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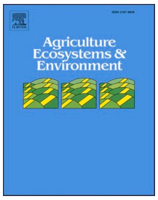

Comprehensive assessment of the effects of nitrification inhibitor application on reactive nitrogen loss in intensive vegetable production systems

Ju Min[a], Haijun Sun[b,d], Herbert J. Kronzucker[c], Yuan Wang[a], Weiming Shi[a,*]

[a] State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China
[b] Co-Innovation Center for Sustainable Forestry in Southern China, College of Forestry, Nanjing Forestry University, Nanjing 210037, China
[c] Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC, V6T 1Z4 Canada
[d] Key Laboratory of Soil and Water Conservation and Ecological Restoration of Jiangsu Province, Nanjing Forestry University, Nanjing 210037, China

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ABSTRACT

Previous studies regarding environmental impacts of nitrification inhibitors (NIs) in intensive vegetable production systems mainly focused on the fate of individual nitrogen (N) compounds. Due to the influence of various N-dynamic pathways on one another, systematic observations, taking into account all key component processes, must be carried out to achieve practically useful recommendations. As well, the mechanisms of how NI application leads to increasing vegetable yields are not well understood. Therefore, we conducted a field experiment with three leading vegetable crops (lettuce, celery, and tomato), and two urea N input rates, without (N1, N2) or with a nitrification inhibitor, 2-chloro-6-(trichloromethyl)-pyridine (CP) (N1 + CP, N2 + CP), to evaluate the comprehensive effects of CP on reactive-N emission in these intensive vegetable production systems, with a focus on leaching, ammonia (NH_3) volatilization, and nitrous oxide (N_2O) emission, and to clarify the possible mechanisms by which CP affects vegetable yield. The results show that CP application significantly decreased ($p < 0.05$) N leaching by 36.9, 26.9, and 28.4 %, soil residual NO_3-N contents by 34.1, 43.7, and 43.9 %, N_2O emission by 46.4, 77.2, and 36.9 %, and significantly increased ($p < 0.05$) NH_3 volatilization by 33.5, 56.3, and 308.1 % in the lettuce, celery, and tomato seasons, respectively, while having no significant effect on yield at the typical N-application rate (N2). Under 60 % of the typical N-application rate (N1), CP addition significantly increased ($p < 0.05$) yield and N-use efficiency (NUE) over the three-season period by 23.9 and 55.1 %, respectively, significantly reduced ($p < 0.05$) N_2O emission by 43.5 %, while having no significant effect on the other three observed N processes. In a lettuce-celery-tomato rotation, compared with the typical N-application rate (N2), 60 % of the typical N-application rate with CP addition (N1 + CP) significantly increased ($p < 0.05$) yield and NUE by 37.1 and 214 %, and decreased ($p < 0.05$) soil residual NO_3-N contents, N leaching, and N_2O emission by 70.9, 51.1, and 69.6 %, respectively, and had no significant effect on NH_3 volatilization. Furthermore, the distribution analysis of N derived from ^{15}N-labeled urea in tomato aboveground suggested that CP application significantly decreased ($p < 0.05$) N allocation to stems and leaves by 12.1 and 9.7 %, and significantly increased ($p < 0.05$) N allocation to fruits by 31.2 %, averaged over 60 % and 100 % N treatments. Application of CP increased N storage in fruits and benefited yield.

1. Introduction

The total vegetable production area in China has significantly increased over the past three decades. The total cultivated land area for vegetables has increased to 22.3 million hectares, equivalent to 13.4 % of the total national crop plantation area (National Bureau of Statistics of China, 2017). As elsewhere, nitrogen (N) fertilization is a widespread practice in the management of vegetable fields to ensure good yield and quality of marketable produce (Agostini et al., 2010). Over the past 30 years, N fertilizer application rates have increased dramatically in agricultural systems in China, and the annual synthetic N-fertilizer input has exceeded 1000 kg N ha^{-1} to meet the high requirement presented by

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* Corresponding author.
E-mail address: wmshi@issas.ac.cn (W. Shi).

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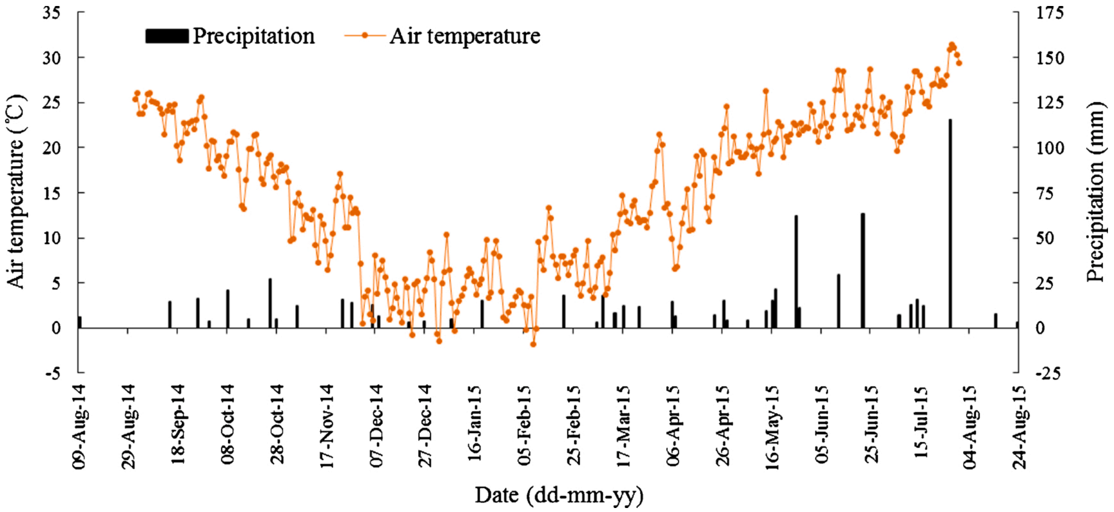

Fig. 1. The air temperature and precipitation during the lettuce, celery, and tomato cultivation periods.

three-to -four annual crop harvests in some cases (Shi et al., 2009). However, agronomic nitrogen use efficiency (NUE) is typically below 30 % (Yan et al., 2014; Coskun et al., 2017), particularly in intensive vegetable systems, where NUEs of only 12–18 % are the norm (Song et al., 2009; Li et al., 2017). The massive releases of the excessive N greatly disturb the natural biogeochemical cycle of N and result in severe environmental problems to water, air and soil (Xiong et al., 2006; Min et al., 2012a,b; Min et al., 2016; Shen et al., 2016; Coskun et al., 2017). Therefore, maintaining crop production while reducing the detrimental effects of N application is an urgent priority for global food security and environmental sustainability (Galloway et al., 2008; Fowler et al., 2013; Min and Shi, 2018).

Urea is widely used as the source of N in vegetable cultivation, due to its cheap price and rapid effects. However, microbial nitrification in the soil converts most urea-N into highly mobile nitrate (NO_3–) within 2–3 weeks of application (Huber et al., 1977). Most NO_3– is lost by leaching, associated with low N retention in soil and consequently low NUE for vegetable crops. Usage of nitrification inhibitor (NI) can suppress soil nitrification, and the use of 2-chloro-6-(trichloromethyl)-pyridine (CP, N-serve), dicyandiamide (DCD), and 3,4-dimethylpyrazole phosphate (DMPP) have received considerable attention in recent years (Di and Cameron, 2005; Subbarao et al., 2006; Chen et al., 2010; Sun et al., 2015; Chen et al., 2019). Soil amendment involving NIs can significantly mitigate nitrous oxide (N_2O) emission (Cui et al., 2011; Scheer et al., 2014; Fan et al., 2018; Lam et al., 2018), reduce NO_3^- leaching (Cui et al., 2011; Wang et al., 2019), although can promote ammonia (NH_3) volatilization (Soares et al., 2012; Zaman et al., 2013; Chen et al., 2015). Qiao et al. (2015) assessed how NIs affect both hydrologic and gaseous N losses and plant NUE by a meta-analysis. Reactive-N losses from an intensive vegetable field include the pathways of NO_3– leaching, NH_3 volatilization, and N_2O emission, which all must be quantified to enable a full assessment of the environmental effects of N-management strategies. However, previous studies regarding the effects of NI application on crop productivity and environmental impacts have rarely considered all forms of the N-loss cascade within the same field settings, or over multiple seasons. Most studies were designed to evaluate one or two pathways of N loss impacted by NI addition, such as N_2O emission (Watanabe, 2006; Zhang et al., 2015), NH_3 volatilization (Ni et al., 2014; Soares et al., 2012), and N leaching (Wang et al., 2019). Due to the mutual influence of the fluxes in the various pathways upon one another, a systematic, comprehensive analysis is required. Some studies have shown that the use of NIs can improve NUE in rice, wheat, and other leading crops (Sun et al., 2015; Zhang et al., 2015; Chen et al., 2019). Vegetable cultivation under plastic shed conditions is often characterized by multiple harvests within a year (a high cropping index) and high N-application rates (Zhu et al., 2011), significantly greater than those for cereal crops. In our previous study, a four-year field trial demonstrated that decreasing chemical N input by 40 % did not reduce vegetable yields but mitigated N loss, while increasing NUE (Min et al., 2012a). Chen et al. (2019) found that a one-third reduction in the conventional N-fertilizer rate, when combined with CP application, was recommended to mitigate N_2O emissions and maintain vegetable yields. Addition of DCD reduced NO_3^- leaching by 58.5 % and 36.2 %, and the N_2O emission factor by 83.8 % and 72.7 % in the alfisol and fluvisols soils under intensive vegetable production, respectively (Cui et al., 2011). Wang et al. (2019) reported that combined addition of urease-inhibitor plus DCD could decrease N leaching by 23.5 % in a typical vegetable field in northern China. However, the effects of NIs in terms of environmental impact from the multiple pathways in any given vegetable season at different N levels are not well understood. Moreover, whether the application of NIs to vegetable fields can enhance NUE, and thereby improve yields in each vegetable season, as well as its related mechanisms, is not known.

Therefore, we conducted a field experiment involving three major vegetable crops (lettuce, celery, and tomato) grown throughout the year, over three seasons, and two N-application rates, with and without CP addition, to assess the full set of effects of NI application on reactive-N losses to environment under intensive management. For tomato, we also monitored the distribution of ^{15}N-labeled urea in vegetable shoots, leaves, and fruits, to explore the possible mechanism by which NIs affect yield under intensive vegetable cultivation conditions. The specific objectives of this study were: 1) to quantify the impacts of CP application on reactive-N losses via leaching, NH_3 volatilization, and N_2O emission; 2) to identify the appropriate N-application rate, when combined with CP, to both improve yield and reduce adverse environmental effects; and 3) to explore the mechanism by which CP addition affects vegetable yield.

2. Materials and methods

2.1. Experimental site

The field experiment was conducted at a representative intensive vegetable farm (31°14′ N and 119°53′ E) in Yixing, Jiangsu Province, China from September 2014 to July 2015. This region has a sub-tropical monsoon climate and the mean annual air temperature and rainfall are 15.7 °C and 1100 mm, respectively. The air temperature and precipitation during the study period are shown in Fig. 1. The greenhouse was covered with a plastic sheet throughout the year. The soil was classified as an *Anthrosol*, consisting of 8.3 % sand, 76.4 % silt, and 15.3 % clay,

Table 1

Cultivation, experimental design, and application rates of synthetic N fertilizers, organic manure, and nitrification inhibitor CP.

Treatments [a]	Growth period (mm/dd/yy)	Growth days	Days between the planting and topdressing	Application amount of N fertilizers (kg N ha^{-1})			CP
				Total	Basal fertilizer	Topdressing fertilizer	
Lettuce							
N0		110	61	0	0	0	0
N1				162	81	81	0
N1 + CP	09/16/14 01/04/15			162	81	81	0.39
N2				270	135	135	0
N2 + CP				270	135	135	0.65
Celery							
N0		108	63	0	0	0	0
N1				180	90	90	0
N1 + CP	01/14/15 03/30/15			180	90	90	0.43
N2				300	150	150	0
N2 + CP				300	150	150	0.72
Tomato							
N0		103	35	0	0	0	0
N1				180	90	90	0
N1 + CP	04/16/15 07/28/15			180	90	90	0.43
N2				300	150	150	0
N2 + CP				300	150	150	0.72

[a] Urea (N content: 46 %) was used as N fertilizer, and all treatments received 78 kg N ha^{-1} of organic manure, 150 kg K_2O ha^{-1} and 120 kg P_2O_5 ha^{-1} as basal fertilizer at the initiation of each of the vegetable growth seasons, respectively.

with an initial pH (1:1 soil to water) of 5.58, an electrical conductivity (EC in 1:5 soil to water extract) of 0.28 mS cm^{-1}, a soil organic matter content of 24.9 g kg^{-1}, and a total N content of 1.04 g kg^{-1}.

2.2. *Experimental design and field management*

The NI used was 2-chloro-6-(trichloromethyl)-pyridine (CP), produced by the Aofutuo chemical company, Shaoxing, Zhejiang Province, China (http://aftchem.company.lookchem.cn); mixing rate of CP was approximately 0.24 % of the applied urea-N, in a mass ratio. The following five treatments with three replications were evaluated: 1) no urea N (N0), 2) a traditional urea-N rate of 270 kg N ha^{-1} for lettuce, and 300 kg N ha^{-1} for celery and tomato (N2), 3) a traditional urea-N rate with CP (N2 + CP), 4) 60 % of the traditional urea-N rate of 162 kg N ha^{-1} for lettuce, and 180 kg N ha^{-1} for celery and tomato (N1), 5) 60 % of the traditional urea-N rate with CP (N1 + CP). The seedlings of lettuce, celery, and tomato were transplanted at the beginning of their respective growing seasons. The fifteen plots (each plot measured 7.0 m × 2.5 m) were arranged in a randomized complete block design, and fifteen microplots were designed as subplots distributed in each plot during the tomato season; these were used for determination of the distribution of ^{15}N from labeled urea in aboveground tomato organs. An ^{15}N-microplot experiment was carried out, and polyvinyl chloride plastic columns were inserted to a soil depth of 50 cm, protruding 10 cm above the soil, and the inner diameter of the columns was 50 cm. The ^{15}N-labeled urea (^{15}N abundance was 10 %) was provided by the Shanghai Research Institute of Chemical Industry. The treatments are shown in Table 1. Basal fertilizers were broadcast evenly onto the soil surface by hand and then incorporated into the soil with shallow plowing before vegetable seedlings were transplanted. The two topdressings were broadcast without incorporation. The vegetable soils were immediately irrigated after each fertilization.

2.3. *Sampling and measurements*

2.3.1. *Plant and soil sampling and measurements*

Vegetables were manually harvested from the whole of each plot to determine yield for each season. The total N concentration of plants was determined according to Chen et al. (2013), and NUE was calculated as the percentage of applied fertilizer N recovered in above-ground biomass minus that of the N0 treatment. The ^{15}N abundance was analyzed using an isotope ratio mass spectrometer (MAT-251, USA, with analytical error ±0.02 %). Four soil cores (3.3 cm diameter) to a 20 cm depth were taken from each plot following lettuce, celery, and tomato harvest. Fresh soil samples were mixed thoroughly and sieved through a 5-mm nylon screen. 10-g subsamples were extracted by shaking with 100 mL 2 mol L^{-1} KCl for 1 h. The filtrate was stored at −20 °C in a freezer until analysis for NO_3-N and NH_4-N concentrations with a continuous flow analyzer (Skalar Corp., The Netherlands).

2.3.2. *N leaching measurements*

Nitrogen leaching samples were collected using lysimeters embedded in the subsoil in each plot at a 0.5-m depth one year before the experiment began, as described by Min et al. (2011). The plots were separated by 0.8 m PVC plates buried belowground, according to the highest groundwater table in the experimental area during the summer. The leaching water from each lysimeter was collected with a PVC tube, and a 200-mL sample was taken, filtered, and frozen for NO_3-N, NH_4-N, and total-N analyses. The total volume of water in the lysimeter was recorded, and then the excess water in the container was emptied prior to subsequent collections. Water samples were collected every 7–10 days from the lysimeter.

2.3.3. *Ammonia volatilization and nitrous oxide emission measurements*

Ammonia volatilization was measured with a modified continuous airflow enclosure method (Kissel et al., 1977). Measurements of NH_3 volatilization were taken twice daily, in the morning (8:00 to 10:00) and in the afternoon (13:00 to 15:00), immediately after fertilizer application. The air was continuously pumped (rate: 15–20 chamber volumes per minute) and allowed to flow through NH_3 absorbent (H_3BO_3 (2% v: v) + mixed indicators of methyl red, bromocresol green, and ethanol) for each measurement. Daily measurements continued until there was no difference in NH_3 volatilization between the N-treated plots and the control. Cumulative NH_3 volatilization load was calculated by the sum of daily emissions over the observation period. Total NH_3 losses under the treatments with N applications were calculated by subtracting the cumulative NH_3 losses from the N0 treatment from the cumulative NH_3 losses under the other treatments.

Nitrous oxide emissions were measured using the closed chamber technique described by Xing et al. (2002). Each static chamber covered an area of 0.6 × 0.7 m and its height could be adjusted from 0.6 to 1.2 m depending on the heights of the plants. N_2O fluxes were measured in triplicate, usually once per week throughout each vegetable growth period. Measurements were carried out more frequently after fertilization and irrigation. Boxes were kept on the plants continuously for 1 h during the daytime (9:00–10:00 am) when sampling. Four samples at 15

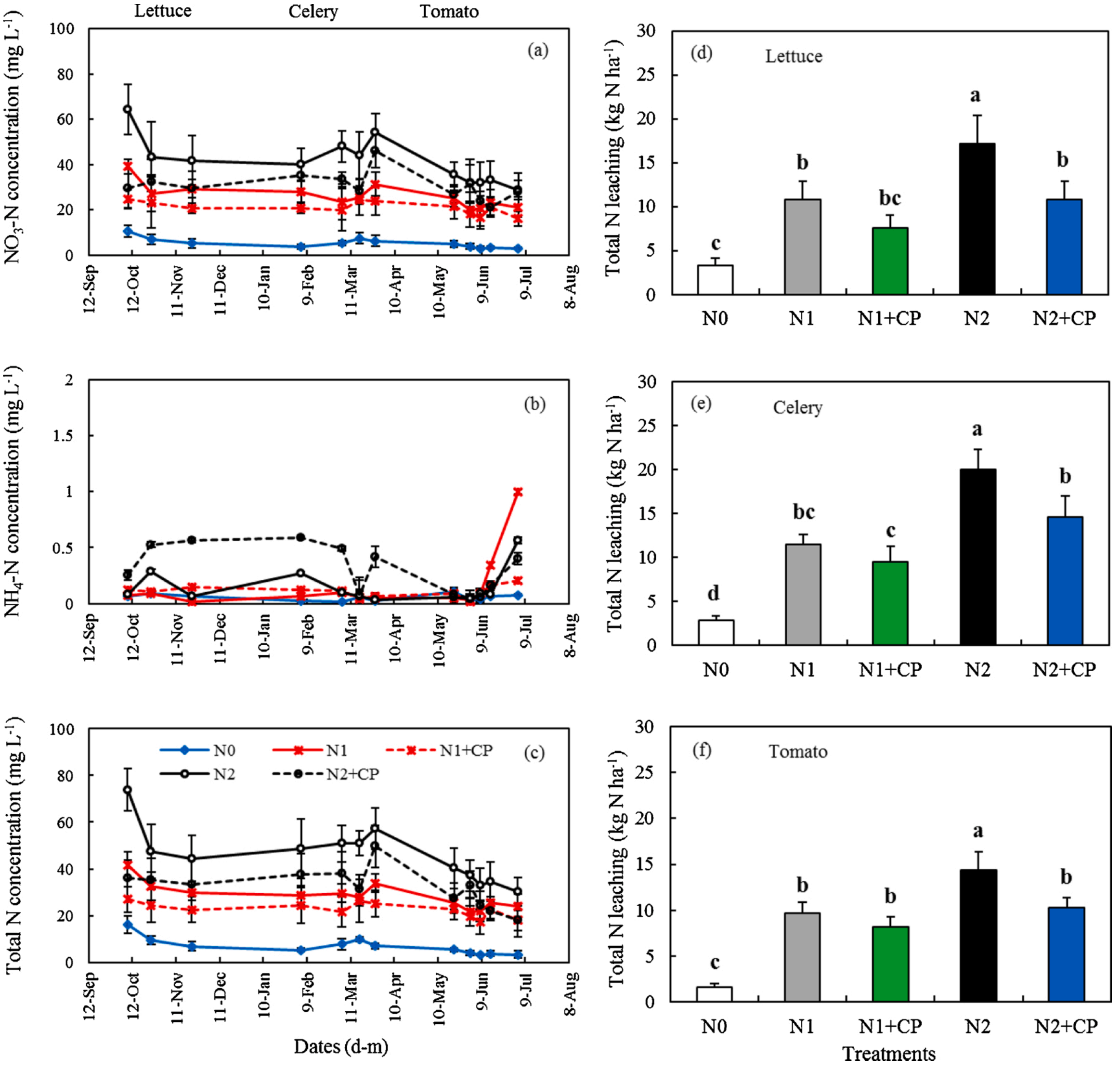

Fig. 2. The impact of CP application on a) NO_3^--N, b) NH_4^+-N, and c) total N concentration of leachates for each sampling, and d) NO_3^--N, e) NH_4^+-N, and f) total N-leaching losses in lettuce-celery-tomato rotation.

min intervals were analyzed for N_2O concentration by a gas chromatograph (Agilent Technologies 7820A). Temperatures inside and outside of the chamber were measured at the same time as the gas sample was collected. Cumulative seasonal N_2O emissions were calculated from the individual fluxes and the time between the measurements.

2.4. Statistical analyses

Data were subjected to analysis of variance (one-way or two-way ANOVA) to determine the significance of the difference between treatments. Tukey multiple comparison tests were conducted to determine the difference between individual treatments (SPSS Ver.16.0 for Windows, SPSS Inc., Chicago, IL, USA).

3. Results

3.1. N leaching

The major form of leached N in each growth season was NO_3^--N, accounting for nearly 90 % of total leached N, while only traces of NH_4^+-N were detected in leachates (Fig. 2). The averaged total N concentration during the three vegetable seasons was 45.7 ± 12.1 mg L^{-1} at the traditional N rate (N2), and this was significantly decreased ($p < 0.05$), by 37.6 % and 50.5 %, at 60 % of the traditional N rate without (N1) and with CP application (N1 + CP), respectively (Fig. 2c). CP application significantly decreased ($p < 0.05$) total N concentration by 29.6 % in the traditional N-rate treatment (N2), and also decreased total N concentration by 20.7 % in the N1 treatment, although the difference was not statistically significant. The cumulative total N-leaching losses in the treatments receiving the traditional N rate (N2) and 60 % of the traditional N rate (N1) were 51.4 ± 7.5 and 31.9 ± 4.3 kg N ha^{-1} in lettuce-celery-tomato rotation, and 36.8 ± 3.9 and 25.3 ± 1.6 kg N ha^{-1} following CP application, respectively (Fig. 2f).

3.2. Ammonia volatilization

The highest cumulative NH_3 volatilization values were 31.9, 26.5, and 23.2 kg N ha^{-1} at a traditional N rate receiving CP treatments (N2 + CP), accounting for 9.2 %, 7.0 %, and 6.1 % of the total applied N in the lettuce, celery, and tomato growth seasons, respectively (Fig. 3). Compared with the traditional N rate (N2), NH_3 volatilization was significantly increased ($p < 0.05$), by 33.5 %, 56.3 %, and 308.1 %, under the treatments receiving CP (N2 + CP) in the lettuce, celery, and tomato growth seasons, respectively. For the treatments receiving a

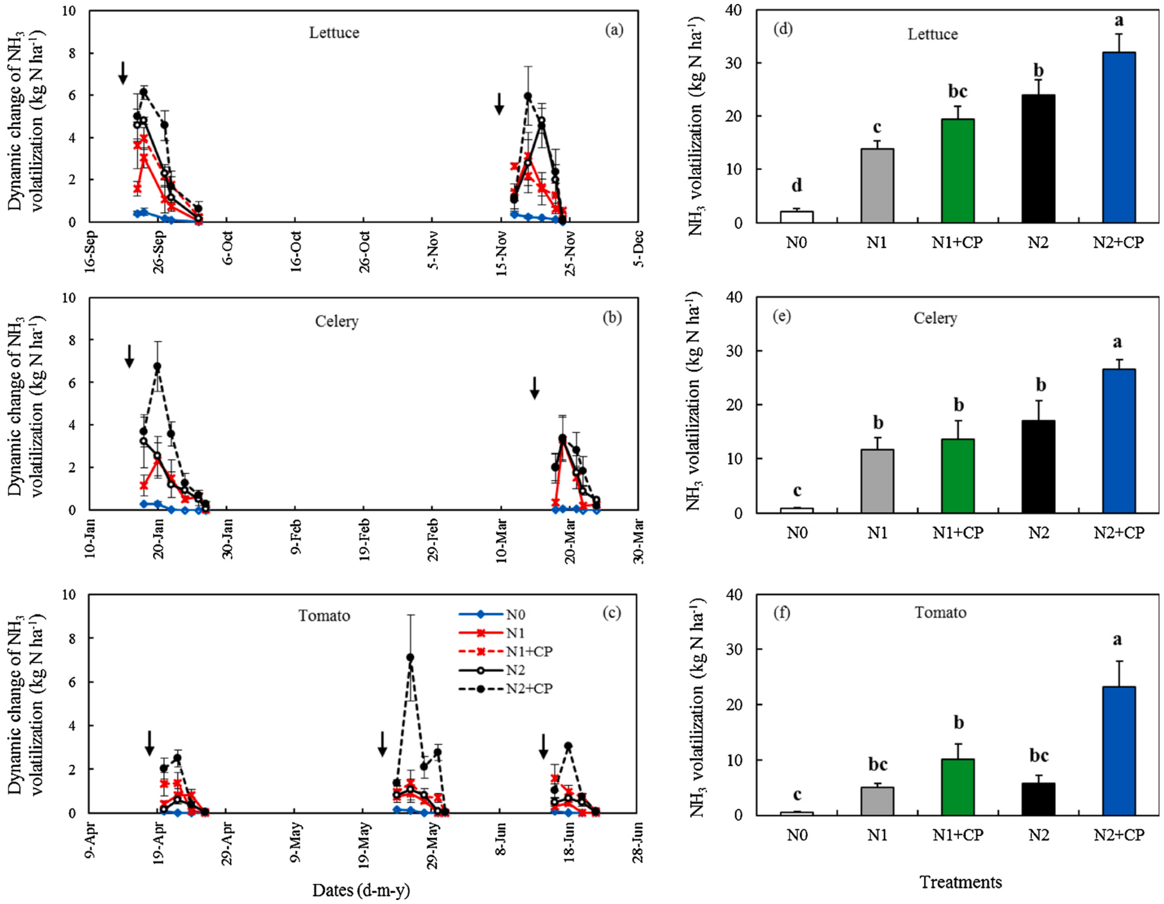

Fig. 3. The impact of application of the nitrification inhibitor CP on the dynamic of NH_3 volatilization in a) lettuce, b) celery, and c) tomato growth seasons, and seasonal cumulative NH_3 volatilization in d) lettuce, e) celery, and f) tomato systems, at different N-application rates.

traditional N rate (N2), N lost at the traditional N rate, expressed as a percentage of applied fertilizer N, was increased by CP from 4.2%–7.4% in a whole lettuce-celery-tomato rotation. The cumulative NH_3 volatilization in the treatment with traditional N rate (N2) was 46.6 ± 8.3 kg N ha^{-1} and increased by 35.1 ± 5.7 kg N ha^{-1} as a result of CP. Compared to the treatment with 60 % of the traditional N rate (N1), the corresponding treatment with CP (N1 + CP) increased NH_3 volatilization by 5.7 ± 0.7, 1.9 ± 1.2, and 5.2 ± 2.0 kg N ha^{-1} in the lettuce, celery, and tomato growth season, respectively (Fig. 3).

3.3. Nitrous oxide emission

N_2O emission rate reached 772, 394, and 3320 μg N m^{-2} h^{-1} at the traditional N rate (N2) during the lettuce, celery, and tomato growth season, respectively. The peak values for the N_2O emission rate at the traditional N rate with CP (N2 + CP), the lower N treatments without (N1) and with CP (N1 + CP) were 21–72 %, 6–54 %, and 68–84 % lower than that recorded at the traditional N rate (N2) (Fig. 4a, b and c). The total N_2O emission in the lettuce-celery-tomato rotation was 5.9 and 11.5 kg N ha^{-1} at the traditional N rate with and without CP (Fig. 4d, e and f). Decreasing the N-fertilizer rate to 60 % of the farmers' usual rate significantly decreased the total N_2O emission by 47.5 %. 72.1 % lower N_2O emissions were found when combining the 60 %-N input and CP (N1 + CP), in comparison to 100 %-N input (N2) (Fig. 4).

3.4. Vegetable yield and N-use efficiency

The yields of lettuce, celery, and tomato at the traditional N rate (N2) were 18.9, 50.7, and 75.9 t ha^{-1}, respectively (Fig. 5). The data also show that the yields of the three vegetables tested were not reduced remarkably when decreasing traditional N-application rates by 40 % (N1). Interestingly, CP application significantly enhanced ($p < 0.05$) lettuce and celery yields in the treatments receiving 60 % of the traditional N rate (N1), by 33.5 % and 22.5 %, respectively. In the tomato growth season, CP application significantly improved ($p < 0.05$) the tomato fruit yields in both treatments receiving 60 % of traditional N rate (N1+CP) and the traditional N rate (N2+CP). N1+CP produced 36 % and 23 % more yield than their counterparts N1 in the lettuce and celery season, respectively. N1+CP and N2+CP produced 16 % and 18 % higher fruit yield than the counterparts N1 and N2 in the tomato season, respectively. Under 60 % N input, the average vegetable yield with CP application increased by 23.9 %. The highest yields under all treatments in each season were 39.9 t ha^{-1} for lettuce, 65.8 t ha^{-1} for celery, and 94.0 t ha^{-1} for tomato, which were recorded under the treatments of 60 % of the traditional N rate with CP (N1 + CP) (Fig. 5).

The NUEs of lettuce, celery, and tomato under the traditional N rate (N2) were 6.0 %, 14.6 % and 11.5 %, respectively (Table 2). When decreasing the traditional N rate by 40 % (N1), NUEs were, on average, increased by 126 % (by 235 % for lettuce, 81 % for celery, and 62 % for tomato) without CP (N1), and by 256 % (by 428 % for lettuce, 142 % for celery, and 198 % for tomato) with CP (N1 + CP), respectively. Compared to the treatments with 60 % N input (N1), the combination with CP (N1 + CP) significantly increased ($p < 0.05$) NUE by 58 % for lettuce, 34 % for celery, and 84 % for tomato. In addition, N2 + CP also significantly increased ($p < 0.05$) NUE of tomato by 56 %, compared to N2.

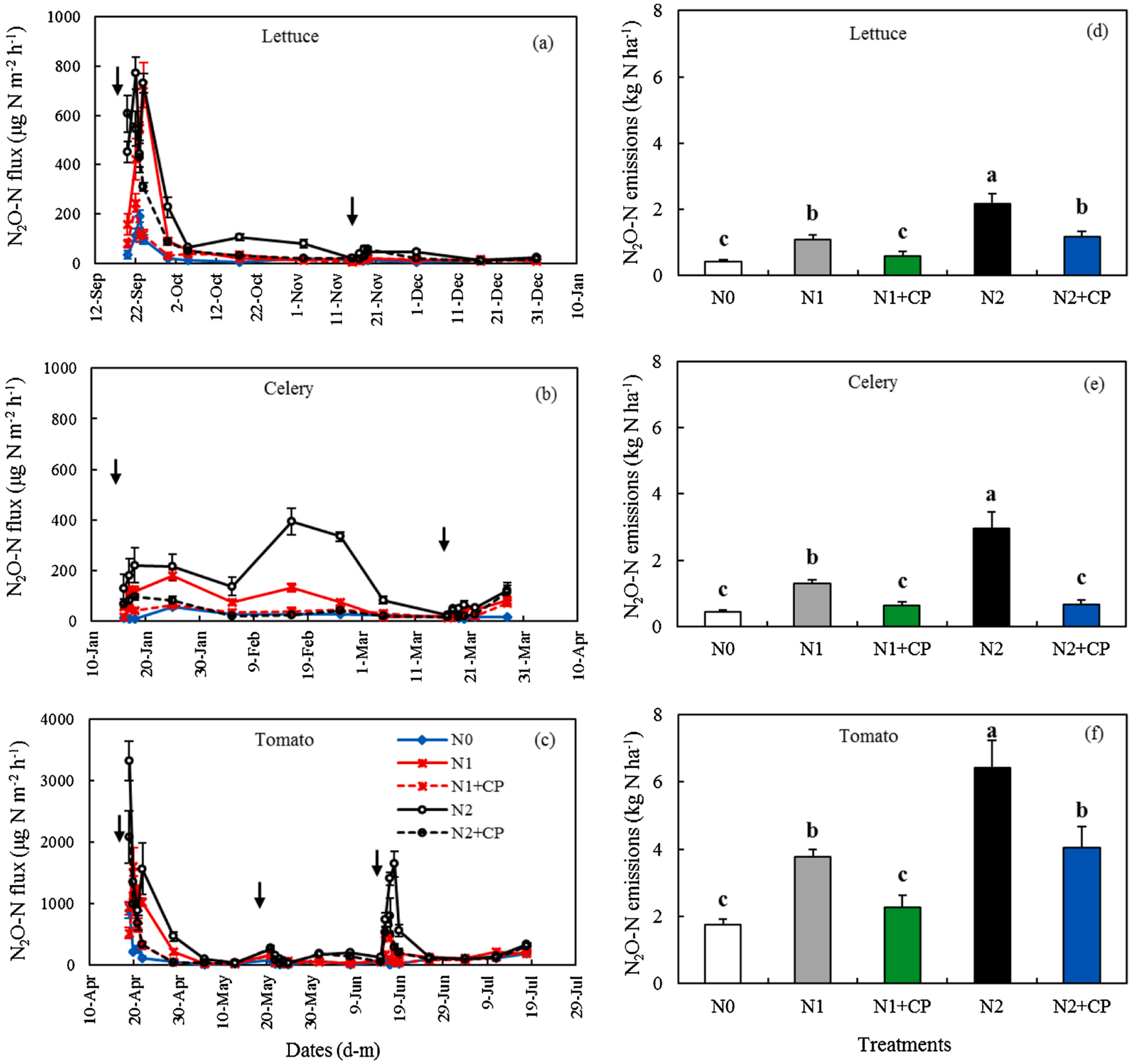

Fig. 4. Seasonal dynamics of N_2O emission flux and cumulative emission rates from soil treated with urea N receiving CP during the lettuce a)/d), celery b)/e), and tomato c)/f) growth seasons, respectively. Arrows denote the timing of N-fertilizer application.

3.5. Soil residual nitrogen

Compared to the traditional N rate (N2), 60 % of traditional N rate without (N1) and with CP (N1 + CP) significantly decreased ($p < 0.05$) soil residual NO_3^--N, by 55.2 % and 67.1 % for lettuce, 53.1 % and 72.4 % for celery, and 52.7 % and 71.1 % for tomato, respectively (Table 3). Relative to the traditional N rate (N2), the soil residual NO_3^--N contents were significantly decreased ($p < 0.05$) when receiving CP treatment (N2 + CP), by 34.1 %, 43.7 %, and 43.9 % over the lettuce, celery, and tomato growth seasons, respectively. Meanwhile, compared to the 60 % N input (N1), soil residual NO_3^--N contents were decreased by CP, although the difference was not statistically significant. Treatment with CP increased soil residual NH_4^+-N contents by 68–113 %, but the effect was significant only under the traditional N rate (N2) (Table 3).

3.6. Total yield and whole-system assessment of environmental impacts of lettuce-celery-tomato rotation

The effects of N input level and CP application on vegetable productivity and environmental impacts across the whole lettuce-celery-tomato rotation are summarized in Table 4. Compared with treatments receiving the traditional N rate (N2), treatments with 60 % of the traditional N rate (N1) did not reduce the total vegetable yield but did significantly reduce ($p < 0.05$) soil NO_3^--N, N leaching, NH_3 volatilization, and N_2O emission, by 53.3 %, 38.1 %, 34.5 %, and 46.1 %, respectively, and it significantly increased ($p < 0.05$) NUE by 102.5 %. For the traditional N rate, the use of CP significantly reduced ($p < 0.05$) soil NO_3^--N, N leaching, and N_2O emission, by 42.1 %, 30.7 %, and 48.7 %, and significantly increased ($p < 0.05$) soil NH_4^+-N content and NH_3 volatilization by 85.7 and 75.1 %. At 60 % of the traditional N rate, CP application significantly increased ($p < 0.05$) total vegetable yield and NUE by 21.3 and 55.1 %, while significantly reducing ($p < 0.05$) N_2O emission by 43.5 %. On average, the percentages of urea-N loss via N

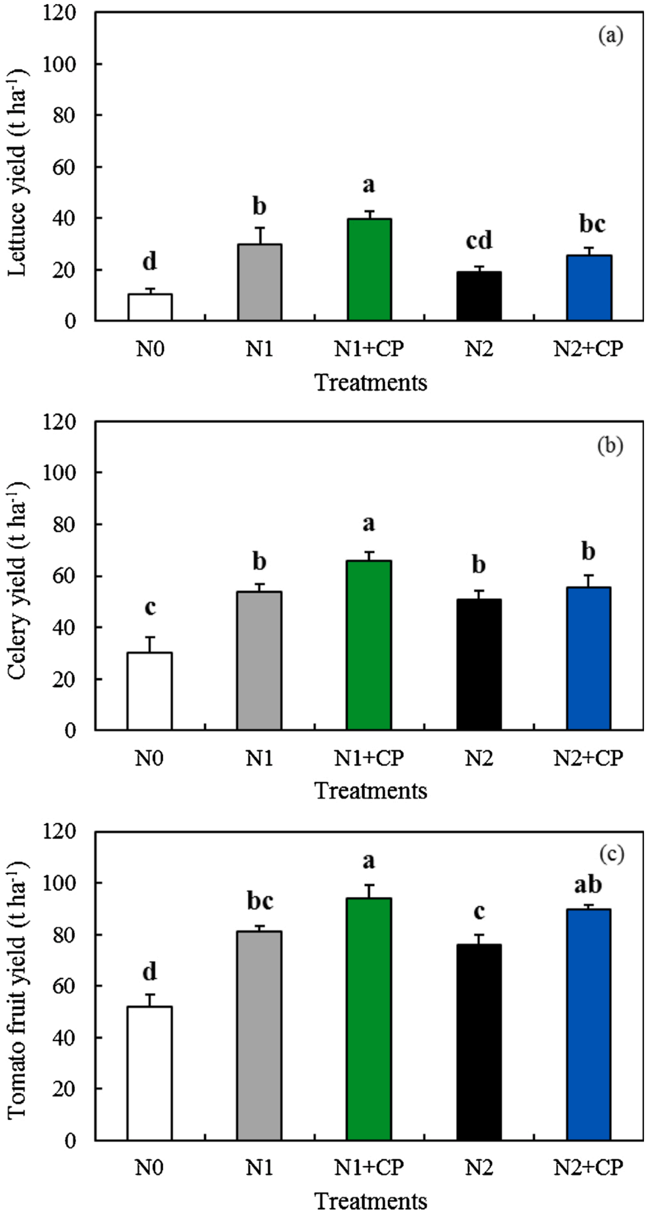

Fig. 5. Marketable yields of a) lettuce, b) celery, and c) tomato under different N rates with and without CP. *Error Bars* represent the SD of means, with four replicates per treatment, and the same letter denotes no significant difference according to Tukey's multiple-comparison test at the 5% level.

Table 2
The impact of CP application on nitrogen-use efficiency (NUE) over the lettuce, celery, and tomato growth seasons.

Treatments	Nitrogen use efficiency (NUE) (%)		
	Lettuce	Celery	Tomato
N1	19.9 ± 6.3 b	26.4 ± 3.0 b	18.6 ± 4.1 b
N1 + CP	31.4 ± 0.7 a	35.3 ± 1.3 a	34.1 ± 1.3 a
N2	6.0 ± 1.2 c	14.6 ± 2.0 c	11.5 ± 1.8 c
N2 + CP	10.5 ± 1.9 c	16.3 ± 2.8 c	17.9 ± 0.5 b

NUE was calculated as the difference in N uptake between the plot receiving N and the control (no N addition). Data are the means ± SD (n = 3); different letters in the same column indicate significant differences according to Tukey's multiple-comparison test at the 5% level.

Table 3
The impact of CP application on soil-residual NO_3-N and NH_4-N contents in the plough layer (top 0–20 cm) following lettuce, celery, and tomato harvests.

Treatments	Lettuce		Celery		Tomato	
	NO_3-N (mg kg^{-1})	NH_4-N (mg kg^{-1})	NO_3-N (mg kg^{-1})	NH_4-N (mg kg^{-1})	NO_3-N (mg kg^{-1})	NH_4-N (mg kg^{-1})
N0	26 ± 8 c	3 ± 1 c	27 ± 5 c	3 ± 1 c	23 ± 4 d	6 ± 1 c
N1	113 ± 29 b	6 ± 1 c	250 ± 56 b	10 ± 3 c	265 ± 44 bc	12 ± 3 c
N1 + CP	83 ± 15 bc	13 ± 4 c	147 ± 39 bc	16 ± 4 c	162 ± 31 cd	15 ± 5 c
N2	252 ± 52 a	30 ± 4 b	533 ± 96 a	55 ± 11 b	560 ± 82 a	51 ± 13 b
N2 + CP	166 ± 31 b	64 ± 11 a	300 ± 70 b	92 ± 24 a	314 ± 73 b	88 ± 21 a

leaching were reduced by 21.9 and 30.8 %, N_2O emission was reduced by 33.3 and 50.0 %, and NH_3 volatilization increased by 38.7 and 74.5 %, following CP applications in 60 % (N1) and 100 % (N2) of the typical N-application rate, respectively (Table 4).

4. Discussion

4.1. The positive effects of CP application on environment

NO_3^--N has a negative charge and is, therefore, not strongly adsorbed to mostly negatively-charged soil particles, and NO_3^--N can easily be lost via leaching, which is furthermore promoted by rainfall and/or artificial irrigation (Agostini et al., 2010). A major effect of NIs is to increase the proportion of mineral N present in the NH_4^+ form rather than as NO_3^- for several days following urea application (Aulakh et al., 2001), which increases the soil NH_4^+-N content resulting in lower NO_3^--N leaching. Application of CP significantly decreased ($p < 0.05$) N leaching at a higher N rate of 270/300/300 kg N ha^{-1} in the lettuce/celery/tomato production system (Fig. 2f). NO_3-N concentrations were significantly positively correlated with the abundance of ammonia-oxidizing bacteria (AOB) rather than ammonia-oxidizing archaea (AOA) (Dai et al., 2013). AOA abundance generally did not respond to N applications, whereas that of AOB was sensitive to N applications (Di et al., 2009). AOB communities were dramatically stimulated when the N-loading rate reached 600–1200 kg N ha^{-1}, creating a high soil-N environment (Jia and Conrad, 2009). According to Dai et al. (2013), DCD can significantly reduce the AOB *amoA* gene copy numbers, especially at high N-application rates. These reported mechanisms might support the highly positive effect of CP on reducing NO_3^--N leaching at a higher N rate in the current work.

It has been reported that leaching is the primary N-loss pathway in intensive vegetable -growing systems receiving high inputs of chemical N fertilizer (Song et al., 2009; Min et al., 2011), which was verified in our work (Table 4). Excessive N-fertilization rates in intensive vegetable-growing fields in southern China has resulted in serious environmental problems (Jin et al., 2005; Ju et al., 2007; Min and Shi, 2018), and, therefore, decreasing N leaching in vegetable systems is urgent. In the current study, CP application significantly decreased ($p < 0.05$) N leaching by 30.7 % at a traditional N rate (N2), similar to the 38.1 % reduction in N leaching that was achieved at a 60 % of traditional N rate (N1) in a whole three-vegetable rotation (Table 4). Similarly, Cui et al. (2011) reported a 36.2–58.5 % reduction of NO_3^--N leaching with addition of DCD in intensive vegetable production systems in China. A meta-analysis showed that NIs in combination with fertilizers reduced NO_3^--N leaching on average by 47 % (DCD), 59 % (DMPP), and 32 % (other NIs) (Qiao et al., 2015). These findings illustrate the potential of CP application as an environmental strategy to reduce NO_3^--N leaching in intensive vegetable production systems. Further field studies, conducted under a wider range of soil types and involving other vegetable

Table 4
The evaluation of the effects of N input and CP application on productivity and environmental impacts in a lettuce-celery-tomato rotation.

Treatments	Yields (t ha^{-1})	NUE %	Soil NO_3-N content (mg kg^{-1})	Soil NH_4-N content (mg kg^{-1})	Leaching (kg N ha^{-1})	NH_3 volatilization (kg N ha^{-1})	N_2O emission (kg N ha^{-1})
N1	165 ± 12 b	22 ± 4 b	209 ± 42 b	9 ± 2 c	32 ± 4 b (6%) [a]	31 ± 5 b (6%)	6 ± 0.5 b (1.1 %)
N1 + CP	200 ± 12 a	34 ± 1 a	131 ± 26 b	15 ± 4 c	25 ± 4 b (5%)	43 ± 9 b (8%)	4 ± 0.6 c (0.8 %)
N2	146 ± 10 b	11 ± 2 c	448 ± 75 a	45 ± 9 b	52 ± 7 a (6%)	47 ± 8 b (5%)	12 ± 1.6 a (1.4 %)
N2 + CP	171 ± 10 b	15 ± 2 bc	260 ± 56 b	81 ± 19 a	36 ± 5 b (4%)	82 ± 10 a (9%)	6 ± 0.9 bc (0.7 %)

[a] The values in parentheses are the proportions of urea-N loss in total N leaching, NH_3 volatilization and N_2O emission.

Table 5
Net global warming potentials (GWPs) as affected by CP amendment in a whole year of the lettuce-celery-tomato rotation, based on calculations of both direct N_2O emission and indirect emission following deposition of volatilized NH_3.

Treatments	Direct decreased N_2O (kg N ha^{-1}) [a]	Indirect N_2O (kg N ha^{-1}) [b]	Net N_2O (kg N ha^{-1}) [c]	Net (CO_2-equivalent, kg ha^{-1}) [d]
N1	6.2	0.54	6.74	3158
N1 + CP	3.5	0.62	4.12	1930
N2	11.5	0.85	12.35	5784
N2 + CP	5.9	1.08	6.98	3270

[a] The value was calculated by the formula: N_2O emission in the treatment without CP - the N_2O emission in the treatment with CP.
[b] The value was calculated by the formula: the NH_3 loss × 1% + N leaching × 0.75 % (IPCC, 2006).
[c] The value was calculated by the formula: Direct decreased N_2O + indirect N_2O.
[d] The IPCC GWPs factors for N_2O is 298 in the time horizon of 100 years (IPCC, 2006). The net effect (CO_2-equivalent) was calculated by the formula: net (N_2O) × 44/28 × 298.

crops, are required to demonstrate the feasibility of full-scale application of CP to reduce NO_3^--N in intensive production systems.

The response of NH_3 volatilization to NI addition varies with type of NI, crop, and soil (Qiao et al., 2015). For example, DCD increased NH_3 volatilization by 4–16 % (Soares et al., 2012), whereas DMPP had no significant impact (Li et al., 2009). In addition, application of DCD increased NH_3 volatilization in wheat by 7% but had no effect in rice (Banerjee et al., 2002). Decreasing the N-application rate by 40 % (N1) from the traditional N rate alone reduced NH_3 volatilization by 35 % (Table 4). Application of CP significantly increased NH_3 volatilization by 75.1 % under the traditional N rate, but had no effect at the 60 % level of the traditional N rate (N1) (Table 4). Soil acidification is more serious in intensive vegetable fields receiving a higher N rate (Shi et al., 2009; Chen et al., 2019). According to previous reports, CP generally increases the soil pH (Li et al., 2015; Fan et al., 2018; Chen et al., 2019), which could result in NH_3 volatilization increases for simple Henderson-Hasselbalch reasons. A dramatic rise in soil pH under a high N rate is expected to lead to high NH_3 volatilization. Furthermore, NO_3-N accumulation may exacerbate soil acidification (Shi et al., 2009). Soil NO_3-N content reached the highest in the three seasons of the rotation at a high N rate (Table 3), which explains the lower NH_3 volatilization in the tomato season under the traditional N rate. NH_3 volatilized from more alkaline soil patches can be toxic to plants in gaseous form (Coskun et al., 2013) or redeposited and lead to accumulation of toxic ammonium ions (Glass et al., 1997; Britto et al., 2001). Corresponding measures to reduce NH_3 volatilization such as the application of urease inhibitors (Lam et al., 2017; Adhikari et al., 2020) or deep application of fertilizer (Liu et al., 2015) should be considered when NIs are used in intensive vegetable field.

In the present study, tomato cultivation in spring-summer resulted in higher N_2O emissions than lettuce cultivation in autumn and celery cultivation in winter (Fig. 4d-f), which was consistent with prior observations performed in the same area (Min et al., 2012b). N_2O emissions from vegetable soils were strongly related to temperature in the different growing seasons, and the average soil temperatures during the periods were 12.7 °C, 7.2 °C, and 22.7 °C for lettuce, celery, and tomato systems, respectively (Fig. 1). Results from this study clearly show that N_2O emissions can be significantly ($p < 0.05$) reduced by CP and by a reduction in N application. A mitigation effect of CP on N_2O emissions has also been observed by Zhang et al. (2015) and Chen et al. (2019). Inhibition of ammonia monooxygenase by NI directly decreased nitrification rate and thereby reduced soil NO_3^- concentrations as the substrate for denitrification (Chen et al., 2019). Hence, the two main pathways of N_2O production in vegetable soils are inhibited and thus lead to decreases of direct N_2O emissions (Ruser and Schulz, 2015).

According to the IPCC (2006), ~0.75 % (0.05–2.5 %) of leached N and 1% (0.2–5 %) of the NH_3-N deposited from the atmosphere to the soil is converted to N_2O through nitrification and denitrification (Mosier et al., 1998; de Klein et al., 2006), which is referred to as indirect N_2O emission from N leaching and NH_3 deposition. Based on the default value, we estimated that CP increased indirect N_2O emission over a whole year by 0.08 and 0.23 kg N ha^{-1} at 60 % and 100 % applications of traditional urea N, respectively (Table 5). The net emission of CO_2-equivalents from farming activities can be decreased by decreased N_2O emission (Robertson et al., 2000). The benefit of CP in mitigating direct N_2O emissions is reduced, however, when indirect emissions associated with the additional volatilization of NH_3 are taken into account. We preliminarily estimated that CP overall might decrease net GWPs by 2,514 kg CO_2-equivalents ha^{-1} at traditional N rates (N2), roughly equal to that achieved by decreasing the traditional N rate by 40 % (N1), which, in turn, lead to a reduction of 3,854 kg CO_2-equivalents ha^{-1} over a whole year (Table 5). Thus, the net effect of CP in decreasing the overall GWP was still beneficial in intensive vegetable systems, consistent a previous report for a rice production system (Sun et al., 2015). Therefore, the positive effects of CP on the environmental impacts are reflected in the reduction of GWPs, N leaching, and NO_3– accumulation in the soil at the high N rates. Therefore, the appropriate use of NIs can reduce the cost of pollution and mitigation of climate change and contribute significantly to the sustainable use of intensive vegetable production.

4.2. *Nitrification inhibitor use maintained high yield*

Our current study indicates that the traditional N-application rate used by local farmers is excessive, and an N rate of 162, 180, and 180 kg N ha^{-1} is sufficient to maintain lettuce, celery, and tomato yields, respectively. CP application improved the yields of lettuce, celery, and tomato, on average by 23.9 %, at a lower N rate of 162/180/180 kg N ha^{-1} in the field (Fig. 5), which is consistent with a study on cereal crops (Rose et al., 2018). The main reason was that NI effects did not manifest under a high-N application rate as N supply is not the limiting factor for crop growth under such conditions, while, at a reduced N application rate, the effects of NIs on crop yield would be significant. In the present study, we observed that the application of CP increased soil NH_4-N content and slightly decreased soil NO_3-N content (Table 3). Given that NO_3-N is more readily lost via leaching, the change in the major form of soil inorganic N reduce N loss through leaching (Fig. 2). The continuously higher soil NH_4-N content observed under the CP-added treatments was also beneficial for the growth and N assimilation of the crops (Liu et al., 2013), and therefore we observed both higher vegetable yield

Table 6
Distribution of N from ^{15}N-labeled urea in aboveground organs in tomato.

Treatments	Labeled urea N uptake /(kg·ha^{-1})				Distribution of labeled urea N /%		
	Stem	Leaf	Fruit	Total	Stem	Leaf	Fruit
N1	5.1 ± 1.7 a	7.6 ± 2.8 a	3.8 ± 0.5 bc	16.5 ± 5.0 ab	31.1 ± 0.3 a	45.8 ± 0.6 a	23.1 ± 0.1 d
N1 + CP	5.5 ± 1.8 a	7.7 ± 2.3 a	6.7 ± 2.1 ab	19.9 ± 6.2 ab	27.6 ± 0.3 b	38.9 ± 0.4 c	33.5 ± 0.3 b
N2	3.1 ± 0.6 a	4.6 ± 1.4 a	3.4 ± 0.8 c	11.1 ± 2.8 b	27.7 ± 0.2 b	41.2 ± 0.5 b	31.1 ± 0.3 c
N2 + CP	5.3 ± 0.8 a	8.6 ± 3.3 a	8.0 ± 2.2 a	21.8 ± 6.3 a	24.1 ± 0.1 c	39.4 ± 0.5 c	36.5 ± 0.3 a

and NUE in the CP treatments in the current work. Some studies have suggested a contribution by increased soil pH (Li et al., 2015; Fan et al., 2018) and the consequently retarded soil acidification and release of toxic aluminum ions (Zhang et al., 2015). In our study, we furthermore monitored the distribution of N derived from ^{15}N-labeled urea in tomato aboveground, and found that CP application significantly increased ($p < 0.05$) the delivery of N derived from fertilizer to the fruit, i.e. CP addition significantly decreased ($p < 0.05$) N allocation to stems and leaves by 11.3 and 15.1 % in the N1 treatment and by 13.0 and 4.4 % in the N2 treatment, respectively, and significantly increased ($p < 0.05$) N allocation to fruits by 45.0 and 17.4 % in the N1 and N2 treatments, respectively (Table 6). Therefore, CP increases N storage in the fruit and benefiting yield. At traditional N rates, the use of CP had no effect on yields of lettuce and celery, but it significantly increased ($p < 0.05$) tomato fruit yield, by 17.9 % (Fig. 5). Therefore, CP application can more efficiently increase yield at relatively lower N rates, a trend that has also been previously demonstrated (Sun et al., 2015; Chen et al., 2019). Since tomato is the last season of the rotation, many factors including absorption of N by lettuce and celery and soil-residual N in first two seasons could affect N transfer in the tomato season and, thus, future research will consider these factors.

Farmers usually apply excessive fertilizer to ensure a high yield in intensive vegetable production systems, and NUE, on average, was only 10.7 % in the current work (Fig. 5), in agreement with other reports (Zhu et al., 2005; Ju et al., 2006; He et al., 2007). In the three-vegetable rotation, NUE was significantly increased ($p < 0.05$) by CP (by 58 %, 34 %, and 84 % in lettuce, celery, and tomato, respectively) when 162/180/180 kg N ha^{-1} was applied (Table 2). This impact is similar to the impact of other NIs (including DCD and DMPP), which increased NUE by 34–93 % (Qiao et al., 2015). Higher NUE not only enhances plant productivity, but also reduces adverse environmental impacts of N fertilizers. When more N is recovered by the crop, less applied N fertilizer is lost to the environment. The highest yields among the treatments for the various vegetable seasons were recorded under the treatments receiving 60 % of the traditional N rate with CP (N1 + CP). The average by which vegetable yield increased with CP was 24 % (Fig. 5), indicating that CP amendment is an effective and practical way to maintain high vegetable production while saving on N fertilizer. Future studies should evaluate different types of N fertilizer on various types of soil before the full-scale applications of NIs can be endorsed and maximal economic and environmental benefits can be achieved under intensive vegetable systems in agriculture.

5. Conclusion

Our field experiment with three major vegetable crops (lettuce, celery, and tomato) in rotation showed that 60 % N input, which decreased the N rate by 40 % from the traditional N rate, did not result in yield losses, but reduced N leaching, NH_3 volatilization, N_2O emission, and residual soil NO_3^--N. In addition, 60 % N input with CP application significantly increased ($p < 0.05$) yield and NUE. Promotion of N transfer from stems and leaves to fruits at the later stage of tomato growth is one of the mechanisms explaining higher yield under CP-added treatments. For the traditional N rate, CP had approximately the same effect as the 60 % N input treatment in terms of decreasing N leaching, N_2O emission, and soil-residual NO_3-N, but had a negative effect on NH_3 volatilization. Our findings suggest that whole-process evaluation is essential to the assessment of the potential of NIs to reduce environmental impact due to the complex processes of N conversion and flux in soil-crop systems.

Declaration of Competing Interest

The authors declare no conflict of interest.

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