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

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Abstract

Expansion in global rabbit populations and in the number of rabbits raised for consumption necessitates assessment of the environmental impact and sustainability of rabbit production systems. This study undertook two evaluations: utilization (animal efficiency) of organic matter (OM) and nitrogen (N) produced from feed during rabbit fattening, and emission of ammonia (NH_3), carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) from rabbit manure during its storage and land application in laboratory-simulated conditions. Results demonstrated manure contained approximately 30 and 65% of the OM and N in the daily feed intake, respectively. Additionally, rabbit manure was shown to produce sizeable NH_3 and GHG emissions. Specifically, cumulative N losses from storage and subsequent land application averaged 32.4 (NH_3) and 2.2% (N_2O) of excreted TN; cumulative proportions of OM lost as CO_2 and CH_4 averaged 51.3 and 0.4%, respectively. Finally, while manure incorporation into the soil effectively abated NH_3 emissions, it also showed its potential to increase N_2O losses, a potent greenhouse gas (GHG). Future research should focus on identifying appropriate emission mitigation measures. Accurate field-scale measurements are also needed to

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

Keywords

Nutrient excretion, Ammonia, Greenhouse gases, Environmental impact.

1. Introduction

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

Intensification and specialization of livestock farming systems is widely recognized as a major cause of environmental pollution (FAO, 2013) mainly due to the increased amount of manure produced by animals per unit of utilized agricultural area. Two topics of major importance are surface and groundwater nitrate contamination as a result of runoff and soil leaching, and atmospheric impacts from ammonia (NH_3) and greenhouse gas (GHG) emissions, namely carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) (Capri et al., 2009; Sanz-Cobena et al., 2017).

Ammonia and GHG production and release are influenced by the physical-chemical characteristics of the manure (especially pH, dry matter - DM, organic matter - OM, total nitrogen – TN, and total ammoniacal nitrogen - TAN), which in turn are determined primarily by feeding factors (such as feed intake and diet digestibility, protein content and solubility, fiber content and degradability, presence of antinutritional substances), and to a lesser extent by animal age, housing system, environmental conditions, and production stage (e.g., Monteny et al., 2001; Ndegwa et al., 2008; Gerber et al., 2013; Sheppard et al., 2013). However, information on the nature of manure production and N excretion from rabbit production systems under specific conditions in Italy is scarce, especially from the fattening period. Similarly, even though ammonia and GHG emissions may originate from any or all stages of rabbit manure management (i.e., production, storage, and application), the relative share of NH_3 and GHG emissions associated with the rabbit sector has yet to be precisely determined due to a lack of data. Most available emission data refer to those released from buildings during rearing (Estellés et al., 2010a; Estellés et al., 2010b; Calvet et al., 2011; Méda et al., 2014). Only a few data on emissions from rabbit manure storage (Estellés et al., 2014) have been reported, whereas little or none exists on emissions from application to land.

What makes investigation and quantification of these N processes important at this time is the convergence of three facts. One, widespread expansion of the rabbit livestock farming and their increasing numbers make it necessary to assess the environmental impact in relation to the production, storage, and utilization of manure as a crop fertilizer. Two, information is available on NH_3 and GHG emissions from rabbit manure managements in estimates produced by the Italian national inventory from a combination of default emission factors and personal communications from researchers and sector experts (Valli and Condòr, 2011; ISPRA, 2016). Three, rabbits have a particular digestive strategy (coprophagy) that may affect hindgut fermentation and potentially, gaseous production from manure.

To this end, this study investigated two related processes of rabbit production. The first aim evaluated the efficiency of the use of OM and N during rabbit rearing and fattening under intensive conditions. The second explored, under controlled laboratory conditions, the NH_3, CO_2, CH_4, and N_2O emissions from rabbit manure during its storage and subsequent land application by analysing and comparing two manure managements: 1) storage followed by application to the soil surface (S+SA), and 2) storage followed by application to the soil surface with incorporation into the soil at six hours after surface application (S+MI).

Scenario S+SA represents the traditional and most common rabbit manure application practice, whereas S+MI represents a technique increasingly adopted by Italian farmers to reduce the environmental impacts of NH_3 emissions. This relatively new practice aligns with current national regulations (DL 152/2006) that recommend incorporating solid manure into the soil within 24 hours of broadcasting.

2. Materials and methods

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

2.1. Animals, their housing, and their diet

The manure was collected from a rabbit farm (Carmagnola, Torino, Italy; 44°53′ N, 7°41′ E, at an altitude of 240 m a.s.l.), in which 120 rabbits (weaned crossbred Grimaud × Hycole, 35 days of age, 935 g live weight - LW) were reared under semi-controlled environmental conditions (temperature 23±5 °C, light length >8 h) in individual California type cages (30×30×40 cm; 0.12 m^2 $head^{-1}$) for 55 days (90 d of age, 3275 g LW). The animals were fed a standard pelleted diet *ad libitum* (Table 1) and had free access to clean drinking water.

2.2. Preparation of samples for analyses

During a four-day period (at 42 to 45 days of age; Perez et al., 1995), feed intake was measured and recorded for each of 12 randomly selected rabbits (1:1 sex ratio) housed in individual metabolic cages that separately collected faeces and urine (Perez et al., 1995). Feed intake was measured by weighing daily the individual feed administration and the remnant feed. Losses of feed during the rearing period were considered negligible, as the pellet form of the feedstuff enabled the rabbits to feed efficiently and prevented feed falling from the manger to the wire-mesh floor of the cage. Faeces and urine samples were also collected from the same individuals during the same period. Each sample was placed in a two-layer plastic bag to prevent moisture loss, and immediately frozen at -20 °C. Each sample was then thawed, mixed thoroughly, pooled, and then ground in a homogenizer (Tecator, Herndon, VA). Representative sub-samples were then taken and weighed in an aluminium foil pan, dried in a draft oven at 80 °C to a constant weight, and then stored for later chemical analysis. The excreta (urine and faeces) produced by the same group of rabbits were also collected each day for six days (48-53 days of age) and maintained at 4 °C for the gaseous emission trials. Each individual rabbit raised in each cage represented one replicate.

2.3. Feed and manure pH measurement and chemical analysis

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

2.4. Determination of Organic matter balance and digestibility

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

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

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

OM digestible = Feed Intake (kg) x Feed OM% x Feed OM apparent digestibility %

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

2.5. Nitrogen balance and crude protein digestibility

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

N excretion = N intake – N gain

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

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

2.6. NH_3 and GHG emissions from storage and simulated land application of rabbit manure

After the six-day collection period, the faeces and urine excreted from each selected rabbit were accurately mixed. Then, 0.50 kg sub-samples of the 12 animal-specific manure mixtures were placed in 1.5 dm^3 vessels (∅ 0.113 m) and stored for 35 days in a climate controlled room at 20 ± 0.5 °C to simulate farm storage before field spreading. The 20 °C temperature used in this study was chosen to approximate the annual average air temperature of most rabbit production areas in Italy. In practice, the duration of rabbit manure storage in Italy can vary and be for as long as 2–3 months. Bulk density of the investigated animal-specific manure mixtures was measured with three replicates by using a cubic box with an inner volume of 1 dm^3. Following the recommendations of the EN 13080 (2002) Standard, the box was completely filled with randomly collected manure sub-sample and carefully levelled without compaction. The bulk density (ρ, in kg dm^{-3}) was then determined after measuring the total mass (kg) of manure introduced into the box. The average bulk density of the investigated manure mixtures was found to be 0.60 kg dm^{-3}, a value that agrees well with the weight to volume ratio of 0.62 suggested by official guidelines (Regione Piemonte, 2007) for rabbit manure heaps stored under typical farm conditions in Italy. Emissions of NH_3, CO_2, CH_4, and N_2O from each stored manure sample (n=12) were measured by a dynamic chamber method using a gas trace analyser (described in 2.7. section). Measurements were performed and recorded three times per week for the first two weeks, twice weekly for the next two weeks, and once during the final week, for a total of 11 times during the 35-day experimental period.

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

2.7. Simulated land application trial

After storage, replicate manure samples were collected and used in a simulated land application experiment. Each experimental unit consisted of a cylindrical glass vessel (volume = 1.5 dm^3; ∅ 0.113 m) filled with 1 dm^3 of loam soil (USDA, 1999). The loam soil used in the experiment had 48.4 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 kg^{-1}. The soil samples came from a farm field cultivated mainly with maize for grain in rotation with wheat, and were representative of arable soil types of the western Po River plain (Northern Italy). After collection, the soil was sieved through a 4 mm screen and stored in moist form, in the dark, at 4 °C prior to trial start. The bulk density (1160 g dm^{-3}) of the undisturbed soil was achieved by shaking the vessels until the required soil volume was reached.

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

At the time of manure application, the gravimetric soil moisture content equalled 198 g kg^{-1}, corresponding to 52.5% water-filled pore space (WFPS) based on the bulk density of the soil and assuming particle density equalled to 2.65 g dm^{-3} (Zavattaro and Grignani, 2001). The application experiment continued for 35 days, during which NH_3, CO_2, CH_4, and N_2O gas emission measurements were taken at the following time intervals (days): immediately after manure application (t=0), at 0.25 (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. The trial was suspended when two consecutive measurement sessions of the emission fluxes for all investigated gases from the manure-amended soils SA and MI failed to be significantly different than those recorded from the control (i.e., unfertilized soil). During the experimental period the soil moisture content was kept constant (198 g kg^{-1}), gravimetrically adjusted for each individual jar, always after a flux measurement session.

Gaseous emissions from both the storage and simulated land application trials were measured with the dynamic chamber method using a gas trace analyser (1412 Photoacoustic Multi-gas Monitor, Innova Air Tech Instruments) per the procedures described by Dinuccio et al. (2008; 2013a). Briefly, prior to emission measurements, each vessel was sealed with a lid equipped with two ports—an air inlet and outlet. The inlet was connected and sealed to a pump and flow meter. In all experiments the air exchange was adjusted by the flow meter to guarantee a complete air exchange per minute, according to the experimental set-up adopted by Balsari et al. (2008) to assess farmyard manure heap NH_3 emissions using a large open dynamic chamber technique. Specifically, the airflow rate was set to 0.04 $m^3\ h^{-1}$ for storage and to 0.03 $m^3\ h^{-1}$ for the land application trials. After lid placement, the headspace was ventilated for 10 minutes with compressed air to create an airflow through the dynamic chamber and to achieve steady conditions. Gas sampling was performed for the ensuing 10 minutes; after which the lid was removed and the procedure repeated with the next vessel. The NH_3, CO_2, CH_4, and N_2O readings (mg m^{-3}) by the photoacoustic analyser were converted into fluxes that left each vessel as follows:

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

where: F (mg $m^{-2}\ h^{-1}$) is the gas flux, Q is the airflow rate ($m^3\ h^{-1}$) dosed to the vessels, C_{in} (mg m^{-3}) is the air inlet gas concentration, C_{out} (mg m^{-3}) is the vessel air outlet gas concentration, and A (m^2) is the emitting surface area of the vessel. In the land application trial, net NH_3, N_2O, CO_2, and CH_4 emission fluxes were calculated as the difference between emission fluxes recorded from the amended soils and those from the control.

The cumulative emissions (g mg^{-2}) of each gas recorded during the complete measurement period were calculated (Pampuro et al., 2016) and expressed in CO_2-eq (IPCC, 2013) using conversion factors of 1, 28, 265, and 2.65 for CO_2, CH_4, N_2O, and NH_3, respectively.

Finally, using the emission data recorded during the experiment and after accounting for OM and N excretion, it was possible to estimate the emission factors (EFs) of NH_3, N_2O, CO_2, and CH_4 for the full rabbit raising period using traditional rabbit manure management practices for storage and land application. The investigated gas EFs were expressed as follows: 1) grams of N lost as NH_3 and N_2O per kg of excreted TN (i.e., gNH_3-N kg^{-1} excr. TN; gN_2O-N kg^{-1} excr. TN); grams of OM lost as CO_2 and CH_4 per kg of excreted OM (i.e., gCO_2 kg^{-1} excr. OM; gCH_4 kg^{-1} excr. OM); grams per year per livestock unit (LU) (g $year^{-1}$ LU^{-1}). The latter calculation assumed six 55-day fattening cycles per year.

2.8. Statistical analyses

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

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

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

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

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

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

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

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

3. Results and discussion

3.1. Manure composition: fresh versus stored

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

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

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

3.2. Organic matter and nitrogen balances

During the rabbit raising period, 144.4 g head^{-1} (SEM 8.06) of fresh urine and faeces were produced daily. The faecal balance analyses (Table 3) found it contained approximately 30% of the OM and 53% of the N consumed in daily feed. The N concentration in urine and faeces averaged 0.3% (SEM 0.03) and 1.3% (SEM 0.04) on fresh basis, respectively. Organic matter and N balances, as well as the N partition value, in rabbit excreta are crucial inputs to estimation of the gaseous emissions linked to rabbit manure management. Carbon dioxide and CH_4 are primarily formed by microbial degradation of OM in faeces (Estellés et al., 2010b, 2014), while most of the N in the rabbit diet is excreted as urea in urine, which is rapidly converted by the microbial enzyme urease to ammonium (NH_4^+), which is a precursor in the pathway to NH_3 and N_2O emissions. Because urease is not usually present in urine (Montes et al., 2013), its presence in manure was assumed to be associated with its having come in contact with rabbit faeces.

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

3.3. NH_3 and GHG emissions from storage of rabbit manure

Fig. 1 shows average emission fluxes for all investigated gases recorded at each reading during the storage trial. Ammonia and N_2O emissions were low when storage began, but increased rapidly over time. Peak rates of 315.0 mg NH_3 m^{-2} h^{-1} and 1.8 mg N_2O m^{-2} h^{-1} were recorded on day 7, and then fell rapidly to result in average values of 249.1 mg NH_3 m^{-2} h^{-1} and 0.7 mg N_2O m^{-2} h^{-1} at the end of the storage period. In contrast, the highest CH_4 emissions occurred very early on (day 1: 132.2 mg m^{-2} 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 CH_4 flux rate was less than 20.0 mg m^{-2} h^{-1}. Average CO_2 flux rates ranged from 5594.9 on day 2 to 6680.4 mg m^{-2} h^{-1} on day 29, decreasing only slightly at the end of the storage period.

Overall, NH_3, N_2O, CH_4, and CO_2 emission fluxes during the experimental 35-day storage period averaged 231.8, 0.9, 43.6, and 6296.9 mg m^{-2} h^{-1}, respectively. In rabbit manure storage tests conducted in the laboratory using dynamic chambers, Estellés et al. (2014) measured N_2O, CO_2, and CH_4 fluxes at magnitudes similar to those we recorded. However, NH_3 emission results were noticeably lower (average of 26.4 mg m^{-2} h^{-1}) than those found in our study, which can be mainly attributed to the approximately two-fold increase in manure N content exhibited in this experiment (Table 2), compared to that (16.7-18.6 g N kg^{-1} DM) found by Estellés et al. (2014). Total N content of manure influences NH_3 emissions (Kulling et al., 2003) during storage because significant biodegradation of organic nitrogen compounds into ammonium (Petersen and Sørensen, 2008) generally occurs, such that NH_3 is generated and emitted from the manure surface. The higher NH_3 emission fluxes found in our study might also be explained by the differences in experimental conditions. In particular, in our study the headspace air in the dynamic chambers was exchanged once min^{-1}, which was approximately 14-fold the rate (0.07 times min^{-1}) used by Estellés et al. (2014). According to Montes et al. (2009), increasing the rate of air exchange over a manured surface may increase the mass transfer coefficient of NH_3, and thereby enhance the NH_3 volatilisation process. In our case, we used an air flow rate based on the work of Balsari et al. (2007) that was representative of the area.

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

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

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

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

Cumulative gas emissions recorded during the simulated land application experiment demonstrated they were affected (P<0.01) by both treatment and sampling date (Table 5). Ammonia volatilization rates peaked (591.8 mg NH_3 m^{-2} h^{-1}) immediately after manure application, and then rapidly dropped with time (Fig. 2). As expected, MI significantly reduced NH_3 emissions at rates in line with the basic 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 NH_3 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 NH_3 volatilization from MI relative to SA continued up to day 4; thereafter, the NH_3 emission flux differences were no longer significant ($P>0.05$). Furthermore, after 14 days, NH_3 emission from manure-amended soils resulted as negligible, regardless of soil treatment. As for total NH_3 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 NH_3 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 NH_3 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 NH_3 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 NH_3 losses recorded during the first six hours after manure application accounted for 37.8 (SA) and 65.3% (MI) of total NH_3 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 NH_3 emissions from rabbit manure application is to incorporate this manure into the soil as quickly as possible. The effectiveness of MI to reduce NH_3 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%) NH_3 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).

On the other hand, manure application with incorporation tended to produce higher N_2O, CO_2, and CH_4 emissions, which most likely relates to the increased contact of manure with soil particles that enhance microbial degradation (Flessa and Beese, 2000; Velthof et al., 2003; Thorman et al., 2007). The nature and source of organic matter, temperature, humidity, and oxygen availability are all important factors that are known to affect microbial activity and related gaseous emissions. For example, soil-produced methane comes from methanogenesis under anaerobic conditions (Oertel et al., 2016) and methane emission rates correlate positively with soil humidity (Tate, 2015). Another example is seen in the favourable conditions that result for N_2O production when oxygen concentrations are low and WFPS >50% as a by-product of denitrification (reduction of nitrate to dinitrogen) (Van der Weerden et al., 2012).

Average emission fluxes from MI remained consistently higher until days 25 (N_2O, CO_2) to 28 (CH_4), but then reduced to the emission levels observed for SA within 30 days of manure incorporation. Furthermore, at the end of the experiment, emission fluxes of all investigated gases measured from manure-amended soils SA and MI were not statistically different (P>0.05) than those recorded for the control (i.e., unfertilized soil), which averaged 0.1 mg NH_3 m^{-2} h^{-1}, -0.1 mg N_2O m^{-2} h^{-1}, -0.4 mg CH_4 m^{-2} h^{-1} and 570.0 mg CO_2 m^{-2} h^{-1} (Fig. 2).

Calculated cumulative emissions were 37.0% (N_2O), 57.3% (CH_4), and 34.8% (CO_2) higher for MI than for SA. Despite these values, no significant (P>0.05) differences in cumulative N_2O losses were observed between the two application methods, with the exception of days 1, 9, 11, and 14 during the 35-day experimental period when the average N_2O emission fluxes were found to be significantly (P<0.01) greater for MI than for SA. These results agree with those of other studies (Webb et al., 2004; Thorman et al., 2005; Thorman et al., 2007), in which no consistent effect was documented for the effect of solid manure (pig, cattle, or poultry) incorporation on N_2O emission.

It is important to note that some evidence has indicated that manure application NH_3 emission reduction practices may actually promote N_2O production and emission due to the increased soil mineral N pool potentially made available for nitrifying and denitrifying microorganisms (Van der

Zaag et al., 2010). In fact, a review of experimental data by Webb et al. (2010) concluded that some circumstances (such as, injected animal slurry, or essentially liquid manure) might result in increased N_2O emissions, while incorporation of solid manure reduced or had no impact on N_2O emissions. The authors speculated that such contrasting effects relate to the generally higher level of readily decomposable organic compounds in liquid relative to solid manure, which might increase soil denitrification activity, and hence N_2O production. In this study, total N_2O emissions averages ranged from 1.8 to 2.5% of N applied to SA and MI, respectively. These figures range within the <1-3% reported in the literature (Chadwick et al., 2011) for land applied solid manure, but are approximately twice as high as the default value of 1.0% of Tot-N applied with manure as recommended by the IPCC (2006).

3.5. Cumulative NH_3 and GHG emissions from manure managed during rabbit fattening

Table 6 presents the EFs for the emissions data recorded during the storage and simulated land application trials (Fig. 1-2). When averaged across the analysed scenarios (S+SA, S+MI), cumulative N losses for NH_3 and N_2O were 322.0 and 22.1 g kg^{-1} excreted TN, respectively, and cumulative CO_2 and CH_4 averaged 637.1 and 4.3 g kg^{-1} excreted OM, respectively. For TN and OM excretion data obtained during the rearing period (Table 3), cumulative NH_3, N_2O, CO_2, and CH_4 emissions averaged 206.1, 14.2, 6059.5, and 41.2 g $year^{-1}$ per LU, respectively.

Our results revealed greater NH_3 and CO_2 losses during storage compared to soil application, regardless of the manure application method. Methane losses recorded during storage accounted for 64.3% and 49.0% of cumulative emissions, depending on whether rabbit manure after application was left on the surface (S+SA) or incorporated (S+MI) into the soil. In contrast, on average, 94.5% (S+SA) and 94.5% (S+MI) of cumulative N_2O emissions were recorded during the land application trial. Carbon dioxide resulted as the main GHG emitted during both storage and land application (Fig. 3), averaging 61.5% of the cumulative GHG losses; N_2O ranked second (26.4% of the total), followed by CH_4 (9.6% of the total) and NH_3 (2.6% of the total). Manure incorporation increased cumulative total GHG emissions by an average of 21.5%, mainly due to high CO_2 and N_2O emissions.

Numerous studies have been conducted under laboratory-controlled conditions to examine the potential emission rates associated with a range of manure types and management strategies (Hou et al., 2015; Pardo et al., 2015). Despite the limitations of laboratory experiments, where many factors affecting emission rates under actual field and scale conditions cannot be reproduced, these simulated settings are still widely recognized as helpful to our understanding of the mechanisms influencing emissions from specific manure sources. Indeed, it is preliminary observations done in the lab that inform the design of trials to assess emissions under real farm conditions, to model emissions from the livestock sector, and to develop effective emission mitigation programs. With this in mind, our experiment provides important results because little information exists on gas emissions associated with storage and land application of rabbit manure. In fact, to our knowledge, this is the first study on this topic in Italy.

4. Conclusions

This study represents the first of its kind to simultaneously quantify NH_3, N_2O, CH_4 and CO_2 emissions from two important phases of the manure management chain of rabbit fattening production systems, i.e. manure storage and land spreading. The results improve our understanding of the relative losses and our ability to explore cost-effective mitigation strategies. The experimental results showed that manure contained 31.4 and 64.5% of the OM and N of daily rabbit feed, respectively. Additionally, rabbit manure was shown to produce sizeable NH_3 and GHG emissions. Specifically, cumulative N losses from storage and subsequent soil application averaged 32.4 (NH_3) and 2.2% (N_2O) of excreted TN; cumulative proportions of OM lost as CO_2 and CH_4 averaged 51.3 and 0.4%, respectively. Furthermore, while manure incorporation into the soil effectively abated NH_3 emissions, it also showed its potential to increase N_2O losses. When the results of this study are overlaid on the Italian fattening rabbit count of 70,739,000 heads, we estimate that manure management of this population could return as much as 15,800, 1,150, 460,000, and 3,200 t to the annual budget of NH_3, N_2O, CO_2, and CH_4 emissions, respectively. While the small-scale and controlled conditions of our study limit its extrapolation to rabbit farming systems in Italy, there is merit to several of the observations and conclusions from our research. Improved management could significantly impact the environmental issues related to NH_3 and GHG emission in the sector. Suggested strategies to minimize gaseous losses from animal manure include diet manipulation, covering or compacting manure heaps, and the use of treatment technologies, such as anaerobic digestion and manure acidification (Petersen et al., 2007; Chadwick et al., 2011; Gioelli et al., 2016). Follow-on research work is needed to evaluate the efficacy and feasibility of such emission mitigation measures in rabbit production systems. Related future research includes how to address gases emitted during rabbit production system housing and measurement of commercial rabbit manure storage heaps at the plot-field scale after manure spreading.

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Table 1

Chemical composition of experimental diet (alfalfa meal, 30%; barley meal, 20%; dried beet pulp, 15%; wheat bran, 20%; soybean meal, 6%; sunflower meal, 6%; and vitamin-mineral 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

Table 2

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

Table 3

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

Table 4

Trend analysis of cumulative gaseous emission recorded during storage of fattening rabbit manure (n=12).

	Cumulative emission	SEM	P-value
NH_3 (g m^{-3} manure)	1163.4	85.32	0.000
N_2O (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_2eq (g m^{-3} manure)	61143.2	1951.21	0.000

Table 5

Trend analysis of cumulative gaseous emission recorded during simulated land application (SA: 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_2O (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_2eq (g m^{-2})	SA	483.4 ^{B}	21.59	0.000	0.000	0.000
	MI	2681.8 ^{A}	64.84	0.000		

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

Table 6

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

	Storage	Land application		Total	
	S	SA	MI	S + SA	S + MI
NH_3-N					
g kg^{-1} excr. TN	224.8 (13.05)	123.5 (7.21)	70.8 (8.11)	348.3 (14.31)	295.6 (26.01)
g year^{-1} LU^{-1}	143.9 (8.35)	79.1 (4.62)	45.3 (5.19)	223.0 (9.16)	189.3 (16.65)
N_2O-N					
g kg^{-1} excr. TN	1.0 (0.16)	17.7 (1.00)	24.5 (2.43)	18.8 (1.09)	25.5 (2.31)
g year^{-1} LU^{-1}	0.7 (0.10)	11.4 (0.64)	15.7 (1.56)	12.0 (0.70)	16.3 (1.48)
CO_2					
g kg^{-1} excr. OM	449.8 (15.86)	141.5 (4.96)	233.1 (11.69)	591.3 (14.19)	683.0 (17.07)
g year^{-1} LU^{-1}	4278.1 (150.86)	1345.4 (47.13)	2217.2 (111.15)	5623.5 (134.99)	6495.4 (162.35)
CH_4					
g kg^{-1} excr. OM	2.5 (0.26)	1.4 (0.09)	2.2 (0.08)	3.9 (0.29)	4.8 (0.41)
g year^{-1} LU^{-1}	23.9 (2.51)	13.3 (0.89)	21.28 (0.77)	37.2 (2.76)	45.2 (3.86)

SA: surface application; MI: incorporation into the soil; OM: organic matter; TN: total nitrogen; LU: livestock unit.

Figure captions

Fig. 1

Average emission fluxes of NH_3, N_2O, CO_2, and CH_4 recorded during storage of manure from fattening rabbits. Error bars indicate SEM (n=12).

Fig. 2

Average net emission fluxes of NH_3, N_2O, CO_2 and CH_4, recorded during simulated land application trials for surface application (SA, n=12) and incorporation into the soil (MI, n=12) of fattening rabbit manure, and for non-fertilized soil (Control, n=4). Error bars indicate SEM.

Fig. 3

Estimated cumulative CO_2-eq emissions for the two manure application management scenarios (S+SA, S+MI) of fattening rabbits.

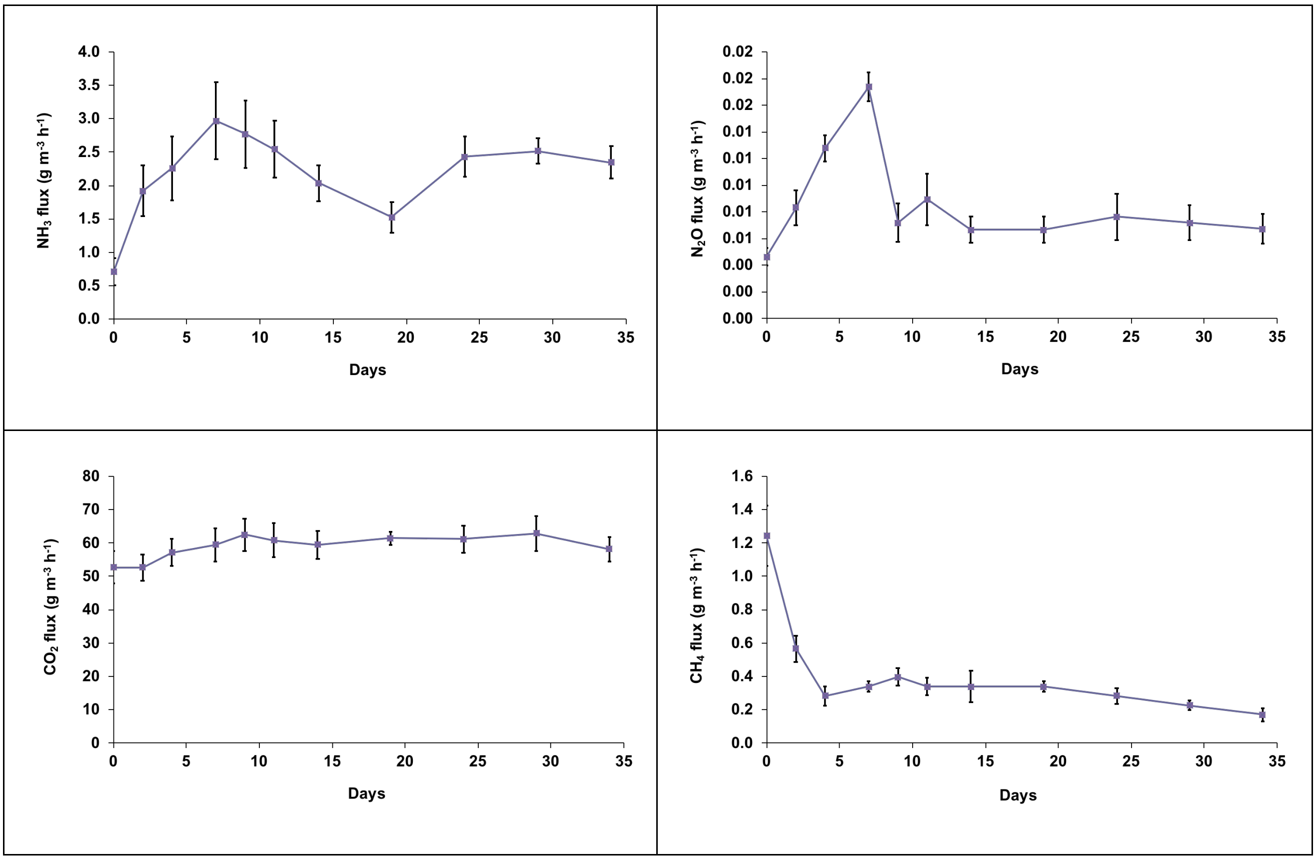

Fig. 1.

732

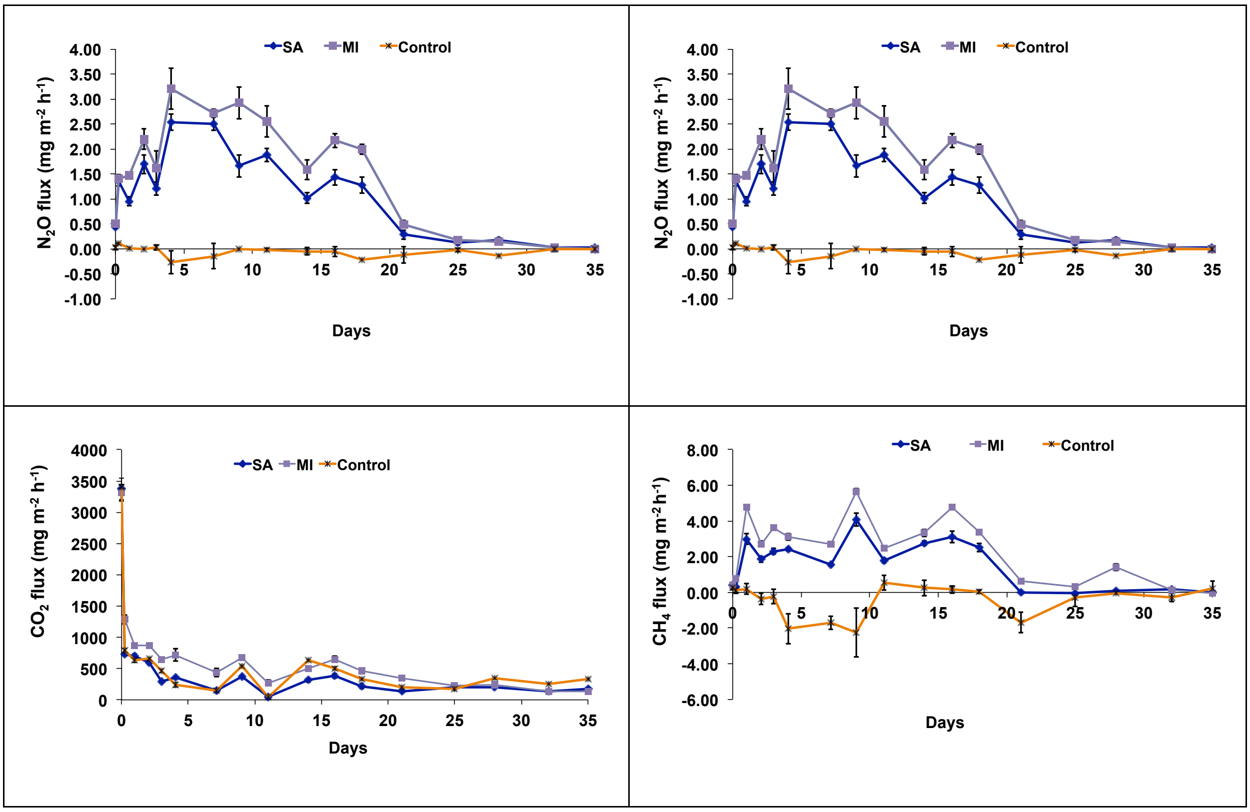
SA
MI
Control
N2O flux (mg m-2 h-1)
Days
SA
MI
Control
N2O flux (mg m-2 h-1)
Days
SA
MI
Control
CO2 flux (mg m-2 h-1)
Days
SA
MI
Control
CH4 flux (mg m-2 h-1)
Days

Fig. 2.

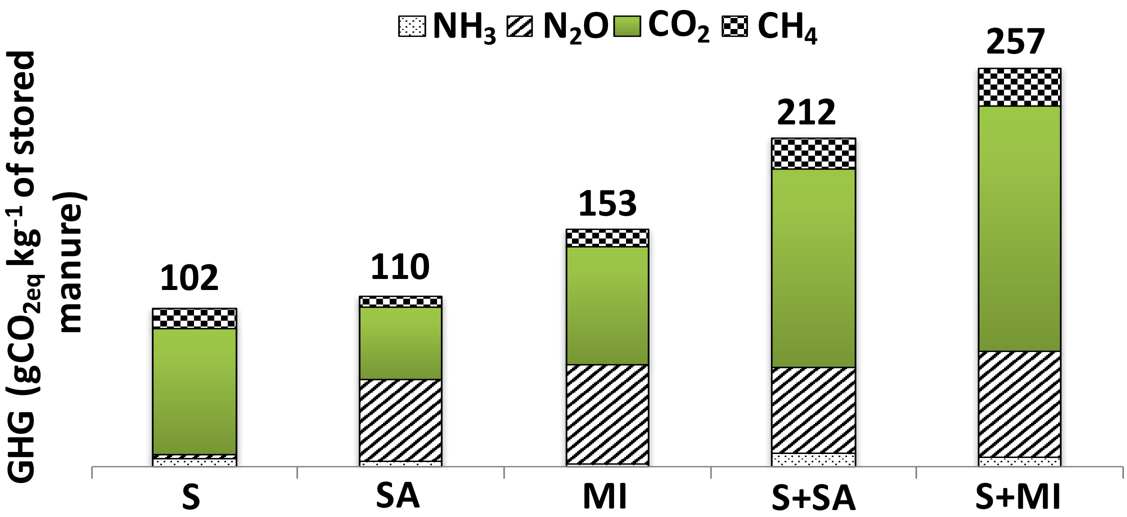
NH3 N2O CO2 CH4
GHG (gCO2eq kg-1 of stored manure)
102
110
153
212
257
S
SA
MI
S+SA
S+MI

Fig. 3.