



Response of N₂O emission to manure application in field trials of agricultural soils across the globe



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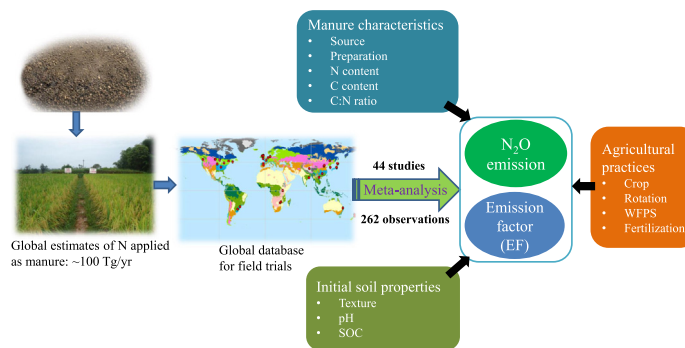
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HIGHLIGHTS

- Manure application significantly increased soil N₂O emission in agricultural land.
- Largest N₂O emissions occurred in soils with raw manure application.
- Soils with 50–90% WFPS contributed the highest N₂O emissions.
- N₂O emission factor induced by manures was similar to N fertilizer and crop residues.

GRAPHICAL ABSTRACT



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ABSTRACT

The response of soil nitrous oxide (N₂O) emission to manure application has been widely reported for laboratory experiments. However, the in-situ effects of manure application on soil N₂O emission from field trials (i.e. real-world conditions) and related mechanisms are poorly understood at the global scale. Here, we performed a meta-analysis using 262 field observations from 44 publications to assess the in-situ effects of manure application on soil N₂O emission and factors regulating N₂O emission (e.g., agricultural practices, manure characteristics and initial soil properties). Our analysis found that manure application significantly increased soil N₂O emission in field trials. The largest N₂O emissions were observed in soils from warm temperate climates, planted with upland non-leguminous crops and using raw manure. Notably, water-filled pore space (WFPS) significantly affected N₂O emission; soils with 50–90% WFPS had the highest N₂O emissions. Initial soil properties (e.g. pH, texture and organic carbon (C)) were generally not significant for predicting N₂O emission, possibly due to changes in soil properties induced by manure additions. Manures with carbon: nitrogen ratios (C:N) of 10–15 and C contents of 100–300 g C kg⁻¹ produced the lowest N₂O emission. The net N₂O emission factor (1.13%) resulting from manure application was similar to additions of synthetic N fertilizer (1.25%) and crop residues (1.06%), suggesting that manure application resulted in a similar N₂O emission to other soil amendments. Our analysis provides a scientific basis for manure management options to minimize N₂O emissions from animal waste disposal on agricultural lands globally.

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1. Introduction

Large amounts of manure are generated globally by livestock farming systems (Thangarajan et al., 2013) and include an estimated global N content of 81.5 to 128.3 Tg yr⁻¹ (Potter and Ramankutty, 2010). In China, annual manure production has rapidly increased from ~1.7 Pg in 1990 to 6.0 Pg in 2015, making it an important resource as an agricultural soil amendment. Manure application to agricultural lands has been demonstrated to improve soil fertility (Steiner et al., 2007). It was also reported that manure application can increase soil N retention and decrease NO₃⁻ leaching to reduce N loss when compared with synthetic fertilizer application ((Zhou et al., 2016). Compared to unfertilized or chemical fertilized soils, manure application also enhance soil C sequestration and thus to increase soil organic carbon (Maillard and Angers, 2014). Therefore manure application is recommended as a beneficial practice to sustain soil productivity. However, high emissions of greenhouse gases, such as N₂O, following manure application have been reported and should not be neglected, as the global warming potential (GWP) of N₂O is ~298 times greater than CO₂ (Intergovernmental Panel on Climate Change (IPCC), 2006; Landman, 2007). Additionally, N₂O can contribute to stratospheric ozone depletion (Ravishankara et al., 2009). Thus, the benefits of manure application for decreasing soil carbon dioxide (CO₂) emission by soil C sequestration maybe offset by increased N₂O emission.

In general, N₂O emission is regulated by both nitrification and denitrification processes (Bateman and Baggs, 2005). Nitrification by autotrophic nitrifiers occurs under aerobic conditions oxidizing NH₄⁺ to NO₃⁻. In contrast, denitrification by heterotrophic denitrifiers transforms NO₃⁻ to nitric oxide (NO), N₂O and nitrogen gas (N₂) under anaerobic conditions using bioavailable C as the electron donor. Several studies have reported the effects of manure properties on soil N₂O emissions along with related mechanisms. Robertson and Tiedje (1987) showed that manures with high inorganic and organic N concentrations can potentially increase soil N₂O emission as NH₄⁺ and NO₃⁻ + NO₂⁻ are reaction substrates for nitrification and denitrification, respectively. In addition, manures with a high C content typically enhance N₂O emissions by serving as a C substrates for denitrifiers (Mori and Hojito, 2012; Zhou et al., 2016; Zhou et al., 2017), and increasing soil microbial activities to rapidly consume oxygen (O₂) and form anaerobic microsites (Zhou et al., 2017). In some cases, manure can accelerate completion of the denitrification reaction by enhancing conversion of N₂O to N₂, especially in soils with intensive irrigation or high rainfall (Mejjide et al., 2007). Furthermore, manures with high C:N ratios may inhibit N₂O emission by stimulating microbial growth and consuming inorganic N (e.g. NH₄⁺ and NO₃⁻) from indigenous soil sources (Mooshammer et al., 2014). In addition, manure treatments, for example compost and digest, change manure physical, chemical and biological properties. These changes will impact manure C and N content, which directly and indirectly regulate nitrification and denitrification processes, resulting in influence of soil N₂O emission after manure application. Lastly, manure application can change soil physicochemical properties (e.g., increasing soil pH or changing gas diffusivity), which indirectly affect microbial activity and N cycling processes (Whalen et al., 2000; Heil et al., 2016).

Soil properties (e.g., soil texture, organic and inorganic C and N contents, pH, etc.) play important roles in regulating N₂O emission. For instance, soil texture and structure strongly affect soil pore size and moisture retention, which determines soil gas diffusion and O₂ availability (Rochette et al., 2010; Lazzano et al., 2016). Low O₂ availability in fine-textured soils would tend to favor growth of denitrifiers, leading to greater N₂O emissions (Bollmann, 1998). Further, low soil C content would suppress denitrification due to the scarcity of C substrates resulting in lower microbial activity. Soils with neutral to higher pH values are generally more suitable for autotrophic nitrifiers and heterotrophic denitrifiers than strongly acidic soils (Xiao et al., 2014). Additionally, agricultural practices (e.g., water management, nutrient

application, and crop type) and climate (air and soil temperature) can influence soil N₂O emission by changing soil structure, C content, pH and microbial activity (Velthof et al., 2003).

Although a number of previous laboratory studies have investigated how manure characteristics, soil properties and controlled environmental conditions affect N₂O emission following manure application, the results and underlying mechanisms from field trials are still contradictory and complicated due to soil heterogeneity and variations in agricultural practices and climate conditions (Pelster et al., 2012). Interactive processes affecting N₂O emission in field trials are very complicated and likely produce different results than laboratory experiments. Thus, a comprehensive analysis is required to synthesize and better understand the factors regulating N₂O emission resulting from manure application. A fundamental understanding is necessary to develop beneficial management practices (BMP) to attenuate N₂O emission associated with manure application. Therefore, we performed a meta-analysis to disentangle the links between N₂O emission and key influencing factors (e.g., manure characteristics, soil initial properties and agricultural practices) that regulate soil N₂O emission following manure application in field studies, as the results from field trials provide a more realistic response to real-world conditions. The objectives of this analysis were to (1) investigate how manure application influences soil N₂O emission fluxes and emission factors in field trials and to elucidate potential regulating mechanisms; and (2) identify important factors related to soil properties, manure characteristics and agricultural practices that regulate N₂O emission fluxes following manure application. Results from this study provide a scientific basis for developing strategies to mitigate soil N₂O emission associated with manure application.

2. Materials and methods

2.1. Data collection

Peer-reviewed articles that reported N₂O emission following manure application in field trials were searched in the Web of Science (Thomson Reuters, Philadelphia, PA, USA). Literature prior to December 2017 with 'manure', 'field', and 'N₂O/nitrous oxide emission' present in the title, keyword or abstract was collected. The following criteria were used to identify the studies for meta-analysis: (i) studies were performed by field trial and with at least 3 replicates; (ii) studies reported soil N₂O emissions in both manure applied treatments and non-manure controls; and (iii) at least one crop season was included at the same experimental site. If multiple growing seasons were available, each growing season was considered as a separate observation. If multiple crops were cultivated at different periods in the same experimental site, each crop type was considered as one observation, as crop type contributes greatly to changes in soil properties. If an experimental site included multiple measurements of N₂O emission, only the final time point was chosen for this meta-analysis. When a treatment was applied as a mixed manure plus mineral fertilizer, the comparison was considered as one observation only if another treatment with the same mineral fertilizer application was set up as a control. In total, 262 observations from 44 publications met these criteria and were included in this analysis.

Cumulative N₂O emission (kg N₂O-N ha⁻¹), sample size and standard deviation in both manure application treatment and non-manure control were extracted. Data Thief software (Bas Tummer, Eindhoven, the Netherlands) was used to extract data presented in figure format. If only the standard error was reported, MetaWin software (Rosenberg et al., 2000) was used to convert standard error to standard deviation. Other information collected from each study including site location, climate condition (annual precipitation and annual temperature), crop type, experimental duration, water management, initial soil properties (e.g., texture, organic C, pH, bulk density and C:N ratio), manure characteristics (e.g., manure type, manure C, manure N and C:N ratio) and application rate. Among these studies, the annual manure

N application rate ranged from 13 to 2486 kg N ha⁻¹ with a median value of 160 kg N ha⁻¹. The experimental duration ranged from <1 month to 19 years. More than twenty types of crops and two cropping system regimes (i.e., rotation and no rotation) were included. Soil properties varied in soil organic C (0.3–450 g kg⁻¹), clay content (4–65%), pH (4.9–8.1), bulk density (0.4–1.7 g cm⁻³), and water-filled pore space (WFPS; 29–100%). Similarly, manure characteristics showed a wide range in properties for C (2.0–501 g C kg⁻¹), N (0.05–82 g N kg⁻¹) and C:N ratio (1.82–48) based on dry weight of manure. The sampling depth across all studies was <20 cm. Manure sources were categorized as farmyard manure (FYM), pig, cattle or poultry. Manure preparation methods were grouped as raw or pre-treated (i.e., composted and digested). In accordance with the generalized climate classification scheme of the IPCC (European Commission, 2012; Maillard and Angers, 2014), climate at each experimental site was grouped into one of three climate zones: cool temperate, warm temperate, and tropical. Details for each category of information are presented in Table 1.

2.2. Meta-analysis

For studies not reporting the standard deviation, a value of 29.2% was assigned for N₂O emission, which was the average value for the standard deviation in our dataset (Skinner et al., 2014). The effects of manure application on soil N₂O emission were evaluated using the natural log of the response ratio (lnR) to define the magnitude of the response effect (Hedges et al., 1999):

$$\ln R = \ln \left(\frac{X_t}{X_c} \right) = \ln(X_t) - \ln(X_c)$$

Where lnR is the effect size of the target variable, X_t denotes the mean value of N₂O emission in the manure applied treatment, and X_c denotes the mean value of N₂O emission in the non-manure control. MetaWin 2.1 software was used to calculate cumulative effect size using a random-effects model and weighted resampling method (Rosenberg et al., 2000). The 95% confidence intervals (CIs) were generated in MetaWin using a bootstrapping procedure with 4999 iterations. The cumulative effect size was significantly positive or negative at p < 0.05 if the 95% confidence interval did not overlap with zero. Random-effects models were used rather than fixed-effects models because random-effects models present more conservative results if observations have high variance heterogeneity.

In order to determine how the attributes of (i) soil initial properties, (ii) manure characteristics, and (iii) agricultural practices influenced N₂O emission following manure application, we conducted a categorical random-model meta-analysis. Each attribute was separated into appropriate categories (Table 1), and the effect size in each category was calculated. For each attribute, the total heterogeneity (Q_T) was partitioned

into within-group (Q_W) heterogeneity and between-group (Q_B) heterogeneity using chi-square distribution with n-1 degrees of freedom. A significance for Q_B (p < 0.05) indicates that the effect sizes are significantly different between various levels of the category. If the 95% confidence intervals from two categories were non-overlapping, the difference between the two categories was significant (Lin et al., 2010). Additionally, we calculated the N₂O emission factor (EF, %) to investigate the net manure effect on soil N₂O emission using the equation:

$$EF (\%) = (E_t - E_c) / M * 100$$

Where E_t is cumulative N₂O emission (kg N₂O-N ha⁻¹) in the manure applied treatment, E_c is cumulative N₂O emission in the non-manure control, and M is the manure application rate (kg N ha⁻¹).

3. Results

3.1. Effects of agricultural practices on N₂O emission

Manure application caused a significant increase in soil N₂O emission (lnR = 1.03, 95% CI: 0.90–1.15) (Fig. 1), regardless of climate regime. Climate regime significantly (p < 0.05) influenced soil N₂O emission. After comparing lnR values in various climate regimes, soils emitted more N₂O in warm temperate (lnR = 1.29) with manure application, which is followed by cool temperate (lnR = 1.04). In contrast, soils in tropical areas were identified with lowest N₂O emission (lnR = 0.74).

Manure application significantly increased soil N₂O emission (lnR = 1.04, 95% CI: 0.92–1.17), regardless of crop type (with the exception of bean and rice) and WFPS (Fig. 2). Crop type had significant (p < 0.05) effects on N₂O emission following manure application. Generally, soils with grass were identified with largest effect size for N₂O emission with manure application (lnR = 1.60), which is followed by maize (lnR = 0.99) and wheat (lnR = 0.74). Soils in rice and bean cropping systems showed similar effect sizes of N₂O emission with manure application (0.13 and 0.09, respectively), which is relatively lower when compared with other cropping systems. There was no significant difference (p > 0.05) in N₂O emission between rotation versus no rotation cropping systems, although N₂O emission was significantly increased by manure application to both cropping systems (lnR = 1.03, 95% CI: 0.91–1.16) (Fig. 2b). N₂O emission increased most in soils with WFPS of 50–90% (lnR = 1.44), which was higher than soils with WFPS <50% (lnR = 1.00, p > 0.05) and WFPS >90% (lnR = 0.62, p < 0.05, Fig. 2c).

Manure application rate had a significant effect on N₂O emission (p < 0.05) with emission increasing at higher application rates (Fig. 3a). The experimental duration had a significant effect on N₂O emission (Fig. 3b). N₂O emission from soils with long-term application of manure (1–5 years) was significantly lower than those with short-term application (i.e., < 3 months) (Fig. 3b).

Table 1

Categorical attributes, observation numbers (n), various levels in each category (L1–L6), heterogeneity between-group (Q_B) in the random-categorical meta-analysis.

	Category	n	L1	L2	L3	L4	L5	L6	Q _B
Manure characteristics	Climate Zone	260	Cool temperate	Warm temperate	Tropical				22.7
	Duration	258	<3 mon	3–12 mon	1–5 yr	>5 yr			28.1
	Manure source	262	FYM	Pig	Poultry	Cattle	Others		7.7
	Manure N (g kg ⁻¹)	218	≤5	5–20	>20				14.3
	Manure C (g kg ⁻¹)	148	≤100	100–300	≥300				21.9
	Manure C:N (mass ratio)	170	≤5	5–10	10–15	15–20	>20		44.6
Manure application regimes	Manure preparation	216	Raw	Pre-treated					27.6
	Manure application rate (kg ha ⁻¹)	259	≤120	120–240	>240				16.8
	Texture	211	Fine	Medium	Coarse				0.1
Soil properties	pH	210	<6.5	6.5–7.3	>7.3				5.6
	SOC (g kg ⁻¹)	218	≤10	10–30	≥30				17.6
	Crop type	253	Bean	Grass	Maize	Wheat	Rice	Others	99.3
	Rotation management	250	With rotation	No rotation					0.3
Agricultural practices	WFPS (%)	164	≤50	50–90	≥90				32.6

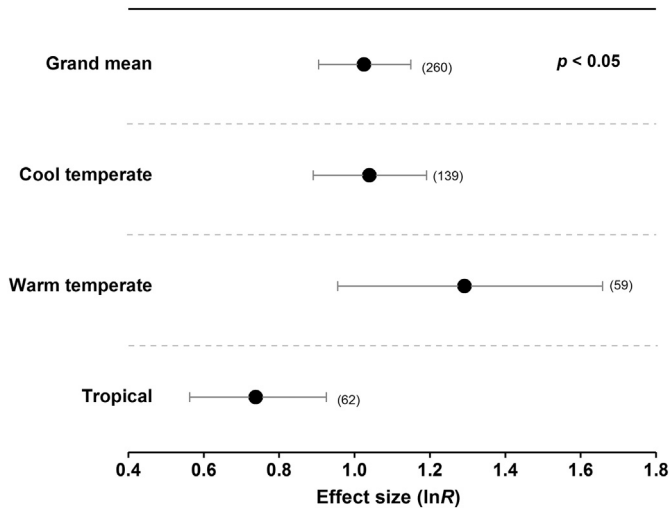


Fig. 1. Effect of climate regimes on changes in soil N₂O emissions following manure application (mean ± 95% confidence interval; number of observations in parentheses). A significant difference in mean effect was observed between various levels if the $p < 0.05$.

3.2. Effect of manure characteristics on N₂O emission

Manure application significantly increased soil N₂O emission, regardless of manure sources, N content, C content and C:N ratio (Fig. 4). The FYM manure produced lower N₂O emission (lnR = 0.64) compared to pig and cattle manure (lnR = 1.02, and lnR = 1.11, respectively), but was not significantly different from other manure types ($p > 0.05$, Fig. 4a). Regarding manure preparation prior to application, both raw and pre-treated manures significantly increased soil N₂O emission (Fig. 4b). However, the increase in N₂O emission from raw manure (lnR = 1.31) was significantly higher than pre-treated manure (lnR = 0.84) ($p < .05$).

Manures with N contents of 5–20 g kg⁻¹ resulted in lower N₂O emission (lnR = 0.88) compared to manures with N content of ≤5 g kg⁻¹ (lnR = 1.05) and > 20 g kg⁻¹ (lnR = 1.38) ($p < 0.05$, Fig. 4c). Manures with C content of 100–300 g kg⁻¹ had significantly lower N₂O emission

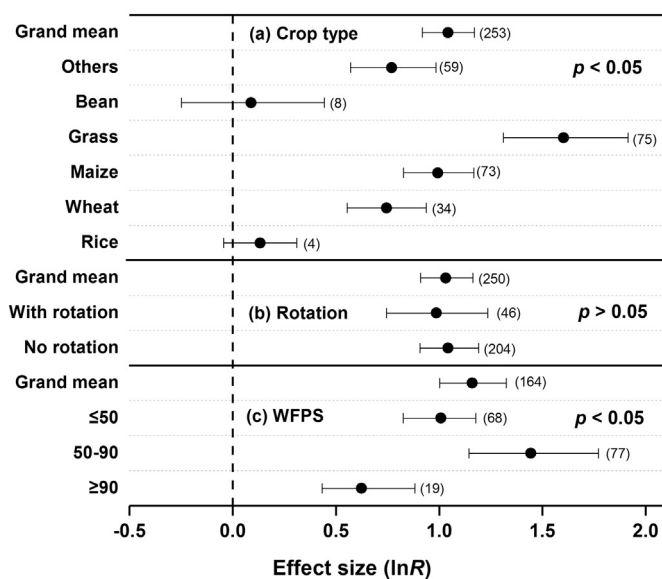


Fig. 2. Effect of agricultural practice regimes, (a) crop type, (b) rotation management and (c) WFPS on changes in soil N₂O emissions following manure application (mean ± 95% confidence interval; number of observations in parentheses). A significant difference in mean effect was observed between various levels if the $p < 0.05$.

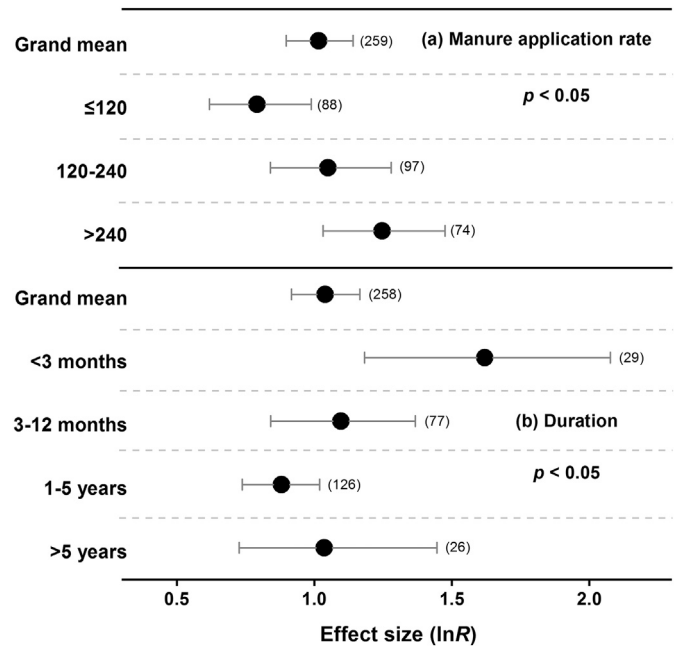


Fig. 3. Effect of manure (a) application rate (kg N ha⁻¹) and (b) duration on changes in soil N₂O emissions following manure application (mean ± 95% confidence interval; number of observations in parentheses). A significant difference in mean effect was observed between various levels if the $p < 0.05$.

(lnR = 0.69) compared to manures with C contents of <100 g kg⁻¹ (lnR = 1.22) and > 300 g kg⁻¹ (lnR = 1.74) ($p < 0.05$, Fig. 4d). Manures with C:N ratio of 10–15 (lnR = 0.57) had lower N₂O emission than

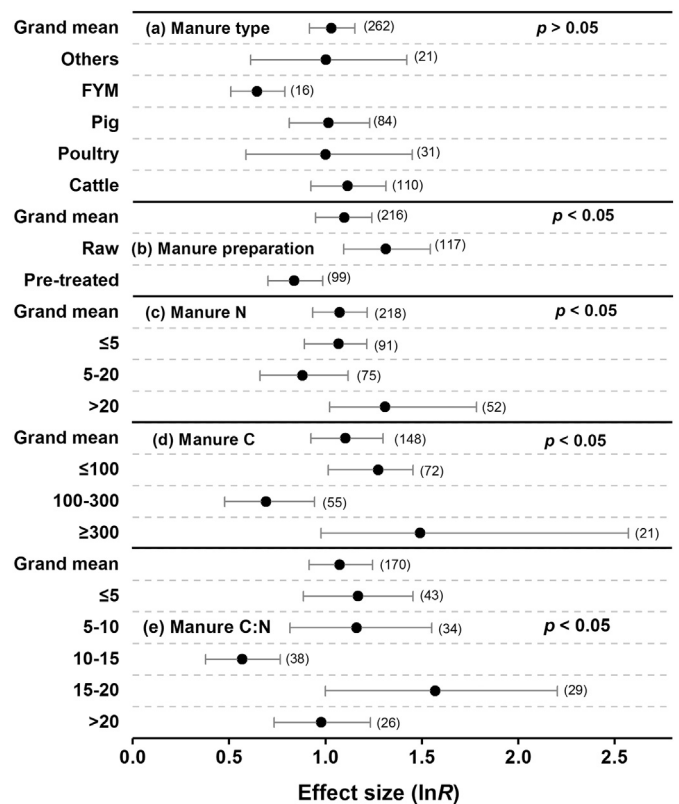


Fig. 4. Effects of manure characteristics, (a) source type, (b) preparation, (c) N content (g N kg⁻¹), (d) C content (g C kg⁻¹), and (e) C:N ratio on changes in soil N₂O emissions following manure application (mean ± 95% confidence interval; number of observations in parentheses). A significant difference in mean effect was observed between various levels if the $p < 0.05$.

manures with C:N of ≤ 5 ($\ln R = 1.17$), 5–10 ($\ln R = 1.16$) and 15–20 ($\ln R = 1.57$) ($p < 0.05$, Fig. 4e).

3.3. Effect of initial soil properties on N_2O emission

Manure application significantly increased soil N_2O emission, regardless of soil texture, pH, or organic C (SOC) (Fig. 5). Soil texture and pH did not significantly affect N_2O emission, whereas SOC had significant effects. The effect size ($\ln R$) of N_2O emission in the soils with medium SOC content (10–30 $g\ kg^{-1}$) (1.25) was greater than low ($<10\ g\ kg^{-1}$) (0.84) and high ($>30\ g\ kg^{-1}$) (0.93) SOC contents (Fig. 5c).

3.4. N_2O emission factor

The overall N_2O emission factor was 1.13% and higher than zero across all comparisons (Fig. 6). The N_2O emission factor in manures with N contents $<5\ g\ kg^{-1}$ was lowest (0.73%), followed by 1.23% for N contents of 5–20 $g\ kg^{-1}$, and 1.70% for N contents $>20\ g\ kg^{-1}$ (Fig. 6). The emission factor for manures with N contents $>20\ g\ kg^{-1}$ was significant higher than those with N contents of $<5\ g\ kg^{-1}$, but was similar to those with N contents of 5–20 $g\ kg^{-1}$. Overall, the emission factor increased as manure N content increased.

4. Discussion

4.1. Effects of agricultural practices on N_2O emission

Climate regimes with contrasting annual temperatures and rainfalls have a strong control on soil microbial activity, and soil microbial activity is highly associated with N_2O production. Our analysis showed that the warm temperate climate produced higher N_2O emission compared to the cool temperate climate (Fig. 1). This is consistent with Cantarel et al. (2012) findings of a strong correlation between increasing N_2O emission with increasing temperature and rainfall. We attribute these results to higher microbial activity induced by higher temperature and rainfall in warmer climates (Fig. 1). The higher microbial activity can increase soil C and N substrate availability by increasing microbial turnover rates (Knorr et al., 2005), or contribute to more anaerobic

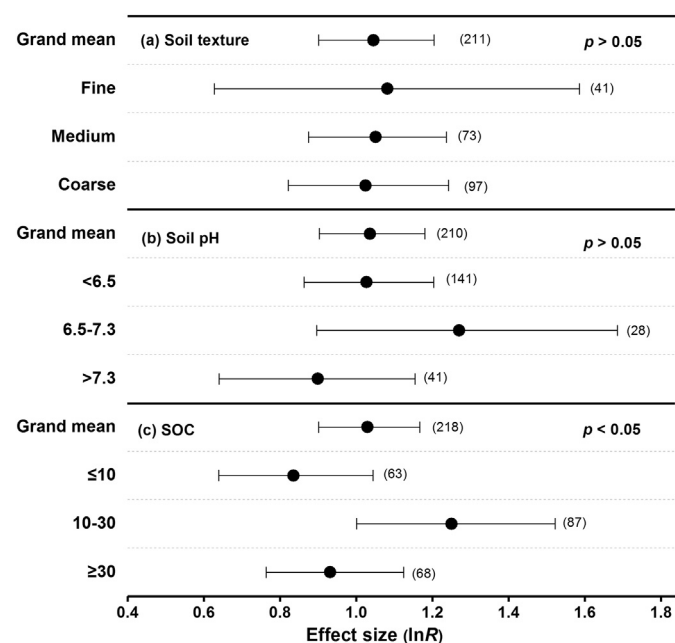


Fig. 5. Effects of initial soil properties, (a) texture, (b) pH and (c) SOC ($g\ kg^{-1}$) on changes in soil N_2O emissions following manure application (mean \pm 95% confidence interval; number of observations in parentheses). A significant difference in mean effect was observed between various levels if the $p < 0.05$.

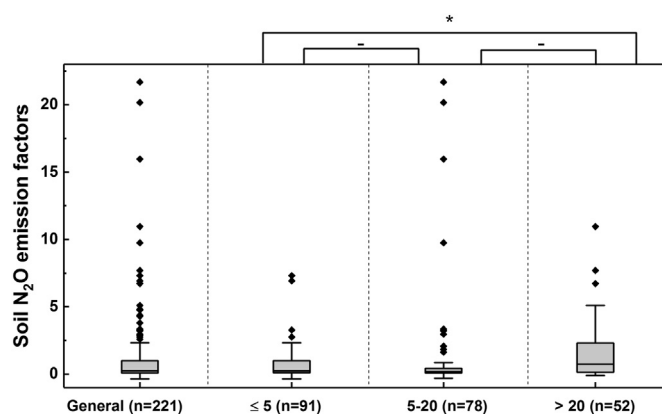


Fig. 6. Influence of manure N content (<5 , 5–20, and $>20\ g\ kg^{-1}$) on the N_2O emission factor (net manure effects) from soil following manure application, illustrated by boxplots. Number of observations is given in parentheses.

microsites by increasing microbial respiration (Kurganova et al., 2012). Increased C and N substrates can supply more essential substrates for N cycling microorganisms. For instance, Song et al. (2018) demonstrated the importance of substrate availability to fast growth of temperature-sensitive N_2O producing microorganisms. The microbiome shift was closely associated with fast N mineralization at warm temperatures, resulting in increased N_2O emissions. The increased microbial mineralization can produce more CO_2 , leading to O_2 depletion (anoxic conditions), and eventually accelerated denitrification (Kurganova et al., 2012).

The tropical zone with the highest annual temperature/rainfall and microbial activities had lower N_2O emission compared to the warm temperate zone (Fig. 1). We attribute this to accelerated completion of denitrification (conversion of N_2O to N_2), C substrate loss and less accumulation of inorganic N. High rainfall may create wet and O_2 limited conditions (especially in soil microsites), which can accelerate completion of the denitrification process by converting N_2O to N_2 (Das and Adhya, 2014). Heavy rains may also transport C/N substrates and N_2O formation deeper into the soil profile, where relatively more N_2O can be consumed before it escapes to the atmosphere. Further, N cycling in tropical systems is generally very efficient between the soil and vegetation, which limits the accumulation of NH_4^+ and NO_3^- in the soil thereby attenuating nitrification and denitrification processes. Hence, lower N_2O emission was observed in tropical compared to warm temperate climates (Fig. 1).

With respect to crop type, our analysis showed that manure application increased N_2O emission in soils of all upland crops, except for beans (Fig. 2). A lack of enhanced N_2O emissions from paddy rice cultivation following manure application was also noted and attributed to: (1) the dominantly anaerobic conditions associated with paddy rice cultivation that limits nitrification and promotes conversion of N_2O to N_2 (Das and Adhya, 2014); and (2) low sample size ($n = 4$) in rice systems may affect the statistical robustness. In general, cultivation of leguminous beans uptakes large amounts of base cations from soils and release H^+ (Tang et al., 1999), leading to lower soil pH and based-cation fertility (Guo et al., 2010). This may inhibit N_2O production, as nitrifiers and denitrifiers prefer relatively neutral or mildly alkaline environments (Čuhel et al., 2010). Additionally, leguminous beans are N_2 -fixers and tend to receive lower manure applications resulting in lower production of N_2O compared to other crops. The WFPS had a significant effect on N_2O emission, with soils having a moderate WFPS experiencing the highest N_2O emission (Fig. 2c). Soils with WFPS at 50–90% appear to provide the optimum conditions for denitrifier activity (Bateman and Baggs, 2005) and N_2O production. At these intermediate WFPS conditions, there is likely some O_2 available to allow nitrification to proceed and the generation of NO_3^- provides substrate for denitrification to occur in adjacent anaerobic microsites. In contrast, the major process

in soils with WFPS <50% is nitrification with denitrification inhibited by the presence of O₂ (Abbasi and Adams, 2000; Gleeson et al., 2010). When the WFPS is >90%, soil porosity is water-saturated, leading to greater conversion of N₂O to N₂ under strongly anaerobic conditions (Bouwman, 1996; Canfield et al., 2010).

It was notable that short-term application of manure (<3 months) produced higher N₂O emission than long-term application (1–5 years) (Fig. 3b). While the exact mechanisms remain unknown, one possible reason is that manure application enhances microbial growth and proliferation (i.e., priming effect) and stimulates soil N cycling by providing more available substrates and generating more anaerobic microsites (Li et al., 2018). Once the N cycling microorganisms adapt to regular manure application, they may become less responsive to further manure applications over time. In addition, regular application of manure may lead to higher microbial biomass and therefore a higher capacity of soil microbial community to retain N, resulting in more uptake of N by the microbial community and less N₂O emission. Another possibility is improved soil abiotic properties resulting from long-term manure application. As manures are applied annually, several soil properties (e.g., soil structure, gaseous diffusion) would be progressively altered to a new steady-state compared to initial soil conditions.

4.2. Effects of manure characteristics on N₂O emission

Zhou et al. (2017) showed no differences in N₂O emissions from different manure sources (i.e., animal species), consistent with the findings of our meta-analysis. Raw manure resulted in higher N₂O emission than pre-treated manure, consistent with the results of Nkoa (2014). In general, raw manure has higher inorganic N and a lower C:N ratio than pre-treated manure (Bernal et al., 2009). Higher inorganic N contents induce higher N₂O emission, as NO₃⁻ and NH₄⁺ are essential substrates for denitrification and nitrification, respectively. Manures with a high C:N ratio would enhance microbial N assimilation (i.e., immobilization), resulting in uptake of inorganic N from indigenous soil sources. The lack of available N substrates (i.e., NH₄⁺ & NO₃⁻) would thereby decrease soil N₂O emission. However, Zhu et al. (2014) demonstrated that manure pre-treatment did not reduce N₂O emissions and Chantigny et al. (2007) showed no difference in N₂O emissions between pre-treated and raw manures. We attribute these contradictory results to factors such as the high heterogeneity of manure, contrasting manure sources and pretreatment methods. In this meta-analysis, we did not specify manure forms (e.g. solid vs liquids, clods vs pellets) or pretreatments for manure (e.g. composted vs digested). Instead, we focused on the in-situ response of N₂O emission to manure application from the perspective of agricultural soil rather than manure source management. As showed in Fig. 4, pre-treated manure showed lower effect size compared to raw manure. Manure treatment, for example, compost and digest, will change the physical, chemical and biological properties of the manure radically, resulting in the difference for N and C content in raw/pre-treated manure and soil N₂O emission after manure application. Thus, a detailed quantitative index of manure characteristics (e.g., manure C, N and C:N ratio) may be more suitable for explaining the mechanisms mediating N₂O emission from soil than qualitative categorical descriptions such as manure preparation and manure type.

The overall increase in soil N₂O emission resulting from manure application was consistently greater than zero (Fig. 4), and the responses of N₂O emission differed with manure characteristics. Different microbial activity and growth induced by different manure characteristics likely account for differences in N₂O emission. In this analysis, manures with the highest N content had the highest soil N₂O emissions compared with manures with medium and low N contents (Fig. 4c). This is in accordance with the consensus that higher inorganic N availability directly enhances nitrification-denitrification processes, resulting in higher N₂O emission. Our analysis also found that manures with medium C content or C:N ratio had significantly lower N₂O emission

compared to those with lower or higher C contents and C:N ratios. Normally, when manures have a C:N ratio < 5 or low C content, they provide ample N for microbial growth and proliferation, resulting in net N mineralization (Probert et al., 2005). Excessive inorganic N produced from mineralization can stimulate soil nitrification and denitrification processes, contributing to increased soil N₂O emission. When the C:N ratio increases, the N content in manure cannot meet the N requirement for microbial growth and proliferation, and the microorganisms will utilize indigenous N (e.g., NH₄⁺ & NO₃⁻) from the soil resulting in microbial N immobilization (Mooshammer et al., 2014). This process competes with heterotrophic denitrification and autotrophic nitrification to utilize the NO₃⁻ and NH₄⁺ substrates, respectively. Further, high manure C:N ratios or C content may initially enhance microbial activity, leading to consumption of O₂ and development of anaerobic conditions (Smith et al., 2014). As a result, denitrification may persist for longer time periods, leading to increasing N₂O emission (Senbayram et al., 2012).

4.3. Effects of initial soil properties on N₂O emission

Soil texture did not significantly affect N₂O emission following manure application (Fig. 5a). This is contradictory with several previous laboratory studies that found higher N₂O emissions from fine-texture soils than coarse-texture soils (Zhou et al., 2017). In general, soil texture strongly affects soil pore distribution, and thereby regulates water and O₂ availability (McTaggart et al., 2002; Singurindy et al., 2006). Soils with coarse textures (high macropore content and high gas diffusion rates) would favor nitrification as the dominant process (Chen et al., 2013). In contrast, denitrification preferentially occur in soils with fine textures (high micropore content and low gas diffusion rates), where O₂ availability is often low (Gu et al., 2013). However, our analysis showed no difference in N₂O emission between soils with coarse and fine textures from field trials (Fig. 5a). This was probably due to the long-term effects of manure application to fields, as continuous and intensive application (e.g. one application per year) can greatly change initial soil properties (e.g., soil structure and bulk density).

Soil pH usually affects the activity of nitrifier and denitrifier microorganisms. In general, nitrifiers prefer neutral to moderately alkaline conditions (Xiao et al., 2013), and heterotrophic denitrifiers are more active in neutral rather than acidic environments (Bárta et al., 2010). Thus, N₂O emission may be expected to be higher in neutral or alkaline soils compared to acidic soils. In contrast, our analysis revealed that the initial soil pH had no significant effect on N₂O emission, contradictory with some previous laboratory studies (Russenes et al., 2016). A potential reason for this discrepancy may result from manure being an effective acidic soil amelioration amendment that can increase soil pH (Walker et al., 2004). After manure application, the final soil pH may be increased to a neutral or alkaline value attenuating possible effects from the initially acidic soil conditions. Given this potential pH buffering and/or soil acidity amelioration effect, the activity of nitrifier and denitrifier communities between initially different pH soils may not be as pronounced as expected based on the initial soil pH values.

Our analysis further found that initial soil organic C content significantly affected N₂O emission and soils with moderate SOC content had the largest N₂O emission. We attributed this to differential C-use efficiency among microorganisms. Soils with low SOC often have low microbial activity (Schnurer et al., 1985), which may lead to low N₂O emission. Soils with high SOC may have their C persevered by chemical/physical protection mechanisms or SOC may have a high C/N ratio resulting in N-limitation for microbes. Additional research is warranted to better understand the role of soil carbon dynamics in N₂O emission.

Overall, initial soil properties were not highly predictive of N₂O emission response to manure application in field trials. As our analysis utilized a global dataset, several interacting factors that regulate N₂O emission within a given site are obscured by combing with data from other regions. Additionally, intensive manure application may substantially alter the initial soil properties, making them non-representative of

post-manure application conditions. In addition, the lack of significant effects of soil properties may be related to many confounding factors in the field trials, which may obscure the individual effect that can be observed in laboratory experiments on N₂O emission with manure application. Compared to field trials, laboratory experiments are typically short-term incubations and receive less cumulative manure application (i.e., manure added only one time vs several applications in many field trials). Therefore, WFPS, which can be controlled and measured during field experiments, is often a better predictor of N₂O emission than initial soil properties, such as soil texture, pH and organic matter. Using real-world data generated from field trials for our meta-analysis was an important distinction of our analysis since laboratory experiments are not able to capture all the complexities and interaction associated with field trials.

4.4. Implications and conclusions

As the meta-analysis was conducted on field studies, providing a more realistic outcome than results from laboratory experiments. As such, the analysis provides guidance for developing beneficial management practices for minimizing N₂O emissions from manure application. The emission factor calculated in this analysis estimates the net effect of manure application on N₂O emission. The overall emission factor following manure application was 1.11%, which was slightly lower than 1.25% for application of synthetic N fertilizer to soils (Bouwman, 1996) and similar to the value of 1% adopted by the IPCC. Similarly, Novoa and Tejeda (2006) reported a N₂O emission factor of 1.06% for plant residues, which was only slightly lower than the emission factor we obtained for manure application. Thus, manure application appears to induce net N₂O emissions similar to other commonly used soil amendments (e.g., mineral fertilizer and crop residues). These findings indicate that manure can be an effective soil amendment with benefits for manure disposal, carbon sequestration, and fertility improvement, while only inducing a moderate additional contribution to N₂O greenhouse gas emission. However, the number of studies included in this study is low and more field experiments are needed to measure N₂O emission after manure application, including various agricultural practices (tillage and irrigation) and soil properties (soil temperature and microbial community). With increasing data availability in recent and future studies, it is important to critically identify the influence and integrated mechanisms involved in N₂O emissions to achieve optimal manure management and agricultural practices for field manure application.

In conclusion, manure application increased soil N₂O emission with emissions being affected by climate, agricultural practices, manure characteristics and some initial soil properties. Compared with synthetic N fertilizer and crop residues, manure application induced a similar net N₂O emission, supporting the use of manure to achieve several beneficial effects, such as enhancement of soil nutrient levels, C sequestration, and soil physical properties. Given the wide range of field trials included in this meta-analysis, the consistent patterns for N₂O emission provide an important understanding for the magnitude of N₂O emissions associated with manure application and factors regulating N₂O emissions across many agricultural systems. We recommend application of pre-treated (i.e. composted or digested) manures with C:N ratios of 10–15 and C contents of 100–300 g C kg⁻¹ to agricultural soils to minimize N₂O emission.

CRedit authorship contribution statement

Fang Xia: Writing - original draft, Conceptualization, Writing - review & editing. **Kun Mei:** Formal analysis, Software. **Yan Xu:** Investigation. **Chi Zhang:** Data curation. **Randy A. Dahlgren:** Writing - review & editing. **Minghua Zhang:** Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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