

Reactive Nitrogen Releases And Nitrogen Footprint During The Life-Cycles of Intensive Vegetable Production Affected By Human Feces Slurry Substitution

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Abstract

Evaluating the sustainability of vegetable production is crucial to secure future food supply. A two-year field study of four different vegetable crops was performed to investigate the effects of inorganic fertilizer and human feces slurry at different ratios on vegetable yields, reactive gaseous nitrogen emissions (GNrEs), reactive nitrogen (Nr) footprint and net ecosystem-economic income (NEEI) by using life-cycle analysis. Four fertilization strategies were studied, including: CK (no fertilization); CF (inorganic fertilization); CHF1 (human feces slurry/inorganic fertilizer, N ratio=1:7); and CHF2 (human slurry/inorganic fertilizer, N ratio=1:3). Results showed that compared with CF treatment, both CHF1 and CHF2 treatments increased the N_2O+NO emissions by 11.8 % and 32.4 % on average, while decreased the vegetable yields by 6.7 % and 7.4 %, respectively. Moreover, the addition of human feces slurry increased the proportions of Nr footprint by 6.6 % (CHF1) and 2.9 % (CHF2) in comparison with CF treatment group. However, although CHF2 treatment significantly increased the values of GNrEs and reactive gaseous nitrogen intensity (GNrI) by 8.4 % and 12.5 %, respectively, in relation to those in CF treatment group, it still increased farmers' income by 16,404 CNY ha^{-1} . These findings suggest that although human feces slurry incorporation could not mitigate Nr releases, the appropriate ratio of inorganic fertilizer and human feces slurry (CHF2) is able to improve net economic income (NEI) and NEEI during intensive vegetable production. Nevertheless, the relationship between combinatorial treatment of inorganic fertilizer and human feces slurry and mitigation of Nr release should be explored further.

Introduction

Since 2004, Chinese government has invested RMB 8.38 billion in the construction and renovation of 21.263 million rural toilets, and the coverage of sanitary toilets in rural areas has increased from 7.5% in 1993 to 78.5% in 2015 (NHFPC, 2016; Cheng et al. 2018). Great achievements have been made in the rural toilet renovation, but the inevitable consequence is a large amount of feces slurry with low reutilization efficiency (Koger et al. 2014). Human feces treated in septic tanks can be used as fast-release fertilizer with considerable amounts of nitrogen, phosphorus and potassium compounds, and the Government has encouraged the *in situ* utilization of feces resource. It is foreseeable that with the rapid development of rural toilets, the proportion of human feces slurry may be increased further.

Vegetable is the second largest crop in terms of planting area (after grain) in China (Li et al, 2019). The annual yield of Chinese vegetables was 76.9 million tons, and the sowing area reached 2.2 million ha, accounting for 13.2% of the global sown area (Wang et al, 2018). In the past, a relatively high economic value of growing vegetables has encouraged farmers to use fertilizers for maximizing crop yields (Li et al. 2019), which may lead to serious environmental problems (Kim et al. 2006). But now, more and more farmers have realized that the sustainable utilization of natural resources, such as human feces slurry, could decrease the use of inorganic fertilizer as well as reduce the production costs (Zhou et al. 2019).

Nowadays, it is well known that intensive vegetable production has become an important source of reactive nitrogen (Nr) releases in China, owing to the large application amounts of N fertilizer and frequent irrigation events (Fan et al. 2017). Approximately 20–50 % of applied fertilizer-N is lost as Nr, such as gaseous emissions of ammonia (NH_3), nitric oxide (NO) and nitrous oxide (N_2O), and lost to water via N leaching and runoff (Xia et al., 2016; Wang et al., 2019). To achieve the maximum crop yield with minimal environmental damage costs (particularly regarding Nr releases), many researchers have proposed the adaptive strategies of inorganic fertilizer substitution (Hillier et al. 2012; Yang et al. 2015). However, different substitute resources and different incorporation ratios might potentially result in variable effects on Nr releases and crop productivity due to diverse fertilizer types and N transformation patterns in agricultural systems (Zhang et al. 2012). More importantly, there are only limited data available on the effects of combination treatment of human feces slurry and inorganic fertilizers at different ratios on Nr losses and crop productivity during intensive vegetable production.

Evaluating the magnitude of the impact of N loss on agroecosystems can generate possible solutions to mitigate climate change and other environmental problems, thus helping to raise awareness in the general public and facilitating decision making with respect to environment-friendly technological development by policy makers. In recent decades, nitrogen footprint has been proposed as a potential indicator to assess how individuals, communities, organizations, or countries contribute to nitrogen pollution through their consumption, and thereby affect the environment and human health. It is widely known as the "total amount of Nr [reactive nitrogen, all other forms than N_2] released to the environment as a result of N consumption" (Leach et al. 2012). In fact, nitrogen footprint has been identified as a critical member of the "footprint family", i.e., a more comprehensive measure of human impact on agroecosystem (Galli et al. 2012; Leach et al. 2012).

In the present study, a two-year field experiment was performed on four different vegetable crops to quantify the amounts of Nr, such as N_2O , NO and NH_3 emissions, after treatment with mineral N fertilizer and human feces slurry in an intensively managed vegetable field in the North China. The aim of this research was to evaluate the impact of different portions of inorganic fertilizer and human feces slurry on average Nr footprints and net ecosystem-economic income (NEEI) associated with the loss of N during intensive vegetable production. We hypothesized that the combination treatment of inorganic fertilizer and human feces slurry at different ratios could affect the proportions of Nr footprint and NEEI during intensive vegetable production.

Materials And Methods

2.1 Experimental area

The field experiment was conducted at a suburban household in Ninghe, Tianjin, China (39°33'N, 117°82'E) from April 30, 2017 to May 10, 2019. According to the Tianjin weather station, this area was dominated by subtropical monsoon climate with an average annual rainfall of 642.8 mm and a mean annual air temperature of 11.2°C. The studied soil was classified as Cambosol (equivalent to Inceptisol in the USDA Soil Taxonomy), and its main compositions included total N: 1.2 g·kg⁻¹, soil organic carbon (SOC): 9.7 g·C·kg⁻¹, cation exchange capacity (CEC): 16.3 cmol·kg⁻¹ and pH: 8.4. Human feces slurry were collected from a household septic tank nearby the experiment site, after 60 days of anaerobic fermentation, the human feces slurry can basically meet the requirements of harmlessness, and the details of the human feces slurry are listed in Table 1.

Table 1
Nutrition and Harmlessness indicators of the tested human feces slurry.

| | Indicator | Unit | Value |
|-----------------------|---------------------------------------|-----------------------|------------|
| Nutrient | pH (H ₂ O _{5:1}) | | 7.9 ± 1.8 |
| | Total nitrogen | (g kg ⁻¹) | 4.9 ± 0.3 |
| | Ammonium content | (g kg ⁻¹) | 2.8 ± 0.2 |
| | Nitrate content | (g kg ⁻¹) | 1.5 ± 0.2 |
| | Total phosphorus | (g kg ⁻¹) | 3.2 ± 0.3 |
| | Total potassium | (g kg ⁻¹) | 6.6 ± 1.1 |
| | Total carbon | (g kg ⁻¹) | 49.5 ± 3.8 |
| Hygiene index | Ascaris eggs mortality | (%) | 100 |
| | Schistosomiasis eggs | - | - |
| | Hookworm eggs | - | - |
| | feces coliforms | - | 0.04 |
| | Salmonella. | - | - |
| - means not detected. | | | |

2.2 Experimental treatments and vegetable management

The experiment was carried out in 3 replicate plots arranged in a randomized complete block design (each plot: 3 × 2.5 m). There were four treatment groups as follows: (i) CK, no fertilization; (ii) CF, inorganic fertilization; (iii) CHF1, combination of inorganic fertilizer and human feces slurry with a N ratio of 7:1; and (iv) CHF2, combination of inorganic fertilizer and human feces slurry with a N ratio of 3:1. In addition, four different vegetable crops were successfully grown in each year, such as fennel (*Foeniculum vulgare Mill.*), Chinese cabbage (*Brassica rapa L.*), spinach (*Spinacia oleracea L.*) and lettuce (*Lactuca sativa L.*), and there was a short fallow after the harvest of each crop. The same vegetable rotation was performed in the second year, indicating that a total of eight vegetable crops were harvested during this field experiment. The fertilizer rates of urea (N = 46.4%), calcium superphosphate (P₂O₅ = 60.7%), potassium chloride (K₂O = 63.1%) and human feces are listed in Table 2. All the fertilizers were incorporated into the soil 3–4 days before sowing. The rates of N application were set according to the local vegetable cropping regimes and fertilizing practices. Meanwhile, the crops in CK group followed the field management practices as similar to those in N-supplied treatment groups. All vegetable fields were plowed before transplanting or sowing. According to the local practices, the application of N fertilizer was often paired with irrigation.

Table 2

Annual fertilizer application rates for the different experimental treatments of vegetables (kg ha⁻¹).

| Treatments | Application date | Vegetable rotation | Applied fertilizers rate (kg ha ⁻¹ yr ⁻¹) | | |
|--|------------------------------|---------------------------|---|-------------------------------|------------------|
| | | | Inorganic fertilizer ^a + human feces slurry ^b | | |
| | | | N | P ₂ O ₅ | K ₂ O |
| CK | Sep.5, 2017; Nov.8, 2017; | Fennel - Spinach - | 0 | 0 | 0 |
| CF | Mar.13, 2018; July.24, 2018; | Chinese cabbage - Lettuce | 800 | 800 | 800 |
| CHF1 | Sep.26, 2018; Nov.3, 2018 | | 700 + 100 | 735 + 65 | 665 + 135 |
| CHF2 | Mar.18, 2019; July.28, 2019; | | 600 + 200 | 669 + 131 | 531 + 269 |
| ^a Inorganic fertilizers were Urea [CO(NH ₂) ₂ , N = 46.4%], calcium superphosphate [Ca(H ₂ PO ₄) ₂ , P ₂ O ₅ = 60.7%] and potassium chloride [KCl, K ₂ O = 63.1%] | | | | | |
| ^b Human feces was applied 20 t ha ⁻¹ yr ⁻¹ and 41 t ha ⁻¹ yr ⁻¹ in the CHF1 and CHF2 treatment, respectively. | | | | | |

2.3 Assessments of NO, N₂O and NH₃ fluxes

The N₂O and NO fluxes in each cultivation plot were determined *in situ* via a static opaque chamber method (Zheng et al., 2008). Briefly, four gaseous samples were collected from the chamber headspace with polypropylene syringes (20 mL capacity) at 0, 10, 20, and 30 min following chamber closure. Approximately 1 L of gaseous sample was pulled into an evacuated sampling bag before closing the chamber; and 30 min later (i.e., after completion of N₂O sampling), another 1 L of gas sample was extracted into new evacuated sampling bag. The air temperature inside the chambers, soil temperature at 10 cm depth and soil moisture at 0–15 cm depth were monitored.

The concentrations of N₂O were determined using an Agilent 7890A gas chromatography system (Agilent Ltd., Shanghai, China) with 2 detectors (ECD and FID). The concentrations of NO were measured by a Thermo model 42i chemiluminescence NO–NO–NO_x analyzer (Thermo Environmental Instruments Inc., Franklin, MA, USA). NH₃ volatilization fluxes were assessed with a continuous air-flow enclosure method (Sun et al., 2017), and the measurement device consisted of a chamber, two flasks a vacuum pump and a vent pipe. The absorbent for NH₃ was 80 mL of 2% w/v boric acid, and the absorption was determined by titration with 0.01 M H₂SO₄ using a mixture of bromocresol green and methyl red (in ethanol solution) as an indicator. NH₃ volatilization was measured once a day after fertilization until no volatilized gas was detectable (about 10 days).

The gaseous reactive nitrogen emissions (GNrEs, kg-N·ha⁻¹) and the reactive gaseous N intensity (GNrI; kg-N·t⁻¹ yield) was calculated using the following equations:

$$\text{GNrEs} = \sum_{j=1}^n FC_{\text{N}_2\text{O}+\text{NO}+\text{NH}_3}$$

$$\text{GNrI} = \text{GNrEs}/\text{vegetable yield}(\text{fresh yield})$$

2.4 Vegetable yield, above-ground N uptake and use efficiency

After reaching physiological maturity, the above-ground parts of each vegetable crop in each plot were weighted and recorded as fresh weight. Subsequently, total N uptake was calculated from the biomass of harvested crop within each plot. The crop biomass was air-dried, and then oven-dried at 65°C for 3 days. After drying, the yield of dry matter was determined. Subsamples were then ground using a ball mill, and their N content was evaluated by a FOSS N analyzer (KT260, Foss Co., Germany). Nitrogen use efficiency (NUE) was calculated using the following equation:

$$\text{NUE} (\%) = \delta \text{TU}_{\text{N-CK}} / \text{TN}$$

where TU_{N-CK} is the difference in the N content of the above-ground crops between N-supplied treatment groups and control group (kg·ha⁻¹); and TN is the total input of fertilizer N (kg·ha⁻¹).

2.5 Estimation of Nr footprint, ecosystem input and ecosystem-economic income

The Nr footprint (g·N·kg⁻¹ food) was calculated as follows:

$$\text{Nr footprint} = \left(\sum_{i=1}^m AI_{i\text{Nr}} + \sum_{j=1}^n FC_{j\text{Nr}} \right) / \text{vegetable yield}$$

where AI_{iNr} indicates the loss of Nr (primarily through NO_x and N₂O emissions) during the harvesting and postharvest handling of agricultural inputs, and the values are shown in Table S1; FC_{jNr} denotes the loss of Nr during farm cultivation (primarily through NO_x and N₂O emissions, N leaching and

runoff, and NH₃ volatilization). The amounts of N₂O and NO emissions were estimated in this field study by using both static chamber and gas chromatography methods, and other Nr species were determined by multiplying the amount of fertilizer by their individual emission factor as described by Ti et al. (2015), i.e., 0.14 and 0.12 for open-air and greenhouse vegetable cropping systems, respectively. The vegetable yields used in this formula were dry matter yields.

The environmental external cost is constituted of the global warming associated with greenhouse gas emissions, soil acidification linked to NH₃ and NO_x emissions, and aquatic eutrophication resulted from NH₃ emission as well as N leaching + runoff (Xia and Yan, 2012). Here, the ecosystem input (CNY ha⁻¹) associated with N loss was assessed using the following equation:

$$\text{environmental external cost} = \sum_{i=1}^n N r_i A * P_i$$

where N_rA (kg N) indicates the released amount of specific Nr forms; and P_i (¥ kg⁻¹ N) is the cost to climate change, ecosystems and human health per kg of specific Nr (Table S2).

Net economic income (NEI; CNY ha⁻¹) and net ecosystem-economic income (NEEI, CNY ha⁻¹) for crop production were determined using the following equations:

$$\text{NEI} = \text{yield income} - \text{input cost}$$

$$\text{NEEI} = \text{NEI} - \text{environmental external cost}$$

where 'yield income' (CNY kg⁻¹) represents the market value of each crop; 'input cost' (CNY kg⁻¹) denotes the cost (per kg) incurred during food production, including procurement of labor and agricultural materials; and 'environmental external cost' (CNY kg⁻¹) indicates the cost (per kg) incurred during food production as a consequence of damage triggered by Nr release.

2.6 Statistical analysis

Statistical analysis was carried out using JMP version 9.0 (SAS Institute Inc., Cary, NC, USA, 2010). All data were normally distributed and had homogeneous variances, hence, the experimental results were analyzed with a parametric test. The statistical comparison the annual and seasonal cumulative emissions of N₂O and NO, vegetable yield, and Nr footprints among different treatment groups was conducted using an one-way analysis of variance (ANOVA). Tukey's multiple range test was employed to assess whether there is any significant difference between the treatment groups at a significance level of < 0.05.

Results

3.1 Reactive gaseous nitrogen emissions

During the vegetable production period, N₂O and NO emissions peaked after the application of N fertilizer, tailed off after one week, and then remained low (Fig. 1a). Notably, the peaks of NO fluxes were lower than those of N₂O (Fig. 1b). The seasonal N₂O emissions occurred mainly in the fennel planting season, which might attributed to the relatively high temperature. The largest NO flux peak occurred in the Chinese cabbage growing season after treatment with CHF2. Despite relatively lower temperature during the planting seasons of spinach and Chinese cabbage, several NO fluxes peaks were still observed, mainly due to a decrease soil moisture that is conducive to NO emissions.

The cumulative reactive gaseous nitrogen emissions generated from different treatments are presented in Table 3. The average seasonal cumulative N₂O, NO and NH₃ fluxes of the four treatment groups ranged from 5.9–17.1 kg·N·ha⁻¹, 1.6–5.4 kg·N·ha⁻¹ and 5.1–12.7 kg·N·ha⁻¹, respectively. Notably, CHF2 treatment significantly increased N₂O emissions by 44.9 % compared to the CF treatment groups, respectively (*p* < 0.05; Table 3). The lowest average cumulative NO emissions were found in CHF1 group among the three N-supplied treatment groups, but the differences were not statistically significant (*p* > 0.05). The lowest NH₃ emission was recorded in CHF2, which was remarkably lower than that in CF (*p* < 0.05). In addition, CHF2 treatment significantly increased GNrEs and GNrI by 8.4% and 12.5% when compared to CF treatment group (*p* < 0.05), and these values were the highest among the three N-supplied treatment groups.

Table 3
Mean annual N₂O, NO and NH₃ emissions, cumulative reactive gaseous nitrogen emissions (GNrEs) and reactive gaseous nitrogen intensity (GNrI) over the two years vegetable cycles.

| Treatments | N ₂ O emissions | NO emissions | NH ₃ emissions | GNrEs | GNrI |
|---|----------------------------|-----------------------|---------------------------|-----------------------|----------------------------|
| | kg N ha ⁻¹ | kg N ha ⁻¹ | kg N ha ⁻¹ | kg N ha ⁻¹ | kg N t ⁻¹ yield |
| CK | 5.9 ± 1.5 c | 1.6 ± 0.2 b | 5.1 ± 0.4 c | 12.6 ± 1.6 c | 0.13 ± 0.01 c |
| CF | 11.8 ± 1.6 b | 5.2 ± 0.9 a | 12.7 ± 1.3 a | 29.7 ± 3.5 b | 0.16 ± 0.01 b |
| CFH1 | 14.5 ± 2.9 ab | 4.5 ± 1.0 a | 10.4 ± 2.8 ab | 29.4 ± 4.1 b | 0.17 ± 0.02 ab |
| CFH2 | 17.1 ± 3.1 a | 5.4 ± 1.4 a | 9.7 ± 1.1 b | 32.2 ± 3.9 a | 0.18 ± 0.02 a |
| Mean ± SD; lowercase letters within the same column indicate significant differences (p < 0.05) | | | | | |

3.2 Vegetable yield and economic benefits

The vegetable fresh yield and economic benefits among all the treatment groups are shown in Table 4. Not unexpectedly, it was found that the three N-supplied treatment groups (CF, CHF1 and CHF2) markedly elevated the yields of vegetable (91.3%, 77.2% and 78.5%, respectively) compared to CK treatment group ($p < 0.05$; Table 4). The highest average yield (190.2 t·ha⁻¹·yr⁻¹) of vegetable was observed in CF treatment, and as a comparison, the two combinatorial treatments with human feces and inorganic fertilizer (CHF1 and CHF2) decreased the average vegetable yields by 7.4% and 6.7%, respectively. However, no significant difference in vegetable yields was noted among all the N-supplied treatment groups.

Table 4
Mean annual vegetable yields (t⁻¹ ha⁻¹) and components of net ecosystem economic income (NEEI) (CNY ha⁻¹) under different fertilizer strategies in intensive vegetable production.

| Treatments | Yield (t ha ⁻¹) | Output (CNY ha ⁻¹) | agricultural input (CNY ha ⁻¹) | | | | Environmental external cost (CNY ha ⁻¹) | | Net economic income (CNY ha ⁻¹) | Net ecosystem economic income (CNY ha ⁻¹) | ratio of the NEI and NEEI (%) |
|--|--------------------------------|-----------------------------------|---|----------------------|-------|--------|--|--------------------|--|--|-------------------------------|
| | | | seed | chemical fertilizers | labor | others | GNrEs | Leaching & running | | | |
| CK | 99.4 ± 10.8b | 301097 | 11835 | | | 3475 | 732 | 0 | 285787 | 285055 | 0.02 |
| CF | 190.2 ± 7.5a | 434520 | 11835 | 18904 | | 3475 | 1618 | 1228 | 400306 | 397460 | 0.05 |
| CFH1 | 176.1 ± 4.7a | 415452 | 11835 | 16418 | 800 | 3475 | 1737 | 1228 | 382924 | 379959 | 0.04 |
| CFH2 | 177.4 ± 10.8a | 444980 | 11835 | 13932 | 800 | 3475 | 1955 | 1228 | 414938 | 411755 | 0.05 |
| The average price of Fennel, Spinach, Chinese cabbage and Lettuce in market were 1.4 CNY kg ⁻¹ , 8.0 CNY kg ⁻¹ , 1.0 CNY kg ⁻¹ and 3.9 CNY kg ⁻¹ during the years 2017–2019, respectively. | | | | | | | | | | | |
| The average price of Fennel, Spinach, Chinese cabbage and Lettuce seeds were 3600 CNY ha ⁻¹ , 3375 CNY ha ⁻¹ , 3510 CNY ha ⁻¹ and 1350 CNY ha ⁻¹ . | | | | | | | | | | | |
| The average price of fertilizer was 4.1 CNY kg ⁻¹ for urea, 3.4 CNY kg ⁻¹ for calcium superphosphate and 5.8 CNY kg ⁻¹ for potassium chloride, respectively. | | | | | | | | | | | |
| The labor means septic tank cleaning, about 200 CNY once for three months. | | | | | | | | | | | |
| The others contained plastic film and pesticide. | | | | | | | | | | | |

Table 4 also shows the total economical balance for the input resources, the output profits, and the environmental cost for different treatment groups averaged over two years. The inputs for agricultural production, such as seeds (34.6–39.4%) and fertilizer (46.4–55.3%), constituted an important part of the total input of the N-supplied vegetable cropping system. Given that most farmers have enough family laborers to work on vegetable production, the labor cost generally included septic tank cleaning only. In addition, compared to CF treatment, CFH2 and CFH1 treatments increased (3.7 %) and decreased (4.3 %) the farmer's economic income, respectively. The environmental external cost associated with the GNrE as well as the leaching and runoff caused by N application should be considered due to their influences on the society. The total environmental external cost varied from 732 to 3183 CNY ha⁻¹ in the four treatment groups, which accounted for 4.6–9.6 % of the total inputs (Table 4).

3.3 N uptake and NUE

Higher N uptake ($0.69 \text{ kg-N}\cdot\text{ha}^{-1}$) was observed in CF treatment group, whereas the addition of human feces could decrease N uptake compared to CF treatment group, but the differences were not statistically significant ($p > 0.05$; Fig. 2a). Taking the amounts of N uptake into considerations, we also quantified the NUE in this study (Fig. 2b). The NUE of all the four vegetable crops ranged from 16.8–20.3% among all the N-supplied treatment groups. In agreement with the results of vegetable yields, CHF1 and CHF2 treatments both decreased the NUE values by 17.2% and 5.4%, respectively, when compared to CF treatment group, but statistical significance was only found in CHF1 treatment group ($p < 0.05$).

3.4 Nr losses and Nr footprint

The estimated total losses of Nr for all the N-supplied treatments varied from 177.6 to 184.1 $\text{kg-N}\cdot\text{ha}^{-1}$, accounting for 22.2–23.1 % of N application rates. The loss of Nr was mainly dominated by N leaching and runoff, with the combined proportions ranging from 71.7 % to 74.3 %. The other (secondary) contributing factors included fertilizers, pesticide and plastic film. N application in all the N-supplied treatment groups significantly increased the losses of Nr when compared to CK group. In comparison with CF treatment group, the human feces substitution treatments slightly ($p > 0.05$) reduced the losses of Nr by approximately 2.3 % in CHF1 and 3.6 % in CHF2.

The Nr footprint of vegetable production varied from 1.7 (CK) to 11.7 $\text{g-N}\cdot\text{kg}^{-1}$ (CHF1). Compared to CK treatment, N application remarkably increased the value of Nr footprint ($p < 0.05$; Fig. 3). In addition, a consistent effect of the combination of inorganic fertilizer and human feces slurry at different ratios was detected in both CHF1 and CHF2 treatment groups. Compared to CF treatment, the proportions of Nr footprint were increased by 4.4 % in CHF1 group and decreased by 0.5 % in CHF2 group ($p > 0.05$).

Discussion

4.1 Effects of different fertilization strategies on GNrEs

The N fertilizer used in the field could increase the initial levels of NH_4^+ and NO_3^- during nitrification and denitrification processes (Cardoso et al. 2017), which in turn regulates the N_2O emissions (Liu et al. 2012). In our experiment, CHF2 treatment exhibited significantly higher cumulative N_2O emissions than CF treatment, which probably due to higher moisture content in the former (since the water content of human feces slurry as high as 95%). This suggests that an increase in soil moisture content may serve as a key driver of N_2O emissions (Uchida et al. 2011; Hu et al. 2017; Feng et al. 2018), and such phenomenon has been observed in previous researches (Pezzolla et al. 2012; Cardenas et al. 2016), particularly when the soil water-filled pores exceeded 60%. Besides, NH_4^+ nitrification has been recognized as an important pathway for N_2O emissions in N fertilizer-amended soils (Skiba et al., 1993). Indeed, the human feces slurry used in this study contained large amounts of ammoniacal nitrogen, which can provide a considerable amount of nitrification substrate. However, urea needs to be mineralized to HCO_3^- and NH_4^+ in soils before denitrification can take place. This may explain why the increase in N_2O losses induced by human feces slurry application may occur more rapidly than that observed for urea fertilizers.

N fertilization is one of the key points of NO emissions (Sanchez et al. 2010). Our results showed that the types of fertilizer used exhibited no remarkable effect on NO emissions. Liu et al (2016) presumed that ammonium nitrate was the most effective for increasing soil NO emissions among different types of inorganic N fertilizers. However, those findings did not provide information about the pathway of NO production. In recent years, numerous investigations have regarded nitrification as the most predominant process for producing NO (Fan et al. 2020). In the present study, NO emission was the lowest among the three gaseous forms of Nr. NO emission could occur in upland crop systems, but was undetectable in anaerobic soils, which might be attributed to the slow diffusion or fast reduction of NO (Russow et al. 2009; Liu et al. 2016).

NH_3 emission is primarily influenced by various abiotic factors, including NH_4^+ concentration, pH and soil texture (Tasistro et al. 2007; Schraml et al. 2016). In our study, treatment with a relatively high proportion of human feces (CHF2) demonstrated a significantly lower NH_3 loss than CF, probably due to (i) higher pH of human feces slurry and (ii) increased infiltration of liquid slurry into soil. Sha and co-workers (2020) have suggested that a deep placement of fertilizers (e.g., ammonium-based fertilizer, liquid manure, etc.) can decrease NH_3 emissions in alkaline soils at high air temperature. An interesting point in the study presented here was that the amounts of $\text{N}_2\text{O} + \text{NO}$ emissions in the three N-supplied treatment groups decreased in the following order: CHF2 → CHF1 → CF, whereas the trend of NH_3 emissions was reversed as follows: CF → CHF1 → CHF2. Similarly, Fan et al. (2017b) found a significant positive relationship between decreased $\text{N}_2\text{O} + \text{NO}$ emissions and increased NH_3 emissions in an incubation experiment of vegetable soils, given the constant amount of the substrates.

Generally, greenhouse gas intensity and global warming potential (GWP) are used to integrate the effects of greenhouse gases. In our research, GNrEs and GNrl were employed to investigate the overall impact of gaseous reactive nitrogen on the environment. Table 3 showed that N_2O and NH_3 were the two dominant components of GNrEs, accounting for 39.7–53.1 % and 30.1–42.8 %, respectively. These ratios are in accordance with Chen et al. (2020) who reported on one-year winter wheat/summer maize rotation system in the Northern Central Region of China. In addition, the high amounts of human feces slurry significantly increased the values of GNrEs and GNrl, indicating that the use of human feces neither decreases the emission of gaseous reactive nitrogen nor increases the yields of vegetables. This poses a substantial obstacle to the future application of human feces slurry.

4.2 Effects of human feces replacing part of nitrogen fertilizer on NUE, vegetable growth and net ecosystem economic benefits

We estimated the NUE in all the N-supplied treatments, and found that the NUE ranged from 16.8–20.3 %, which were higher than the values reported by Li et al (2017). However, the two treatments incorporating human feces slurry decreased the NUE in relation to CF treatment (Fig. 2), indicating that replacing the inorganic fertilizer with human feces slurry could not effectively increase the NUE during intensive vegetable production. This could be attributed to the higher Nr emissions in CHF1 and CHF2 groups than those in CF group. Generally, substituting compound fertilizers (usually by organic matter) markedly elevated plant N uptake and reduced gaseous N losses (Zhou et al. 2016; Zhuang et al. 2019), however, the composition of human feces slurry was different from that of standard organic fertilizer, despite containing a certain amount of organic matter, and human feces usually considering as quick-acting fertilizer rather than organic fertilizer.

In this study, human feces replacing part of nitrogen fertilizer exerted no pronounced effects on the vegetable yield in two years (four successive vegetable seasons), possibly due to the fact that the total N application rates were relatively similar in the three N-supplied treatment groups (Table 4). It has been shown that with NH_4^+ as the dominant nitrogen form, both grain yields and N uptake improved than the conventional fertilizer strategy (Deppe et al. 2016). However, in this study, the vegetable yields were similar between CHF1 and CHF2 treatment groups, but did not differed greatly from CF treatment group. This was probably due to the fact that we applied human feces slurry as base fertilizer before planting/seeding, suggesting that the high NH_4^+ concentration immediately upon application could not induce toxicity (cf. Müller et al., 2006). Indeed, the results of crop yields were in good agreement with the enhanced uptake of nutrients during the growing season (Cardenas et al. 2016).

Emissions of NO_x , N_2O and NH_3 can threaten human health and cause severe diseases (e.g., cataract, skin cancer, etc.), mainly via particle pollution, ground-level ozone pollution, and stratospheric ozone depletion. In China, the total health damage cost related to atmospheric Nr emissions was estimated at US\$19 – 62 billion in 2008, which is much larger than the damage costs in the United States and Europe. According to Gu et al. (2012), agricultural Nr emission is one of the largest sources of health-related damage, accounting for about 50% of total expenditures in China, which are consistent with the results of this study (Fig. 3). However, other reports identified NO_x emissions as the largest source of health-related damage in the United States and Europe (Birch et al. 2015), which is contrary to our findings (Fig. 3), possibly because of the high N inputs during intensive vegetable production (Table 2). Moreover, leaching + runoff was an important contribution to the ecosystem N input in this study, which emphasized the importance of reducing leaching + runoff during intensive vegetable production. In addition, we used the coefficient estimated by Ti et al (2015) related to the total N applied, which might have underestimated the leaching + runoff losses in the two treatment groups with human feces slurry due to its rapid infiltration.

Profitability is often regarded as the main driver for farmers to improve their agricultural practices. Taking the yield income, input costs and environmental cost into consideration, we also quantified the net ecosystem economic benefits among the three N-supplied treatment groups (Table 4). Notably, CHF2 had the highest values of NEI and NEEI, representing a sufficient incentive for farmers to alter their N management strategies, if such information were available for them.

4.3 Nr footprint in crop production

The evaluation of Nr footprint can aid better detection of the 'authentic' environmental hotspots of Nr releases in the national food system (Cheng et al. 2014; Chen et al. 2020). In our research, the Nr footprint ranged from 1.6–11.8 g-N-kg⁻¹ (Fig. 3), which were comparable to the findings in a wheat-maize system in North China Plain (Xu et al. 2020) and different fertilization strategies in vegetable crop rotations in Southeast China (Zhou et al. 2019). In addition, our results indicated that the N leaching and runoff, with a high N application rate, was the dominant contribution to the field Nr footprint, accounting for about 72% of the total Nr footprint. It is commonly known that N fertilizer is an essential source of Nr emissions during crop cultivation (Chen et al., 2014). However, this is inconsistent with Xue and co-workers (2016), who analyzed the Nr footprint of double rice production in Southern China by using the life cycle assessment method, and identified that NH_3 emission from paddy fields could be the main contributor. Typically, the Nr footprint in paddy fields is dominated by NH_3 volatilization, whereas N leaching/runoff is a primary contributor to Nr footprint in the upland ecosystems (Xia et al. 2016). It is worth noting that the amount of N leaching and runoff in our study was estimated by the coefficient obtained from Xia et al. (2017), thereby avoiding possible uncertainty. The three N-supplied treatment groups had the similar Nr footprint values ($p > 0.05$), indicating that all the fertilizer types exert a similar environmental impact.

Conclusion

A major obstacle for solving N-driven environmental issues is the lack of knowledge on complete N budgets, such as the major N fluxes, in agricultural systems, especially those under alternative farm management practices. Our results indicate that the release of Nr is dominated by N leaching and runoff. The human feces slurry substituting for a portion of inorganic fertilizer elevated Nr footprint and environmental cost when compared to CF treatment. However, although with increasing environmental costs, CHF2 treatment increased farmers' net economic income compared to CF treatment. Our study suggests that the application of human feces slurry together with inorganic fertilizer at an appropriate ratio could increase farmers' net income, even though releases more Nr into environment. Therefore, further investigations are required to ensure Nr mitigation in cases of human feces slurry treatment during intensive vegetable production.

Declarations

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Authors Contributions

Bo Yang: Data curation, Writing- Original draft preparation.

Tao Zhang: Conceptualization, Methodology, Software.

Man Zhang: Visualization, Investigation.

Bo Li: Supervision, Writing- Reviewing and Editing.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare no competing financial interests.

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Figures

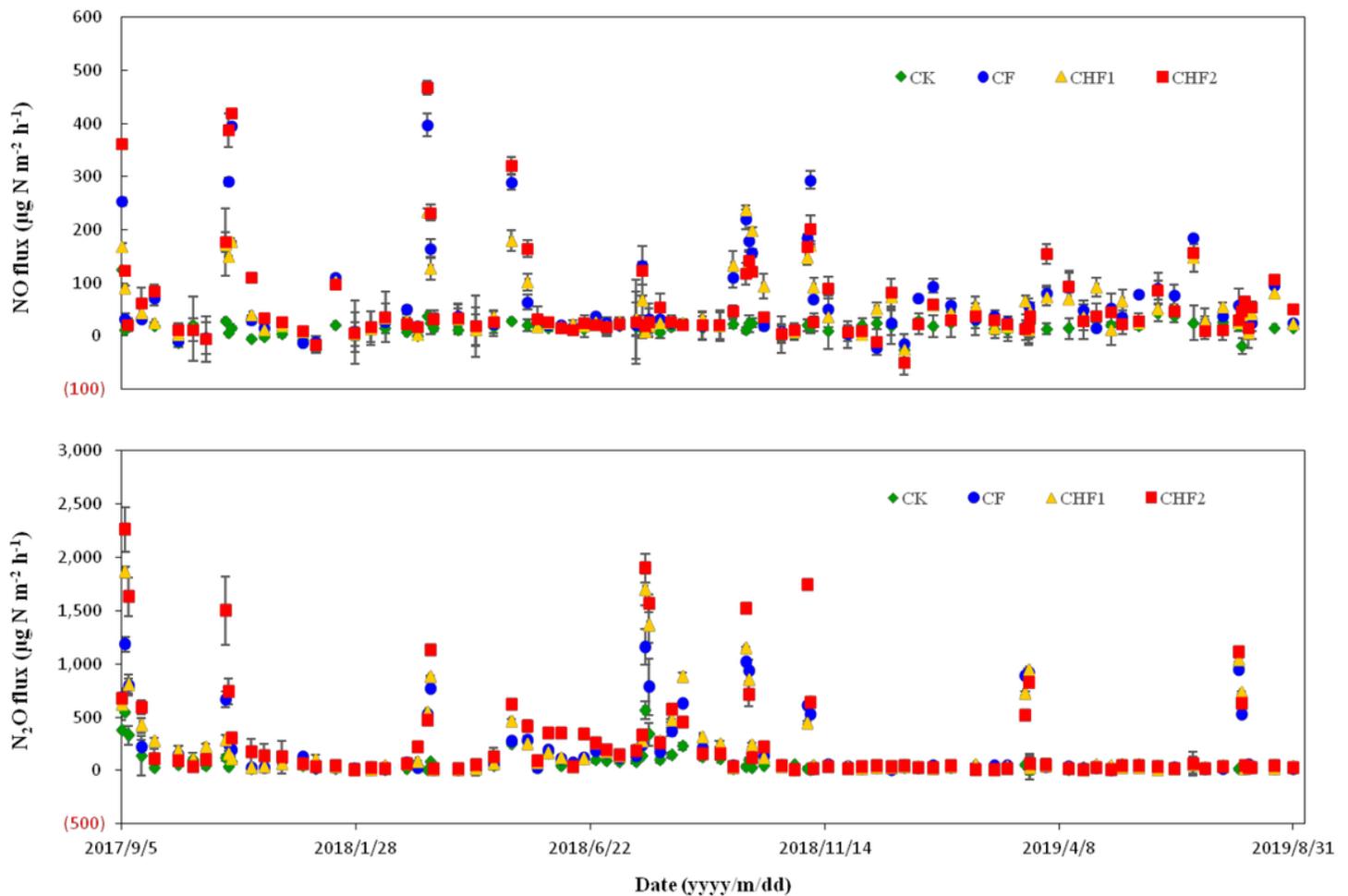


Figure 1

Dynamics of soil NO fluxes (a) and N₂O (b) fluxes under different treatments in intensified vegetable field. CK, no fertilization addition; CF, inorganic fertilization addition; CHF1, human feces slurry/inorganic fertilizer=1:7; CHF2, human feces slurry /inorganic fertilizer=1:3. The bars indicate the standard error of the mean (+SE) for the three replicates of each treatment.

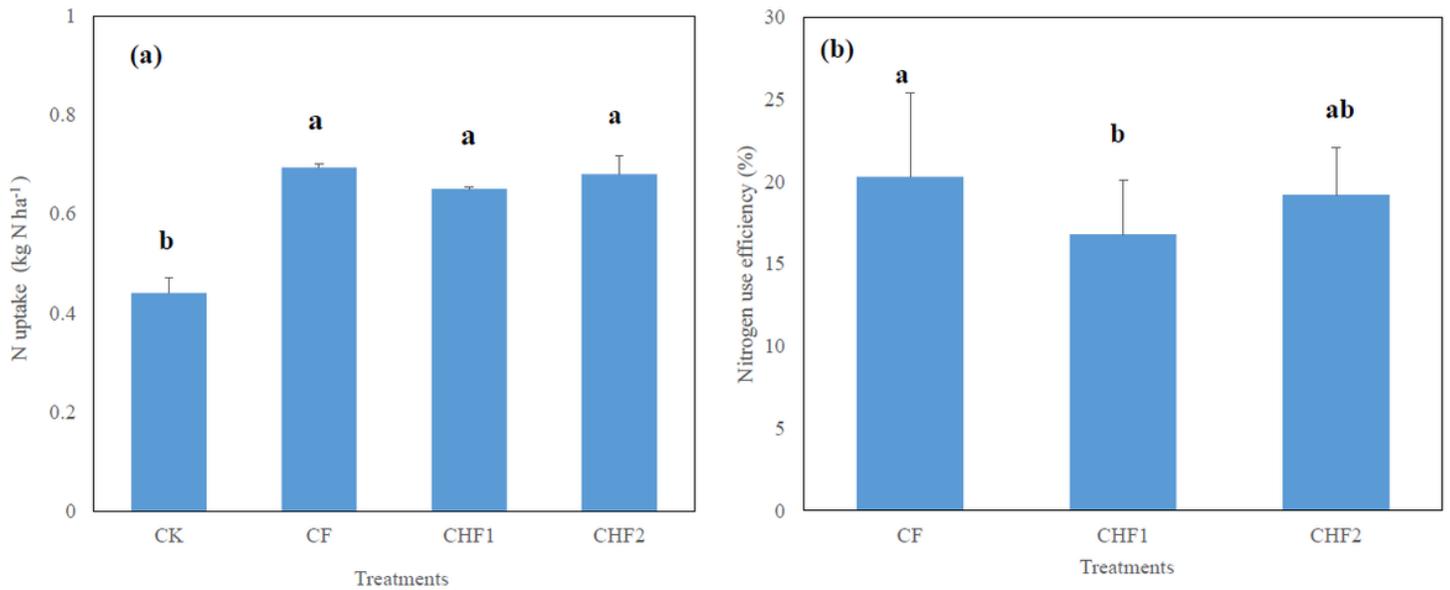


Figure 2
 N uptake (a) and nitrogen use efficiency (b) under different treatments in intensified vegetable field. The bars indicate the standard error of the mean (+SE) for the three replicates of each treatment. See Table 1 for treatment codes.

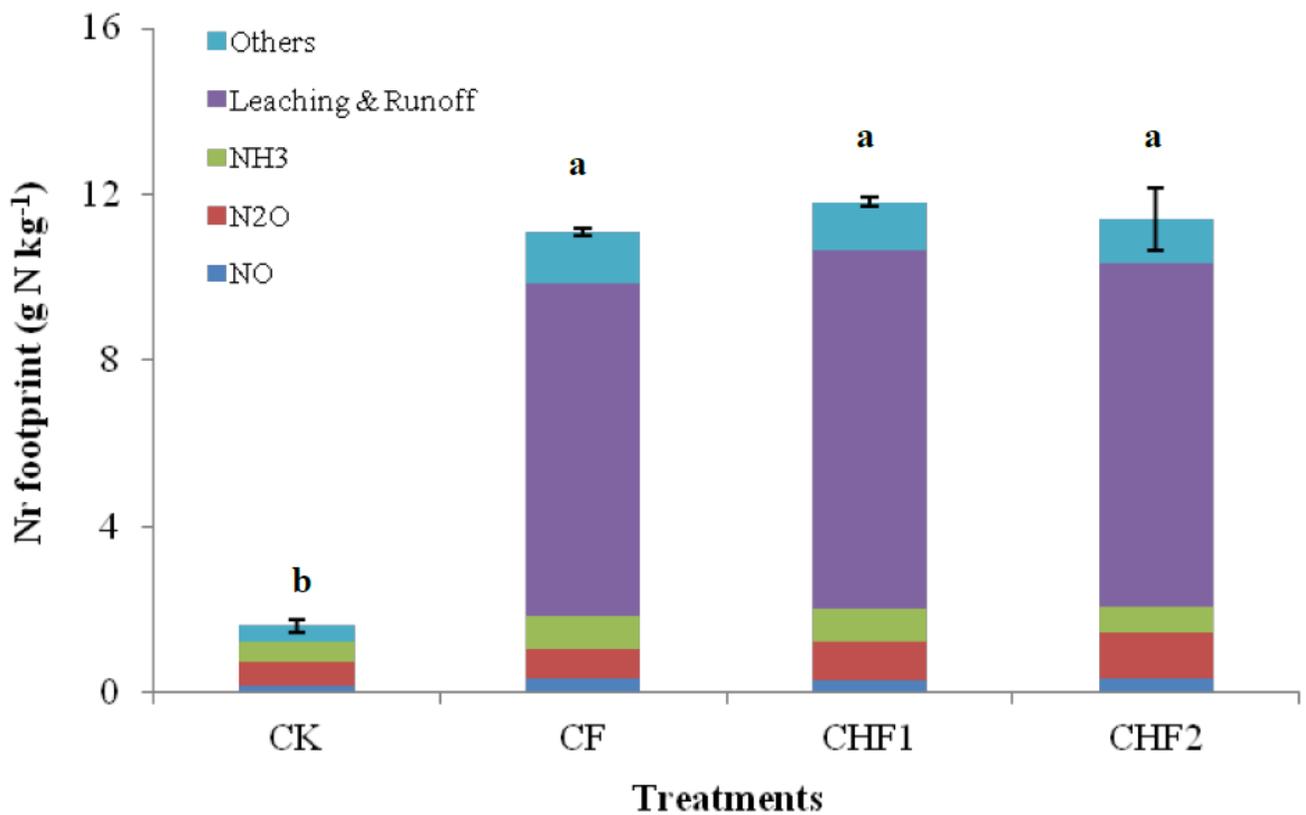


Figure 3
 Contributions of different sources/activities to the Nr footprints of intensive vegetable production. N fertilizer refers to synthetic N fertilizer production, transportation and application; Others refers to the sum of other sources of GHG, such as the production of phosphorus, potassium and their transportation and application. The small letters in each sub figure indicate significant differences according to the Tukey's multiple range test ($p < 0.05$) among all the treatments.

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