Opportunities to improve nitrogen use efficiency in an intensive vegetable system without compromising yield

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Abbreviations: ANUE, apparent nitrogen use efficiency; DMPP, 3,4-dimethylpyrazole phosphate.

Core Ideas

- 50% of the applied nitrogen (N) in intensive vegetable systems is unaccounted for.
- ^{15}N-labelled fertilizer recovery reveals inefficiency of N applied during early crop stages.
- DMPP was ineffective in improving N recovery due to excessive N input.
- Nitrogen inputs can be reduced to reduce N_2O emissions without yield penalty.

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ABSTRACT

Intensive vegetable cropping systems rely heavily on nitrogen (N) inputs from multiple synthetic and organic fertilizer applications. The majority of applied N is lost to the environment through numerous pathways, including as nitrous oxide (N_2O). A field trial was conducted to examine the opportunities to reduce N input in an intensive vegetable system without compromising yield. Treatments applied were control (C, no N), manure (M, 408 kg N ha^{-1} from chicken manure), grower practice (GP, 408 kg N ha^{-1} from chicken manure+195 kg N ha^{-1} from fertilizer) and 2/3 GP (2/3 of the total N input in GP), all with and without 3,4-dimethylpyrazole phosphate (DMPP). Nitrogen recovery in the GP treatment was determined using ^{15}N-labelled fertilizer. Using only manure significantly lowered celery (*Apium graveolens* L.) yield and apparent N use efficiency (ANUE) compared to GP. Reducing N input by 1/3 did not affect yield or ANUE. Use of DMPP increased ANUE despite no yield improvement. More than 50% of the applied N in the GP treatment was lost to the environment, with almost 10 kg N ha^{-1} emitted as N_2O over the season, which was 67 times more than from the Control. Reducing the N input by 1/3 or using manure only reduced N_2O emissions by more than 70% relative to GP. This study shows that there is a clear opportunity to reduce N input and N_2O emissions in high fertilizer input vegetable systems without compromising vegetable yield.

1. INTRODUCTION

Intensive vegetable cropping systems rely on high nitrogen (N) input through regular application of fertilizer and manure, with combined applications in these systems usually supplying >300 kg N ha^{-1}, and often exceeding 600 kg N ha^{-1} in one cropping season (Pfab et al., 2012; De Rosa et al., 2016; Porter et al., 2017). This high input, combined with short growth periods and high inputs of water, poses a risk of low N use efficiency (NUE) and high N loss, including as reactive N (Bai et al., 2014; Lam et al., 2017). Reactive N loss from agriculture causes unwanted economic loss for farmers, and adversely affects environmental quality (Chen et al., 2008) as well as human and ecosystem health, equivalent to hundreds of billions of dollars per year globally (Sutton et al., 2013).

Reactive N loss to the atmosphere can be as ammonia (NH_3) and nitrous oxide (N_2O) (Erisman et al., 2008). Nitrous oxide is a potent greenhouse gas and decreases stratospheric ozone (Erisman et al., 2008). Low uptake of the applied N-fertilizer in agricultural systems, including intensive vegetable systems, is largely responsible for the perturbation of N_2O levels in the atmosphere (Xiong et al., 2006; Erisman et al., 2008). Intensive vegetable production systems pose a high risk of N_2O loss – with high N and water inputs, and seasonal emissions have been reported at more 18 kg N_2O-N ha^{-1} from a crop receiving > 500 kg N/ha (Porter et al., 2017).

Reducing N inputs can reduce N_2O emissions from intensive vegetable systems (Porter et al., 2017; Yao et al., 2017). However, this can also decrease the marketable yield for some vegetable crops (Di Mola et al., 2020), so farmers may be reluctant to adopt this strategy. Use of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) has been effective at reducing N_2O emissions from applied fertilizer-N in vegetable systems (Lam et al., 2018) but the impact on NUE and vegetable yield is not guaranteed (Pfab et al., 2012; Scheer et al., 2017). Rose et al. (2018) suggested that the effectiveness of DMPP should be evaluated considering the potential reductions in fertilizer-N that can be made compared to conventional practice to achieve the same level of yield. It is unclear if using a reduced rate of N combined with DMPP will decrease N_2O emissions and maintain yields in an intensive vegetable production system compared to the conventional N rates. We conducted a field experiment to examine how reducing N input and using DMPP affect crop yield, NUE and

N_2O emissions, compared to the grower standard practice, in an intensive celery production system.

2. MATERIALS AND METHODS

2.1 Experimental site

A field experiment was established for one full growth cycle of a celery crop (12th April to 10th August 2013) in Boneo, Victoria, Australia (38° 21'S, 144° 45'E). The field site was part of a commercial farm with regular production of a variety of vegetable crops, primarily celery, following high levels of fertilizer input. Daily rainfall and mean daily temperature during the experimental period are presented in Supplemental Figure S1. The soil in the area is classified as a Tenosol (Isbell, 2016). The surface soil (0-15 cm) is sand (>91%) with a slightly alkaline pH (7.9, pH_w 1:5) and 6.4 g organic carbon kg^{-1} and 0.8 g N kg^{-1}.

2.2 Experimental design and crop management

The field experiment was a randomized block design with seven treatments and five replicates within a 243 m × 192 m field. Each plot was 1.7 m × 10 m and included 2 rows of celery plants on raised beds. Fertilizer was applied six times during the celery crop as Nitrophoska® (12% N: 6.5% NH_4^+-N and 5.5% NO_3^--N) and chicken manure (12 t ha^{-1}, 3.4% N: 470 mg kg^{-1} NO_3^--N and 4600 mg kg^{-1} NH_4^+-N and 25.8% moisture content) with and without 3, 4-dimethylpyrazole phosphate (DMPP), and as calcium nitrate [$Ca(NO_3)_2$] (Table 1). Manure was surface applied using calibrated farm equipment, and fertilizers were surface applied by hand between the celery rows with application timing and rates following the local growers' practice. Celery seedlings (25 plants per 10 m^2) were transplanted on 12th April and calcium nitrate (39 kg N ha^{-1}) was applied to all plots, except the control and manure only treatment plots. DMPP (0.8% of applied NH_4^+-N) was applied as the commercial product Nitrophoska Entec® or was applied to the surface of the manure after manure application.

Approximately 73 mm of irrigation water was applied over the crop growth period using an overhead sprinkler. At harvest (10th August 2013), 20 celery plants were sampled from each plot by cutting to ground level, weighed to determine fresh biomass yield, and then oven dried at 60 °C to constant weight. Plant samples were analyzed for total N content to determine apparent N use efficiency (ANUE), which was calculated as follow:

$$ANUE = \frac{(plant\ N\ uptake\ in\ fertilized\ treatment - plant\ N\ uptake\ in\ the\ control)}{amount\ of\ N\ fertilizer\ applied} \times 100\%$$

2.3 Recovery of applied N-fertilizer in plants and soil

A ^{15}N microplot study was used to determine N recovery from different fertilizers forms applied in the grower practice (GP) treatment at different growth stages of the celery (Table 1). Three main plots among the five main plots (replicates) in the GP treatment were randomly selected and seven microplots were installed in each of the three main plots (21 in total). The microplots were fertilized regularly as per GP, with ^{15}N labelled fertilizer at four separate events as follows:

(i) One microplot in each of the three main plots received $K^{15}NO_3$ on 12th April (representing calcium nitrate at transplanting) at 39 kg N ha^{-1}.

(ii) Two microplots in each of the three main plots received $^{15}NH_4NO_3$ spiked manure either with DMPP or without DMPP on 6th May (representing manure) at total manure N of 408 kg N ha^{-1}.

(iii) Two microplots in each of the three main plots received $^{15}NH_4{}^{15}NO_3$ either with or without DMPP on 13th May (representing Nitrophoska) at 39 kg N ha^{-1}.

(iv) Two microplots in each of the three main plots received $^{15}NH_4{}^{15}NO_3$ either with or without DMPP on 3rd June (representing Nitrophoska) at 39 kg N ha^{-1}.

Each microplot was enclosed by a 60 cm x 20 cm bottomless rectangular steel frame with a height of 65 cm which was driven 50 cm into the soil at the time of transplanting to cover both bed and furrow areas, and contained a single celery plant. The microplots were established on 12th April 2013 (transplant) and excavated on 10th August 2013, except the treatment receiving $K^{15}NO_3$ on 12th April [(i) above], which was excavated on 5th May 2013 before manure application to assess the fate of N applied at transplanting. On removal of the microplots, above ground biomass and roots were collected and oven dried at 60 °C to constant weight. Soil samples were collected in 15 cm layers (0-60 cm) from the bed and furrow areas and dried at 40 °C. Dried plant and soil samples were fine ground (<50µm) using a tissuelyser, and analyzed for ^{15}N atom% enrichment with an isotope ratio mass spectrometer (Sercon Hydra, Crewe, UK). The recovery of applied ^{15}N in the plant and soil was calculated as described by Malhi et al. (2004).

2.4 Gas sampling, analysis and calculation

Gaseous emissions were measured using static chambers (16 cm diameter and 16 cm height) with screw top lids. The chambers were permanently installed between the plant rows to cover the fertilizer band by inserting 8 cm into the ground. Gas sampling commenced at 1:00 pm every sampling date after the chamber was closed with a gas tight lid. Gas samples (20 ml) were collected from the chamber headspace at 0, 30 and 60 min after the chamber closure, and injected into a pre-evacuated 12 mL Exetainer vial (Labco Ltd.). Gas samples were collected every day for three days immediately after each fertilization event and then every two to four days with twenty-seven sampling events between 13th April and 6th July 2013. Gas samples were analyzed for N_2O by gas chromatography (Agilent 7890A) using an ECD detector. The gas flux was calculated from the linear regression (LR) of N_2O concentration over time due to the sampling intensity and other methodology considerations (Venterea et al., 2013; Venterea et al., 2020). Flux rates were discarded if the regression coefficient was less than 0.90 (Figure S2). Cumulative N_2O flux was calculated by integrating the area under the flux curve.

2.5 Soil and plant analysis

Soil samples (0-15 cm) were collected on the gas sampling dates using a soil corer. Collected samples were dried (40 ^oC) and NH_4^+ and NO_3^- content was determined colourimetrically using a Segmented Flow Autoanalyser (SFA; Skalar SAN++ Holland) after extraction with 2M KCl solution (1:5). Soil moisture and temperature were continuously monitored at 10 cm increments to 60 cm depth using EnviroPro® capacitance probes (EnviroPro, Australia). Total N in dried plant samples was determined by Kjeldahl digestion followed by colourimetric analysis using the SFA.

2.6 Statistical analysis

Statistical analysis to determine treatment effects on N_2O flux was done using a mixed linear model for repeated measures, and treatment effects on other parameters were analyzed using a mixed linear model with treatment as the fixed effect and blocks as the random effects. Regression analysis examined the relationship between soil moisture (at 20 cm depth) and N_2O flux. Statistical analyses were performed using a Mixed Procedure in SAS (version 9.4, SAS Institute Inc., NC, USA). Pearson's correlation was performed to test the relationship between soil NO_3^- and N_2O flux using Proc Corr in SAS. The Shapiro-Wilk test was used to test for normality and homoscedasticity of residuals (Univariate procedure of SAS). The N_2O flux data were log-transformed before the parametric test.

3. RESULTS

3.1 Celery yield and ANUE

Celery yield increased with addition of N as fertilizer and manure relative to the control (Table 2). Reducing input of fertilizer N (GP to 2/3 GP) did not significantly affect yield (2.4 t ha^{-1} at GP and 2.2 t ha^{-1} at 2/3 GP; $p > 0.05$). Adding only manure (M) led to 25% and 18% lower celery yield than the GP and 2/3 GP treatments, respectively. DMPP had no significant effect on celery yield (Table 2). ANUE was less than 8% across all treatments, and the manure only treatment showed 44% and 50% lower ANUE compared to the GP and 2/3 GP treatments, respectively (Table 2). There was no significant difference in ANUE between the GP and 2/3 GP treatments. DMPP significantly increased ANUE only in the manure treatment (Table 2).

3.2 Recovery of nitrogen fertilizer

The recovery of fertilizer-N in the soil-plant system ranged from 43 to 74% (Table 3). Only a small proportion of the N applied as nitrate (7%) was recovered in the plant 24 days after fertilizer addition, with 53% found in the soil and 40% unaccounted for. Around 8% of the N applied as manure was recovered in the celery plant, with 34% recovered in the soil after 96 days. Early (May 13th) application of Nitrophoska application led to significantly more N recovery in the plant (28%) compared to the manure application (8%). Later application of Nitrophoska (3rd June) led to even greater ($p < 0.05$) plant uptake (43-49%) compared to when applied earlier (13th May, 25-29%). Total N recovery (plant and soil) with the use of DMPP was consistently higher than its counterparts, with an additional 9-17% recovered, albeit not significantly ($p > 0.05$, Table 3).

3.3 Mineral nitrogen

Soil NH_4^+ levels in all non-DMPP treatments were similar through May to July (Figure 1a). Application of DMPP led to higher soil NH_4^+ compared to the corresponding treatments after 21st of May through to 23rd of July. The soil NO_3^- concentrations fluctuated over the growing season, increasing to more than 30 mg kg^{-1} soil in the GP treatment following Nitrophoska application before declining again (Figure 1b). The NO_3^--N levels in 2/3 GP were lower than in the GP treatment but followed a similar trend. Application of DMPP did not significantly affect the soil NO_3^- content.

3.4 N_2O emissions

The N_2O flux remained very low (<4 g N ha^{-1} day^{-1}) in the control treatment throughout the experiment (Figure 2). The N_2O flux from the manure only treatments (± DMPP) did not exceed 136 g N ha^{-1} d^{-1} during the experiment. The N_2O flux increased in all the treatments receiving Nitrophoska (GP and 2/3 GP) immediately after each application, regardless of the DMPP treatment (Figure 2). The highest N_2O flux (821 g N ha^{-1} d^{-1}) was observed from the GP treatment on 16th of May, three days after the first Nitrophoska application, coinciding with a rise in soil moisture due to the irrigation and rainfall prior to gas sampling day (Figure 2 and S3). The GP+DMPP (767 g N_2O-N ha^{-1} d^{-1}) also showed high N_2O flux on this day. In the 2/3 GP treatment, the N_2O flux remained below 60 g N ha^{-1} d^{-1} except on the 16 and 18 May when it was 120 and 123 g N ha^{-1} d^{-1}, respectively. There was a significantly positive relationship (p = 0.038) between N_2O flux and soil moisture at 20 cm depth for all treatments, but this only explained 20% of the variability in N_2O emissions, and between N_2O flux and soil NO_3^-, except for the manure without DMPP and the control (Table S1).

Higher cumulative N_2O emissions (over 61 days) were observed in all fertilized treatments compared to the control (Table 2). The manure only and 2/3 GP treatments had significantly lower cumulative N_2O emissions (<4 kg N_2O-N ha^{-1}) than the GP (~10 kg N_2O-N ha^{-1}). There was a trend for reduced N_2O emissions when DMPP was applied with manure and in the GP treatment, but overall DMPP had no significant effect on cumulative N_2O emissions in any of the treatment pairs (Table 2).

4. DISCUSSION

4.1 Effects of fertilization strategies on celery yield, ANUE and N recovery

We observed that addition of N was required to achieve a good celery yield, but this input could be reduced by 1/3rd from the grower standard practice (603 kg N ha^{-1}) without compromising the yield (Table 2), similar to the findings of Chen et al. (2019). The high levels of loss (up to 57% of the applied N) indicate that the grower practice is not sustainable. A concurrent study at the site found that 20 kg N ha^{-1} was lost as NH_3 within a week after chicken manure application (Bai et al., 2014). And we expect leaching losses would contribute to the N loss due to the sandy nature of the soil and the soil moisture fluctuation at 60 cm depth (Figure S3), particularly in May and June when most of the fertilizer was applied and soil moisture levels were high. Using manure as the sole N source led to lower yield than compared to a similar N rate of chemical fertilizer, which differs to the findings of Riches et al. (2016) who observed similar cauliflower yield with manure as with synthetic fertilizer. Nitrogen from manure is slowly mineralized (Figure 1), and it is likely that N supply is too slow to match the N demand of a short-season vegetable crop (Zhuang et al., 2019).

There was a positive effect of the use of DMPP on ANUE, most noticeably in the manure treatment (Table 2). The high N application rate in this study most likely resulted in plant-available N being greater than plant demand. So, even if the DMPP increased soil NH_4^+ compared to the treatments without DMPP (Figure 1a), this was not reflected in the yield or

ANUE. The productivity benefits from DMPP in vegetable systems is unclear with some studies indicating improved crop yield and NUE (Pasda et al., 2001; Zhang et al., 2015) and others not (Pfab et al., 2012; Riches et al., 2016; Scheer et al., 2017). The effectiveness of DMPP depends on several managerial, climatic and edaphic factors (Chen et al., 2010; Gilsanz et al., 2016; Marsden et al., 2017; Scheer et al., 2017), and can also depend on N application rates. For example, Souza et al. (2020) observed a positive effect of DMPP application on potato tuber yield and N uptake when N application rates were below 100 kg N ha^{-1}. A meta-analysis by Rose et al. (2018), however, suggested that the agronomic efficacy of DMPP may only prove beneficial in terms of the extent of fertilizer N input that can be reduced to achieve the same level of yield as in conventional practices.

The ^{15}N recovery results indicate that there is poor N utilization by the plant and a high residual N level in the soil, particularly in the manure treatment (Table 3), which could pose a high risk of post-harvest N loss (Scheer et al., 2017). But, if the residual N is managed well, it could provide benefits to the succeeding crop and lead to reduced N inputs. Use of DMPP increased fertilizer N recovery mainly through higher N recovery in soil (Table 3), indicating that a longer term (multiple growing seasons) view of the benefits of DMPP is worth consideration.

4.2 Effects of fertilization strategies on N_2O emissions

Nitrogen input and soil moisture influenced the flux of N_2O (Figure 2 and S3) as shown with the low N_2O flux from the control treatment, and high emissions from 15-18 May when soil was wetter and nitrate levels higher. The N_2O flux in the manure treatment was lower than with the chemical fertilizers because of the lower contribution of the available N pool from chicken manure that is susceptible to denitrification which has been observed in this (Figure 1) and other studies (Diaz et al., 2008; Pelster et al., 2012). The high cumulative N_2O

emissions (~10 kg N_2O-N ha^{-1}) from the GP treatment in this study was lower than that observed by Zhang et al. (2016) in a similar intensive system.

High soil moisture can diminish the efficacy of DMPP in reducing N_2O emissions because in such a condition denitrification rather than nitrification mediates N_2O emissions (Chen et al., 2010; Menéndez et al., 2012). The high soil moisture during fertilizer application, and the positive correlation observed between N_2O emissions and soil NO_3^- content indicates that N_2O emissions are primarily from denitrification (Table S1). Apart from soil moisture, the choice of a NO_3^- containing fertilizer (Nitrophoska) and its application a week after manure application may have influenced the apparent efficacy of DMPP in reducing N_2O emissions during the peak flux period. Almost half of the N in Nitrophoska is in the form of NO_3^- and hence not affected by the nitrification inhibitor, and this, together with the microbially available C in the manure, likely enhanced denitrification mediated N_2O production (Weier et al., 1993). The observed soil mineral N data also suggests the same. After the Nitrophoska application on 13 May, the soil NH_4^+ levels were consistently higher in the +DMPP treatments than in the comparative -DMPP treatments. However, DMPP was not able to reduce NO_3^- levels during the peak N_2O emissions period (15-20 May, Figures 1b and 2). We demonstrated that N_2O emissions can be reduced significantly by simply reducing the N input from the grower practice rate by 1/3, without affecting yield, and is more effective than DMPP in these excessive N input vegetable systems.

5. CONCLUSIONS

Our study demonstrated that a reduction in N input by 1/3 from the existing grower practice does not have a negative effect on celery yield. Using manure as the sole source of N can decrease celery yield. Application of DMPP was ineffective in improving yield and ANUE, except in manure only treatment. Around 50% of the applied N in the grower practice was lost to the environment. Almost 10 kg N ha^{-1} was lost as N_2O from the grower practice over the two months of celery growing period which is 67 times higher than the control treatment. Application of N-fertilizer shortly after manure application led to the highest N_2O flux. Reducing N input in the grower practice by 1/3 reduced N_2O emissions by 4.5 times. Our study suggests that growing vegetables on these sandy soils with high N input leads to high N loss, including considerable N_2O emissions, and that N input can be reduced without compromising yield. Farmers use excessive amount of N fertilizer in intensive vegetable systems around the world, but limited data is available on N use efficiency from field-based experiments. This single season field experiment shows high N use inefficiency of an intensive vegetable system. Intensive vegetable systems involve high inputs in all seasons, so we expect the N use inefficiency to be of a similar magnitude across seasons but longer-term measurements across multiple crops is needed to work towards developing sustainable production systems.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUPPLEMENTAL MATERIALS

Additional supporting information can be found on the online version of this article.

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Figure Captions:

Figure 1. Soil (0-15 cm) mineral N content: (a) NH_4^+ and (b) NO_3^-. C, Control; M, Manure; GP, Grower Practice; 2/3 GP, 2/3 of the total N input in grower practice. Error bars are ±1 standard error (n=5).

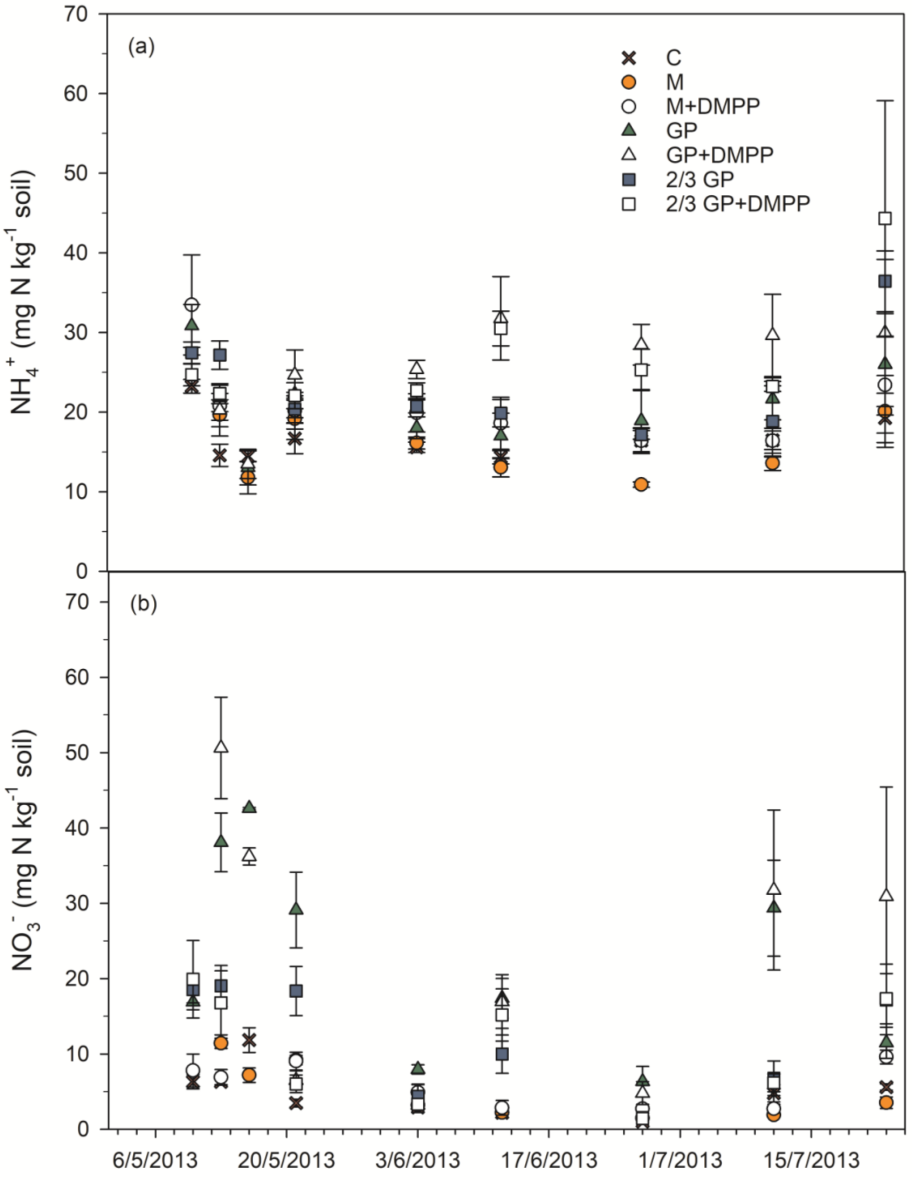

Figure 2. Daily N_2O flux (g N_2O-N ha^{-1}) from May to July 2013. C, Control; M, Manure; GP, Grower Practice; 2/3 GP, 2/3 GP, 2/3 of the total N input in grower practice.

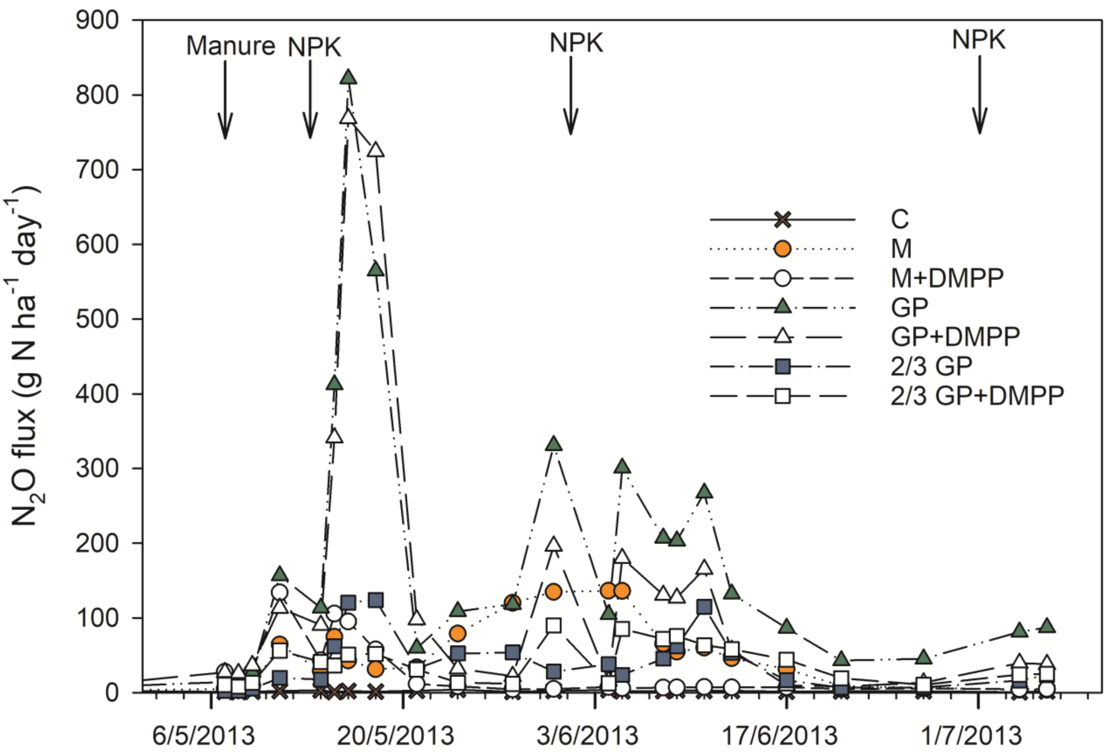

Table 1. Fertilizer forms, application dates and rates (kg N ha^{-1}) in the seven treatments.

Treatments	Fertilization dates and rates (kg N ha^{-1})						
	12 April	6 May	13 May	3 June	1 July	26 July	Total
	$Ca(NO_3)_2$	Manure	NPK*	NPK	NPK	$Ca(NO_3)_2$	
Control (C)	0	0	0	0	0	0	0
Manure only (M)	0	408	0	0	0	0	408
Manure only (M + DMPP)	0	408	0	0	0	0	408
Grower practice (GP)	39	408	39	39	39	39	603
Grower practice (GP + DMPP)	39	408	39	39	39	39	603
2/3 Grower practice (2/3 GP)	39	272	24	24	24	39	422
2/3 Grower practice (2/3 GP + DMPP)	39	272	24	24	24	39	422

*NPK fertilizer is Nitrophoska®

Table 2. Mean (±SE) celery dry matter yield (tonnes ha^{-1}), ANUE (%) and cumulative N_2O emissions (g N ha^{-1}) as affected by treatments. Values followed by different letters within a column are significantly different ($p < 0.05$).

Treatments	Celery yield (t ha^{-1})	ANUE (%)	N_2O (kg N ha^{-1})
Control	1.15±0.09d		0.15±0.02d
M	1.76±0.14c	3.53±0.99b	3.08±1.41bc
M+DMPP	1.93±0.18bc	6.75±1.06a	1.27±0.28c
GP	2.35±0.16a	6.24±0.54a	9.79±3.54a
GP+DMPP	2.27±0.08ab	6.56±0.23a	6.96±1.37ab
2/3 GP	2.15±0.06ab	7.04±0.61a	2.18±0.79c
2/3 GP+DMPP	2.23±0.07ab	8.21±1.38a	2.29±0.54c

Table 3. Recovery of ^{15}N in the plant and soil (±SE of the mean (n=3)) from different forms of N applied at different stages of the celery crop in the grower standard practice (GP) (Table 2). All samples were harvested on 10th of August, except the nitrate treatment which was harvested on 6th of May.

Treatment	Date of ^{15}N application	Days to harvest	Plant Recovery (%)	Soil Recovery (%)	Total Recovery (%)
Nitrate	12th April	24	6.9±0.7c	53.3±10.8a	60.2±11.5ab
Manure	6th May	96	8.6±2.3c	34.3±1.3bc	42.9±1.1b
Manure + DMPP	6th May	96	12.1±1.3c	41.7±0.5ab	54.5±1.7ab
Nitrophoska 1st	13th May	89	28.8±3.3b	21.7±7.6c	49.4±13.0ab
Nitrophoska 1st + DMPP	13th May	89	25.4±2.4b	40.7±7.2bc	66.1±9.4ab
Nitrophoska 2nd	3rd June	68	43.0±2.1a	22.1±12.9c	65.1±12.7ab
Nitrophoska 2nd + DMPP	3rd June	68	49.2±3.8a	24.7±4.1bc	73.9±7.6a