in Italy (Minoli et al., 2015) revealed calculated average EFs by of 55.4% (range 18.5-127.6%), 42.2% (range 5.4-96.3%), and 53.7% (range 49.8-57.6%) of TAN applied for cattle, pig, and poultry solid manures, respectively. Major variables influencing NH₃ emission resulted related to manure characteristics (e.g., pH, DM, TAN/TN), meteorological conditions (wind speed, temperature), soil properties (e.g., pH, cation exchange capacity), and manure application method. Incorporated manure exhibited total NH₃ losses of 3.36 g m⁻² (Table 5), which represents a 42.0% reduction when compared with manure left on the soil surface. However, total NH₃ losses recorded during the first six hours after manure application accounted for 37.8 (SA) and 65.3% (MI) of total NH₃ losses over the entire 35 days of measurements. Our results indicate that the 24-hour interval post-application, as recommended by current Italian solid manure regulations, is far too long. Instead, a more suitable recommendation for reducing NH₃ emissions from rabbit manure application is to incorporate this manure into the soil as quickly as possible. The effectiveness of MI to reduce NH₃ emissions depends primarily on the time interval between soil incorporation and spreading (Sommer and Hutchings, 2001). Plowing pig FYM immediately into cultivated land, compared with surface application without incorporation, effectively decreased (90%) NH₃ losses, while the effectiveness of this practice decreased to 60% when incorporation occurred within four hours of spreading (Webb et al., 2004). Similar results have been reported for cattle (McGinn and Sommer, 2007) and poultry solid manures (Rohde and Karlsson, 2002; Sagoo et al., 2007).

386 On the other hand, manure application with incorporation tended to produce higher N₂O, CO₂, and
387 CH₄ emissions, which most likely relates to the increased contact of manure with soil particles that
388 enhance microbial degradation (Flessa and Beese, 2000; Velthof et al., 2003; Thorman et al., 2007).
389 The nature and source of organic matter, temperature, humidity, and oxygen availability are all
390 important factors that are known to affect microbial activity and related gaseous emissions. For
391 example, soil-produced methane comes from methanogenesis under anaerobic conditions (Oertel et
392 al., 2016) and methane emission rates correlate positively with soil humidity (Tate, 2015). Another
393 example is seen in the favourable conditions that result for N₂O production when oxygen
394 concentrations are low and WFPS >50% as a by-product of denitrification (reduction of nitrate to
395 dinitrogen) (Van der Weerden et al., 2012).

396 Average emission fluxes from MI remained consistently higher until days 25 (N₂O, CO₂) to 28 (CH₄),
397 but then reduced to the emission levels observed for SA within 30 days of manure incorporation.
398 Furthermore, at the end of the experiment, emission fluxes of all investigated gases measured from
399 manure-amended soils SA and MI were not statistically different (P>0.05) than those recorded for
400 the control (i.e., unfertilized soil), which averaged 0.1 mg NH₃ m⁻² h⁻¹, -0.1 mg N₂O m⁻² h⁻¹, -0.4 mg
401 CH₄ m⁻² h⁻¹ and 570.0 mg CO₂ m⁻² h⁻¹ (Fig. 2).

402 Calculated cumulative emissions were 37.0% (N₂O), 57.3% (CH₄), and 34.8% (CO₂) higher for MI
403 than for SA. Despite these values, no significant (P>0.05) differences in cumulative N₂O losses were
404 observed between the two application methods, with the exception of days 1, 9, 11, and 14 during the
405 35-day experimental period when the average N₂O emission fluxes were found to be significantly
406 (P<0.01) greater for MI than for SA. These results agree with those of other studies (Webb et al.,
407 2004; Thorman et al., 2005; Thorman et al., 2007), in which no consistent effect was documented for
408 the effect of solid manure (pig, cattle, or poultry) incorporation on N₂O emission.

409 It is important to note that some evidence has indicated that manure application NH₃ emission
410 reduction practices may actually promote N₂O production and emission due to the increased soil
411 mineral N pool potentially made available for nitrifying and denitrifying microorganisms (Van der

412 Zaag et al., 2010). In fact, a review of experimental data by Webb et al. (2010) concluded that some
413 circumstances (such as, injected animal slurry, or essentially liquid manure) might result in increased
414 N₂O emissions, while incorporation of solid manure reduced or had no impact on N₂O emissions.
415 The authors speculated that such contrasting effects relate to the generally higher level of readily
416 decomposable organic compounds in liquid relative to solid manure, which might increase soil
417 denitrification activity, and hence N₂O production. In this study, total N₂O emissions averages ranged
418 from 1.8 to 2.5% of N applied to SA and MI, respectively. These figures range within the <1-3%
419 reported in the literature (Chadwick et al., 2011) for land applied solid manure, but are approximately
420 twice as high as the default value of 1.0% of Tot-N applied with manure as recommended by the
421 IPCC (2006).

422

423 *3.5. Cumulative NH₃ and GHG emissions from manure managed during rabbit fattening*

424 Table 6 presents the EFs for the emissions data recorded during the storage and simulated land
425 application trials (Fig. 1-2). When averaged across the analysed scenarios (S+SA, S+MI), cumulative
426 N losses for NH₃ and N₂O were 322.0 and 22.1 g kg⁻¹ excreted TN, respectively, and cumulative CO₂
427 and CH₄ averaged 637.1 and 4.3 g kg⁻¹ excreted OM, respectively. For TN and OM excretion data
428 obtained during the rearing period (Table 3), cumulative NH₃, N₂O, CO₂, and CH₄ emissions
429 averaged 206.1, 14.2, 6059.5, and 41.2 g year⁻¹ per LU, respectively.

430 Our results revealed greater NH₃ and CO₂ losses during storage compared to soil application,
431 regardless of the manure application method. Methane losses recorded during storage accounted for
432 64.3% and 49.0% of cumulative emissions, depending on whether rabbit manure after application
433 was left on the surface (S+SA) or incorporated (S+MI) into the soil. In contrast, on average, 94.5%
434 (S+SA) and 94.5% (S+MI) of cumulative N₂O emissions were recorded during the land application
435 trial. Carbon dioxide resulted as the main GHG emitted during both storage and land application (Fig.
436 3), averaging 61.5% of the cumulative GHG losses; N₂O ranked second (26.4% of the total), followed

437 by CH₄ (9.6% of the total) and NH₃ (2.6% of the total). Manure incorporation increased cumulative
438 total GHG emissions by an average of 21.5%, mainly due to high CO₂ and N₂O emissions.
439 Numerous studies have been conducted under laboratory-controlled conditions to examine the
440 potential emission rates associated with a range of manure types and management strategies (Hou et
441 al., 2015; Pardo et al., 2015). Despite the limitations of laboratory experiments, where many factors
442 affecting emission rates under actual field and scale conditions cannot be reproduced, these simulated
443 settings are still widely recognized as helpful to our understanding of the mechanisms influencing
444 emissions from specific manure sources. Indeed, it is preliminary observations done in the lab that
445 inform the design of trials to assess emissions under real farm conditions, to model emissions from
446 the livestock sector, and to develop effective emission mitigation programs. With this in mind, our
447 experiment provides important results because little information exists on gas emissions associated
448 with storage and land application of rabbit manure. In fact, to our knowledge, this is the first study
449 on this topic in Italy.

450

451 **4. Conclusions**

452 This study represents the first of its kind to simultaneously quantify NH₃, N₂O, CH₄ and CO₂
453 emissions from two important phases of the manure management chain of rabbit fattening production
454 systems, i.e. manure storage and land spreading. The results improve our understanding of the relative
455 losses and our ability to explore cost-effective mitigation strategies. The experimental results showed
456 that manure contained 31.4 and 64.5% of the OM and N of daily rabbit feed, respectively.
457 Additionally, rabbit manure was shown to produce sizeable NH₃ and GHG emissions. Specifically,
458 cumulative N losses from storage and subsequent soil application averaged 32.4 (NH₃) and 2.2%
459 (N₂O) of excreted TN; cumulative proportions of OM lost as CO₂ and CH₄ averaged 51.3 and 0.4%,
460 respectively. Furthermore, while manure incorporation into the soil effectively abated NH₃ emissions,
461 it also showed its potential to increase N₂O losses. When the results of this study are overlaid on the
462 Italian fattening rabbit count of 70,739,000 heads, we estimate that manure management of this

463 population could return as much as 15,800, 1,150, 460,000, and 3,200 t to the annual budget of NH₃,
464 N₂O, CO₂, and CH₄ emissions, respectively. While the small-scale and controlled conditions of our
465 study limit its extrapolation to rabbit farming systems in Italy, there is merit to several of the
466 observations and conclusions from our research. Improved management could significantly impact
467 the environmental issues related to NH₃ and GHG emission in the sector. Suggested strategies to
468 minimize gaseous losses from animal manure include diet manipulation, covering or compacting
469 manure heaps, and the use of treatment technologies, such as anaerobic digestion and manure
470 acidification (Petersen et al., 2007; Chadwick et al., 2011; Gioelli et al., 2016). Follow-on research
471 work is needed to evaluate the efficacy and feasibility of such emission mitigation measures in rabbit
472 production systems. Related future research includes how to address gases emitted during rabbit
473 production system housing and measurement of commercial rabbit manure storage heaps at the plot-
474 field scale after manure spreading.

475

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479

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685

686 **Table 1**

687 Chemical composition of experimental diet (alfalfa meal, 30%; barley meal, 20%; dried beet pulp,
688 15%; wheat bran, 20%; soybean meal, 6%; sunflower meal, 6%; and vitamin-mineral
689 supplementation, 3%; n=12).

	Mean	SEM
Dry matter (g 100 g ⁻¹)	89.97	0.20
Organic matter (g 100 g ⁻¹ DM)	93.20	0.17
Crude protein (g 100 g ⁻¹ DM)	17.77	0.12
Ether extract (g 100 g ⁻¹ DM)	3.70	0.15
Neutral detergent fibre (g 100 g ⁻¹ DM)	38.13	0.54
Acid detergent fibre (g 100 g ⁻¹ DM)	20.90	0.85
Acid detergent lignin (g 100 g ⁻¹ DM)	4.53	0.15
Acid insoluble ash (g 100 g ⁻¹ DM)	0.73	0.23
Gross energy (MJ kg ⁻¹ DM)	18.80	0.12

690

691

692 **Table 2**

693 Rabbit manure composition at the beginning and end (i.e., before simulated land application trials)

694 of the storage period (n=12).

	Beginning of storage		Before soil application		P-value
	Mean	SEM	Mean	SEM	
pH	7.44	0.09	8.01	0.05	0.000
Dry matter (g 100 g ⁻¹)	20.89	0.47	27.66	0.47	0.000
Organic matter (g 100 g ⁻¹ DM)	86.36	1.15	82.44	1.10	0.022
Total nitrogen (g 100 g ⁻¹ DM)	3.37	0.09	2.97	0.09	0.006
Total ammoniacal nitrogen (g 100 g ⁻¹ DM)	0.70	0.08	0.67	0.03	0.726
Neutral detergent fibre (g 100 g ⁻¹ DM)	63.23	1.44	61.41	1.19	0.336
Acid detergent fibre (g 100 g ⁻¹ DM)	42.92	0.94	40.51	0.48	0.029
Acid detergent lignin (g 100 g ⁻¹ DM)	12.29	0.29	8.84	0.41	0.000

695

696

697 **Table 3**

698 Organic matter and nitrogen balances per head (n=12).

	Mean	SEM
Daily feed intake (g d ⁻¹)	109.67	2.61
Organic matter feed content (g 100 g ⁻¹ DM)	93.20	0.17
Organic matter daily intake (g d ⁻¹)	91.96	2.22
Organic matter into faeces (g d ⁻¹)	28.82	0.60
Digestible organic matter (g d ⁻¹)	63.13	2.02
Organic matter apparent digestibility (%)	68.63	0.75
Crude protein apparent digestibility (%)	78.60	0.68
Nitrogen daily intake (g d ⁻¹)	3.01	0.03
Nitrogen daily gain (g d ⁻¹)	1.07	0.14
Nitrogen daily excretion (g d ⁻¹)	1.94	0.02
Nitrogen efficiency (%)	35.60	0.37
Nitrogen excretion per live weight gain (g kg ⁻¹)	58.05	1.95

699

700

701 **Table 4**

702 Trend analysis of cumulative gaseous emission recorded during storage of fattening rabbit manure

703 (n=12).

	Cumulative emission	SEM	P-value
NH ₃ (g m ⁻³ manure)	1163.4	85.32	0.000
N ₂ O (g m ⁻³ manure)	6.6	0.93	0.000
CO ₂ (g m ⁻³ manure)	48744.2	1721.77	0.000
CH ₄ (g m ⁻³ manure)	269.8	26.90	0.000
CO ₂ eq (g m ⁻³ manure)	61143.2	1951.21	0.000

704

705

706 **Table 5**

707 Trend analysis of cumulative gaseous emission recorded during simulated land application (SA:
 708 surface application; MI: incorporation into the soil) of fattening rabbit manure (n=12).

	Application method	Cumulative emission*	SEM	Time P-value	Treatment P-value	Treatment x Time P-value
NH ₃ (g m ⁻²)	SA	5.8 ^A	0.36	0.000	0.003	0.002
	MI	3.4 ^B	0.40	0.000		
N ₂ O (g m ⁻²)	SA	0.8	0.05	0.000	0.090	0.005
	MI	1.1	0.08	0.000		
CO ₂ (g m ⁻²)	SA	214.9 ^B	7.72	0.000	0.000	0.000
	MI	724.8 ^A	27.54	0.000		
CH ₄ (g m ⁻²)	SA	1.2 ^B	0.08	0.000	0.001	0.001
	MI	1.8 ^A	0.05	0.000		
CO ₂ eq (g m ⁻²)	SA	483.4 ^B	21.59	0.000	0.000	0.000
	MI	2681.8 ^A	64.84	0.000		

709 *Cumulative emission within the same gas having different superscripts are significantly different
 710 for P<0.01.

711

712 **Table 6**

713 Estimated cumulative losses from the agronomic management (storage + simulated land
 714 application) of fattening rabbit manure (SEM values into brackets) (n=12).

	Storage	Land application		Total	
	S	SA	MI	S + SA	S + MI
NH₃-N					
g kg ⁻¹ excr. TN	224.8	123.5	70.8	348.3	295.6
	(13.05)	(7.21)	(8.11)	(14.31)	(26.01)
g year ⁻¹ LU ⁻¹	143.9	79.1	45.3	223.0	189.3
	(8.35)	(4.62)	(5.19)	(9.16)	(16.65)
N₂O-N					
g kg ⁻¹ excr. TN	1.0	17.7	24.5	18.8	25.5
	(0.16)	(1.00)	(2.43)	(1.09)	(2.31)
g year ⁻¹ LU ⁻¹	0.7	11.4	15.7	12.0	16.3
	(0.10)	(0.64)	(1.56)	(0.70)	(1.48)
CO₂					
g kg ⁻¹ excr. OM	449.8	141.5	233.1	591.3	683.0
	(15.86)	(4.96)	(11.69)	(14.19)	(17.07)
g year ⁻¹ LU ⁻¹	4278.1	1345.4	2217.2	5623.5	6495.4
	(150.86)	(47.13)	(111.15)	(134.99)	(162.35)
CH₄					
g kg ⁻¹ excr. OM	2.5	1.4	2.2	3.9	4.8
	(0.26)	(0.09)	(0.08)	(0.29)	(0.41)
g year ⁻¹ LU ⁻¹	23.9	13.3	21.28	37.2	45.2
	(2.51)	(0.89)	(0.77)	(2.76)	(3.86)

715 SA: surface application; MI: incorporation into the soil; OM: organic matter; TN: total nitrogen;

716 LU: livestock unit.

717

718 **Figure captions**

719 **Fig. 1**

720 Average emission fluxes of NH₃, N₂O, CO₂, and CH₄ recorded during storage of manure from
721 fattening rabbits. Error bars indicate SEM (n=12).

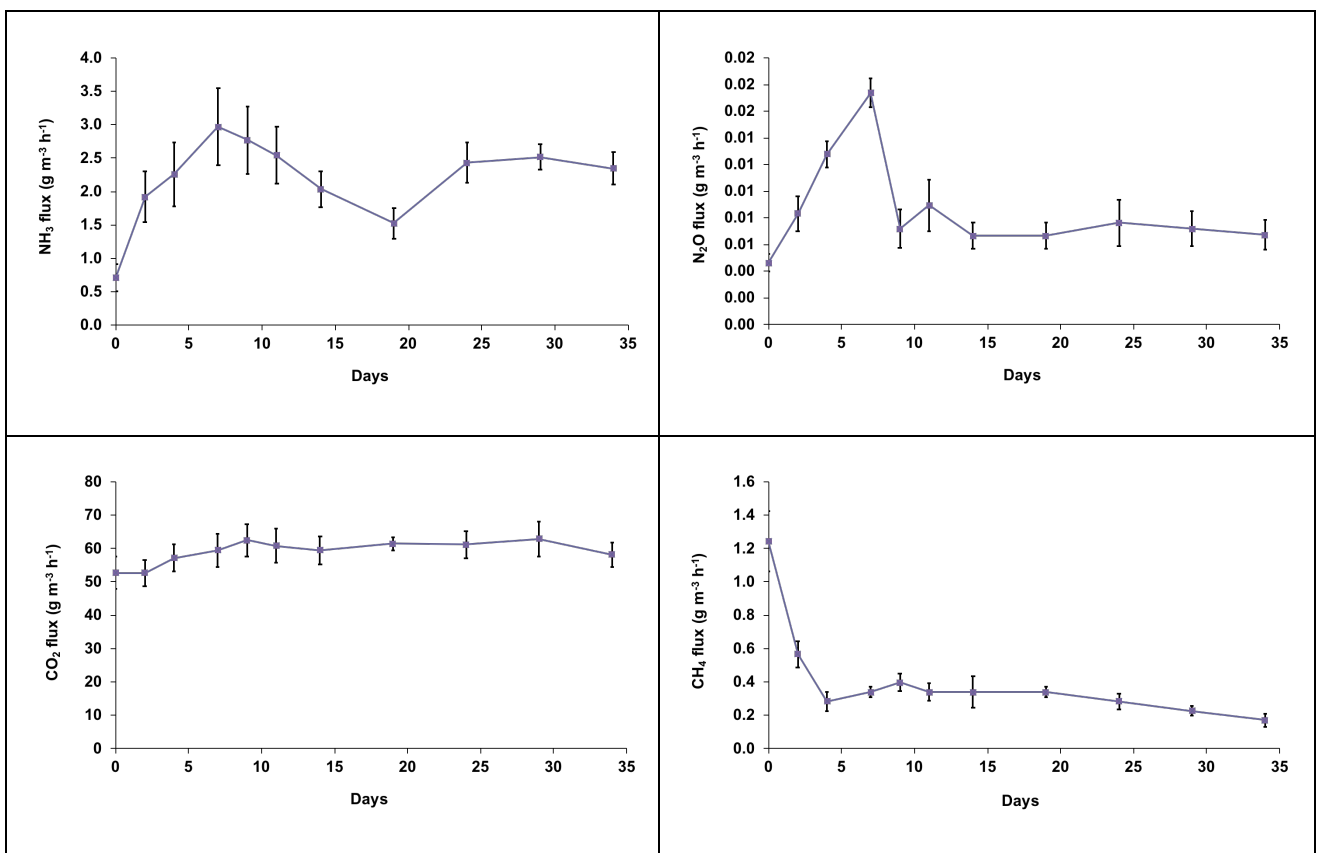
722 **Fig. 2**

723 Average net emission fluxes of NH₃, N₂O, CO₂ and CH₄, recorded during simulated land
724 application trials for surface application (SA, n=12) and incorporation into the soil (MI, n=12) of
725 fattening rabbit manure, and for non-fertilized soil (Control, n=4). Error bars indicate SEM.

726 **Fig. 3**

727 Estimated cumulative CO₂-eq emissions for the two manure application management scenarios
728 (S+SA, S+MI) of fattening rabbits.

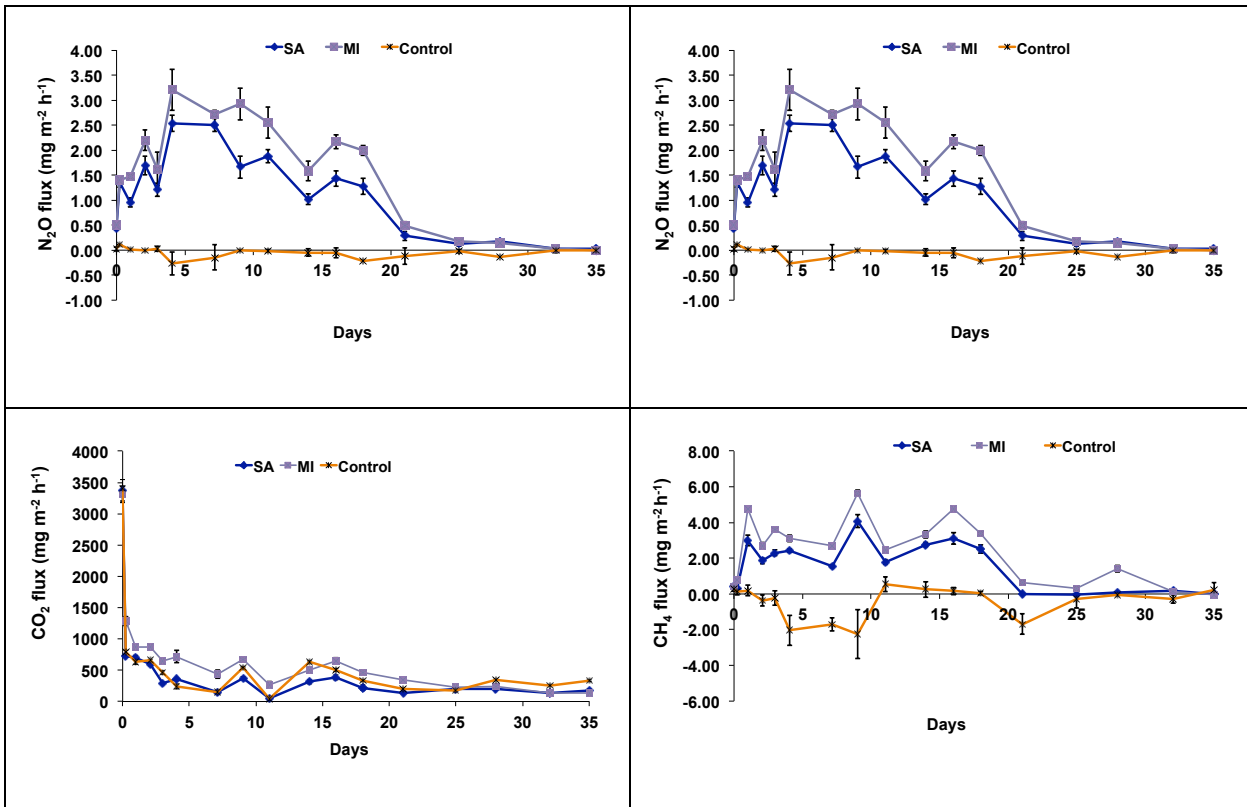
729



730 **Fig. 1.**

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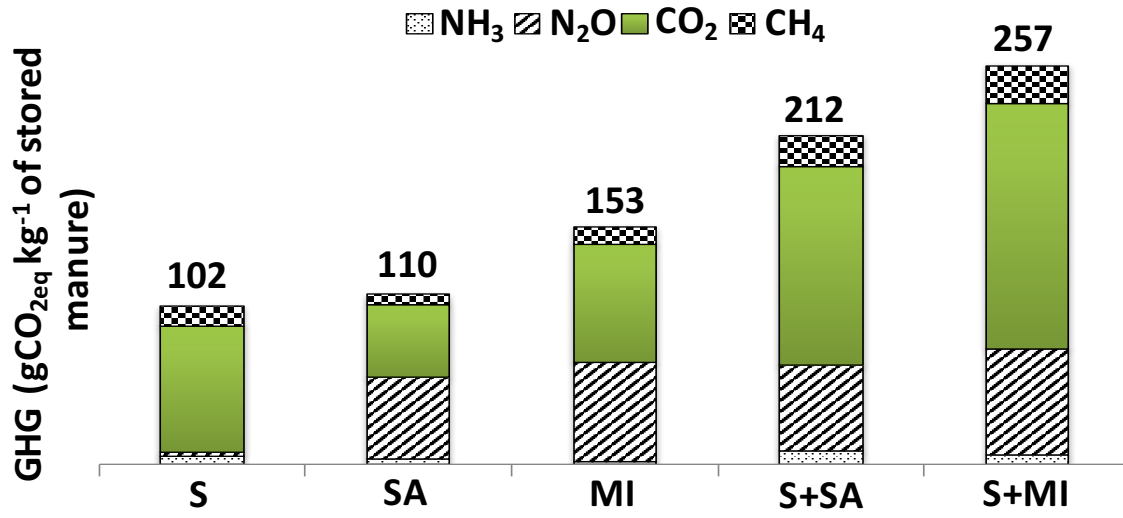


734 **Fig. 2.**

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738

739 Fig. 3.

740