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Strategies to mitigate greenhouse gas emissions in intensively managed vegetable cropping systems in subtropical Australia

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28 **Abstract**

29 The greenhouse gas fluxes and effective mitigation strategies in subtropical vegetable cropping
30 systems remain unclear. In this field experiment, nitrous oxide (N₂O) and methane (CH₄) fluxes from
31 an irrigated lettuce cropping system in subtropical Queensland, Australia were measured using
32 manual sampling chambers. Four treatments were included: Control (no fertiliser), U100 (100 kg N
33 ha⁻¹ as urea), U200 (200 kg N ha⁻¹ as urea) and N100 (100 kg N ha⁻¹ as nitrate-based fertilisers). The
34 N fertilisers were applied in three splits and irrigation was delivered sparingly and frequently to keep
35 soil moisture around the field capacity. The cumulative N₂O emissions from the control, U100, U200
36 and N100 treatments over the 68-day cropping season were 30, 151, 206 and 68 g N₂O-N ha⁻¹,
37 respectively. Methane emission and uptake were negligible. Using N₂O emission from the Control
38 treatment as the background emission, direct emission factors for U100, U200 and N100 treatments
39 were 0.12%, 0.09% and 0.04% of applied fertiliser N, respectively. Soil ammonium (NH₄⁺)
40 concentration, instead of nitrate (NO₃⁻) concentration, displayed a significant correlation with N₂O
41 emissions at the site where the soil moisture was controlled within 50-64% water-filled pore space
42 (WFPS). Furthermore, soil temperature rather than water content was the main regulating factor of
43 N₂O fluxes in the fertilised treatments. The fertiliser type and application rates had no significant
44 effect on yield parameters. Partial N balance analysis indicated that approximately 80% and 52% of
45 fertiliser N was recovered in plants and soil in the treatments receiving 100 kg N ha⁻¹ and 200 kg N
46 ha⁻¹, respectively. Therefore, in combination with frequent and low intensity irrigation and split
47 application of fertiliser N, substitution of NO₃⁻-based fertilisers for urea and reduction in fertiliser N
48 application rates were considered promising mitigation strategies to maintain yield and minimise
49 N₂O emissions during the low rainfall season.

50 **Keywords:** Nitrous oxide, Methane, Vegetable, Subtropical climate, Greenhouse gas mitigation

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52

53 **Introduction**

54 Agricultural manipulation of the soil nitrogen (N) cycle during past decades has caused a significant
55 increase in N₂O emissions (Yan *et al.* 2003; Dong *et al.* 2007). Agricultural soils are the primary
56 source of anthropogenic N₂O emissions, and global N₂O emissions from this source are
57 approximately 3.9 Tg N yr⁻¹ (FAOSTAT, 2014). Nitrous oxide production in soil is mainly through
58 the microbial processes of nitrification (oxidation of NH₄⁺ to NO₃⁻) and denitrification (anaerobic
59 reduction of NO₃⁻ to gaseous NO, N₂O or N₂). The most important factors controlling these processes
60 are soil available carbon (C) and N, temperature, moisture and pH (Dalal *et al.* 2003).

61 Due to continuous decline in soil fertility in agricultural production systems (Dalal and Mayer 1986)
62 and in order to increase productivity to meet the needs of a rapidly increasing human population
63 (Vallejo *et al.* 2005), N fertiliser applications have become essential for sustaining crop production
64 systems. However, the tendency to apply N fertilisers in excess of crop requirements often results in
65 high mineral N concentrations in the surface soil (Wagner-Riddle *et al.* 2007; He *et al.* 2007, 2009),
66 which intensifies N₂O emissions from cultivated soils (Mosier and Kroeze 2000).

67 Vegetable cropping systems are associated with high N fertiliser application rates and intensive
68 cultivation. However, the N recovery from intensively cultivated vegetable fields is only 20 to 50%
69 of the applied N fertiliser (Huang *et al.* 2006), suggesting that large amounts of N surplus to crop
70 requirements can be lost by nitrate leaching, runoff and/or emissions of gases including N₂O.
71 Synchronizing the soil N supply with crop requirements during the growing season and improving
72 fertiliser and irrigation management practices would increase N use efficiency in the soil-plant
73 systems and consequently reduce N losses (Vanlauwe *et al.* 2001; Dalal *et al.* 2003).

74 The atmospheric concentration of CH₄ has increased rapidly in recent years with a concentration of
75 around 1803 ppbv in 2011 (IPCC 2013). Agriculture, especially animal production, is responsible for
76 about 52% of total anthropogenic CH₄ emissions (Smith *et al.* 2008). The production and
77 consumption of CH₄ in soil is mainly driven by the biological decomposition of organic materials in

78 anoxic conditions and biological oxidization in aerobic environments (Mosier *et al.* 1998). It has
79 been reported that well-irrigated vegetable fields could emit CH₄ during wet periods and consume
80 CH₄ during dry periods of the growing season (Jia *et al.* 2012). Striegl (1993) demonstrated that
81 diffusion of atmospheric CH₄ into well-drained agricultural soils is the main factor limiting its
82 biological oxidation rate.

83 Field measurements of N₂O and CH₄ emissions conducted in vegetable production systems are
84 limited. Although subtropical farms of Queensland contribute more than 30% of the Australian gross
85 vegetable value, there is a lack of knowledge about greenhouse gas production and mitigation
86 options in these intensive cropping systems. The main objectives of this study were therefore to: (1)
87 compare different fertiliser management practices (nitrate vs. urea application at different rates) in
88 relation to greenhouse gas emissions, crop yield and fertiliser N use efficiency; and (2) identify the
89 factors regulating N₂O emissions during the cropping season. The underlying hypothesis was that
90 avoiding excessive N application and changing the fertiliser type from urea to nitrate-based products
91 in combination with controlled irrigation would reduce N₂O emissions during the dry cropping
92 season.

93

94 **Materials and methods**

95 *Study site and experimental design*

96 The experimental site was located at Gatton Horticultural Research Station (27° 32' 39" S, 152° 19'
97 38" E) in the Lockyer Valley, one of the key horticultural regions in north-east Australia. The soil at
98 this site is a black Vertosol (Isbell, 2002) and the clay mineralogy of the soil is dominated by
99 smectite (~60%; Dalal and Mayer, 1986). The experimental area has a mean annual air temperature
100 of 20°C and a mean annual precipitation of 772 mm. Soil properties in the 0-90 cm profile
101 summarised in Table 1.

102

103 Table 1: Initial physico-chemical characteristics of the experimental soil

Soil Depth (cm)	Bulk Density (g cm ⁻³)	pH (1:5 water)	Clay (%)	Silt (%)	Sand (%)	CEC (cmol kg ⁻¹)	Exchange-able ^a K (mg kg ⁻¹)	Colwell ^b P (mg kg ⁻¹)	Total OC ^c (%)	Total N (%)
0-10	1.17	7.69	41	24	35	37.9	312	136	1.54	0.11
10-30	1.32	7.79	43	23	34	37.2	207	115	1.53	0.11
30-60	1.41	7.77	55	15	30	41.2	-----	-----	-----	-----
60-90	1.51	7.84	47	15	38	40.1	-----	-----	-----	-----

104 ^a Exchangeable K extracted with 1M NH₄Cl at pH = 7 ; ^b Colwell P with 0.5M NaHCO₃ ; ^c Total organic carbon was determined by
105 pre-treatment of soil samples with sulphurous acid (H₂SO₃) and then dry combustion using a LECO CN analyser (TruMac NO. 830-
106 300-400).
107

108 The field experiment was conducted in an intensively managed vegetable cropping system typical of
109 the subtropical climatic zone and consisted of four treatments: (1) no fertiliser application (Control);
110 (2) urea applied at 100 kg N ha⁻¹ (U100); (3) urea applied at 200 kg N ha⁻¹ (U200); and (4)
111 predominantly NO₃⁻-based fertilisers applied at 100 kg N ha⁻¹ (N100, Table 2). The U200 treatment
112 represents approximately the highest N application rate in the region. The fertilised treatments
113 received three splits of N applications during the growing season to better match N supply with plant
114 demand during the growing season (Table 2). The fertilisers were applied by broadcasting on the
115 surface immediately before irrigation. The experiment was arranged using a randomized block
116 design with four replicates (7.0m × 1.5m plots). Iceberg lettuce (*Lactuca sativa*, var. *capitata* cv.
117 *Kong*) was transplanted on 9th May 2012 and harvested on 16th July 2012.

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123 Table 2: Date, type and application rates (kg N ha⁻¹) of N fertilisers

Treatment	Application Date			Total N Rate (kg ha ⁻¹)
	9/05/2012	4/06/2012	18/06/2012	
Control	0	0	0	0
U100	50 kg N as urea	25 kg N as urea	25 kg N as urea	100
U200	50 kg N as urea	50 kg N as urea	100 kg N as urea	200
N100	27 kg NH ₄ -N + 23 kg NO ₃ -N as Nitrophoska ^a	25 kg N as Ca(NO ₃) ₂	25 kg N as KNO ₃	100

124 ^a Nitrophoska Blue Special: Incitec Pivot, Australia; containing 6.5% NH₄⁺-N and 5.5% NO₃⁻-N

125

126 The irrigation regime aimed to keep the soil moisture content close to field capacity using a sprinkler
 127 irrigation system, as this was considered to be optimal for growth and less inductive to N losses.
 128 Therefore, several light irrigations were applied throughout the lettuce growing season according to
 129 soil moisture content, except for the 4th June when heavy rainfall was received just after the
 130 irrigation (Fig. 1a).

131

132 *Soil sampling and analysis*

133 Prior to fertiliser application, soil samples were collected to a depth of 90 cm in four increments of 0-
 134 10 cm, 10-30 cm, 30-60 cm and 60-90 cm to determine the physicochemical properties of the
 135 experimental site (Table 1). This sampling was also repeated after crop harvest to provide
 136 information about N leaching and the N budget for different treatments. During the growing season,
 137 soil samples were taken once per fortnight at two depths (0-10 cm and 10-30 cm) from all plots (4
 138 treatment × 4 replications). The sampling location was marked to avoid resampling the same spots.

139 Field-moist samples were transported to the laboratory and kept at 4°C until mineral N extraction
 140 (within 48 h). The soil samples were extracted with 2 M KCl at a soil to solution ratio of 1:5. The
 141 NO₃⁻-N and NH₄⁺-N concentrations in the extractant were determined using colorimetric techniques

142 (Rayment and Lyons 2011). Total extractable mineral N was calculated as the sum of NO_3^- -N and
143 NH_4^+ -N concentrations. All results were expressed on an oven-dry basis. Soil pH was measured in a
144 1:5 volumetric suspension of soil in distilled water (Rayment and Lyons 2011).

145 Before planting, and one week prior to the harvest, three replicate undisturbed soil cores were
146 collected using stainless steel cylinders (10 cm in diameter and 10 cm in length) to measure surface
147 soil bulk density. The cores were dried for 48 h at 105°C and weighed. Bulk density was calculated
148 from the oven dry soil weight divided by the core volume. Field capacity (FC%) was determined
149 using the method described by Cassel and Nielsen (1986).

150

151 *Measurement of N_2O and CH_4 fluxes*

152 In order to investigate the effect of the crop on gas fluxes, two different types of manual gas
153 sampling chamber were used: a) cylindrical polyethylene chambers (23.5 cm diameter and 12 cm
154 height) installed between plants and b) cubic chambers (50×50×50 cm) with stainless steel frames,
155 opaque PVC panels and a circulating fan (Wang *et al.* 2011) installed over a growing lettuce plant.
156 Each treatment was instrumented with eight cylindrical and four cubic chambers. The chambers were
157 relocated every 3 weeks to minimise spatial variability in fluxes and to minimise the impact of crop
158 roots exclusion in the cylindrical chambers during the growing season.

159 Gas fluxes were measured 3 times per week between 9:00 and 11:00am local time to minimize the
160 effect of diurnal variation on flux patterns. This schedule is reported to be the optimum timing for
161 intermittent manual flux measurements in the field with lowest deviation in the seasonal cumulative
162 emissions (Liu *et al.* 2010; Wang *et al.* 2011; Deng *et al.* 2012). Gas samples were collected from the
163 chamber headspace approximately one hour after chamber closure using a 25 mL gas-tight syringe
164 and immediately transferred to pre-evacuated 12 mL glass vials (Exetainer, Labco Ltd, High
165 Wycombe, UK).

Gas samples were analysed for N₂O and CH₄ concentrations within 5 days of sampling using a gas chromatograph (Varian CP-3800, Varian Inc., Middleburgh, the Netherlands) as described by Wang et al. (2011). Standards were injected after every ten samples to monitor instrument precision. Gas fluxes were calculated from the increases in the N₂O and CH₄ concentrations during the sampling time. Linearity tests on gas concentration increases were performed on a subset of sampling occasions during the growing season for all treatments by taking samples after chamber closure every 30 min for 1.5 hours. In general, N₂O and CH₄ fluxes showed a linear trend over the measurement period. The emissions for the days without gas sampling were estimated using the arithmetic mean of the two closest measurements. The cumulative seasonal emissions were calculated by summing up the daily flux measurements.

176

Measurements of environmental factors

Climate parameters including daily rainfall and hourly air temperature during the entire monitoring period were obtained from an on-site automatic weather station. Immediately after planting, soil moisture and temperature were logged hourly using moisture probes (Theta Probe, Delta-T Devices Ltd., Cambridge, UK) installed at 7-13 cm depth and temperature probes (Measurement Engineering Australia Ltd., SA, Australia) installed at 5-7 cm depth inside and outside a cylindrical and a cubic chamber. Moisture probe readings were calibrated against simultaneous measurements of soil water contents using the oven drying method. The calibrated volumetric moisture content was then converted to water-filled pore space (WFPS) using the following equations:

$$\text{WFPS (\%)} = \text{Volumetric soil water content (\%)} / (1 - \text{Soil bulk density (g cm}^{-3}\text{)} / 2.65) \times 100\% \quad (1)$$

$$\text{Volumetric soil water content} = \text{Gravimetric soil water content (\%)} \times \text{Soil bulk density (g cm}^{-3}\text{)} \quad (2)$$

where 2.65 g cm⁻³ was assumed to be soil particle density.

189

190 *Nitrogen balance*

191 To aid understanding of the N use efficiency of applied fertilisers, the N balance for each treatment
192 was calculated using equation (3), as described by Patil et al. (2001).

$$193 \quad N_{\text{balance}} = (N_{\text{crop}} + N_{\text{min harvest}}) - (N_{\text{min planting}} + N_{\text{fer}} + N_{\text{irri}}) \quad (3)$$

194 where N_{crop} = total N uptake in the lettuce biomass (kg N ha^{-1}), $N_{\text{min harvest}}$ = soil mineral N in the 0–90
195 cm depth immediately after harvest (kg N ha^{-1}), $N_{\text{min planting}}$ = soil mineral N at 0–90 cm depth before
196 planting (kg N ha^{-1}), N_{fer} = total N from applied fertiliser (kg N ha^{-1}), and N_{irri} = total N from irrigation
197 water (kg N ha^{-1}).

198 Lettuce yield was determined by manually harvesting and weighing 12 lettuce heads per replicate
199 and multiplying by the plant population ($60,000 \text{ plants ha}^{-1}$). The lettuce sub-samples for each of the
200 four replicate plots were dried at 65°C for 72 hours to measure dry matter content and then ground
201 ($<1 \text{ mm}$) before analysing for total N content using an Isotope Ratio Mass Spectrometer (Sercon
202 Hydra 20-22, Sercon Europa EA-GSL). Lettuce heads and wrappers were measured separately.

203 *Statistical analysis*

204 Statistical analysis was performed using the SPSS 19 software package. Differences at $P \leq 0.05$ were
205 considered statistically significant and variables were tested for normality of distribution. Soil
206 ammonium concentration data had a square root transformation to meet the normal distribution
207 requirement for statistical analysis. Stepwise multiple linear regression analysis was used to identify
208 relationships between soil/environmental factors and N_2O emission fluxes.

209 **Results**

210 *Seasonal pattern of environmental conditions and mineral N dynamics*

211 Total rainfall and irrigation were 127mm and 137mm respectively during the 68-day cropping
212 season. The WFPS of the soil at field capacity was 57% (Fig. 1a). The mean daily WFPS ranged

213 from 50% to 64%, with a seasonal mean of 57% (equal to field capacity) in the 7-13cm depth. The
 214 mean daily soil temperature at 5-7cm depth ranged from 11.3°C to 18.1°C and air temperature from
 215 11.1°C to 19.6°C during the growing season, with an average of 14.6°C for soil and 14.9°C for air
 216 temperature. The results showed a similar temporal pattern for air and soil temperatures during the
 217 investigation period (Fig. 1b).

218

219

220 Fig.1. Rainfall, irrigation and soil moisture (WFPS) at 7-13 cm depth (a) and daily mean air and soil
 221 temperatures at 5-7 cm depth (b) during the lettuce growing season.
 222

223 The concentrations of NO_3^- -N and NH_4^+ -N in the top 0-10 cm and the 10-30 cm layers indicated
 224 moderate mineral N leaching from the surface soil (Fig. 2). There were distinctive differences in

225 mineral N distribution patterns following the application of nitrate-based and urea-based fertilisers,
 226 especially in the top 0-10 cm soil. Soil $\text{NH}_4^+\text{-N}$ concentrations increased sharply after fertilisation
 227 with urea (Figs 2a and b) and then decreased rapidly. The concentrations of $\text{NH}_4^+\text{-N}$ in the N100
 228 treatment (including 27 kg $\text{NH}_4^+\text{-N/ha}$ in the first application) were consistently lower compared to
 229 those in the urea treatments. Mineral N concentrations in all fertilised treatments at the end of the
 230 experiment declined to low levels similar to those in the control treatment, regardless of the amount
 231 of fertiliser applied.

232
 233 Fig.2. Soil $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations in the 0-10 cm (a and c) and 10-30 cm (b and d) depth
 234 intervals in all treatments. The black arrows indicate the timing of fertiliser application. Control = no
 235 fertiliser; U100 = 100 kg N ha^{-1} as urea; U200 = 200 kg N ha^{-1} as urea; N100 = 100 kg N ha^{-1} mainly
 236 as nitrate-based fertilisers.

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 238
 239 *N₂O and CH₄ fluxes*

240 The daily and cumulative N_2O emissions during the lettuce growing period are summarised in Figure
 241 3. Several emission peaks occurred following irrigation or rainfall events in the fertilised treatments.
 242 After the first N fertiliser application, the N_2O fluxes from the fertilised plots initially increased, and

then gradually decreased to the pre-fertilisation level. The magnitude of these fluxes was higher in the U100 treatment than in the N100 treatment although the concentrations of NO_3^- -N were higher in the latter. The results showed no significant differences between the chambers placed between lettuce plants and the chambers that contained a lettuce plant inside for the whole period of experiment. Thus, the lettuce plants did not have any significant effect on N_2O emissions.

The Control, U100, U200 and N100 treatments had cumulative N_2O emissions of 30, 151, 206 and 68 $\text{g N}_2\text{O-N ha}^{-1}$, respectively, during the experimental season. The cumulative N_2O emissions were significantly higher for the fertilised treatments compared to the Control. During the growing season, the N100 treatment reduced total N_2O emission compared to the U100 treatment by 55% ($P < 0.01$). The U100 treatment also reduced the total N_2O emission by 27% in comparison to the U200 treatment ($P < 0.05$).

Fig.3. Daily (a) and cumulative (b) N_2O emissions from different fertiliser treatments in the lettuce field. The black arrows indicate the timing of fertiliser application. Control = no fertiliser; U100 = 100 kg N ha^{-1} as urea; U200 = 200 kg N ha^{-1} as urea; N100 = 100 kg N ha^{-1} mainly as nitrate-based fertilisers.

The CH_4 fluxes were minor during the early growing stages and higher emissions were recorded during the late stages of the growing season (Fig. 4). The Control, U100, U200 and N100 treatments respectively emitted 20.5, 6.2, 6.8 and 16.8 $\text{g CH}_4 \text{ ha}^{-1}$ during the experiment's season, which were not significantly different at $P = 0.05$. Similar to N_2O emissions, the results from different chamber types did not show any significant difference in CH_4 fluxes during the experimental period.

Fig.4. Daily (a) and cumulative (b) CH₄ emissions from different fertiliser treatments in the lettuce field. Control = no fertiliser; U100 = 100 kg N ha⁻¹ as urea; U200 = 200 kg N ha⁻¹ as urea; N100 = 100 kg N ha⁻¹ mainly as nitrate-based fertilisers.

Relationships of N₂O fluxes with environmental factors and soil mineral nitrogen contents

For each treatment, stepwise linear regression was used to model N₂O fluxes using soil NO₃⁻-N and NH₄⁺-N concentrations, WFPS and soil temperature as variables. The stepwise regressions indicated that the seasonal variations in N₂O emissions from the control were positively correlated with soil moisture:

$$\text{N}_2\text{O flux for Control (g N}_2\text{O-N ha}^{-1} \text{ d}^{-1}) = -7.112 + 0.126 \text{ WFPS (\%);} \quad (4)$$

$n = 48, r = 0.30, P < 0.05.$

In the urea treatments, soil NH₄⁺-N concentration and soil temperature were the most important factors controlling the magnitude of N₂O emissions:

$$\text{N}_2\text{O flux under U100 (g N}_2\text{O-N ha}^{-1} \text{ d}^{-1}) = -5.170 + 0.444 \text{ Soil T (}^\circ\text{C)} + 0.244 (\text{NH}_4^+)^{1/2} (\text{mg N kg}^{-1}); \quad (5)$$

$n = 48, r = 0.61, P < 0.05$

$$\text{N}_2\text{O flux under U200 (g N}_2\text{O-N ha}^{-1} \text{ d}^{-1}) = -4.018 + 0.361 \text{ Soil T (}^\circ\text{C)} + 0.303 (\text{NH}_4^+)^{1/2} (\text{mg N kg}^{-1}); \quad (6)$$

$n = 48, r = 0.54, P < 0.05$

In the N100 treatment, soil NH₄⁺-N concentration appeared to be the only dominant factor regulating N₂O fluxes and there was no significant effect of any environmental factor:

$$\text{N}_2\text{O flux under N100 (g N}_2\text{O-N ha}^{-1} \text{ d}^{-1}) = 0.012 + 0.269 (\text{NH}_4^+)^{1/2} (\text{mg N kg}^{-1}); \quad (7)$$

$n = 48, r = 0.48, P < 0.01$

288 *Direct N₂O emission factors*

289 Using the N₂O emission from the control treatment as the background emission, the proportions of
 290 the N₂O emissions attributable to N fertiliser application were calculated to be 80%, 86% and 57%
 291 for U100, U200 and N100 treatments, respectively. The direct emission factors [(N₂O-N from
 292 fertilised treatment - N₂O-N from control)/fertiliser-N*100%] for U100, U200 and N100 treatments
 293 were 0.12%, 0.09% and 0.04% respectively. These emission factors over 68 days are substantially
 294 lower than the IPCC default emission factor of 1.0%. The results indicate that the nitrate fertiliser
 295 form had the lowest direct emission factor and the higher urea application rate decreased the direct
 296 emission factor.

297 *Lettuce yield*

298 Total fresh yield of lettuce increased by more than two-fold with N fertiliser application (P<0.05;
 299 Table 3). There was no difference in yield between the nitrate-based fertiliser and urea. Reducing
 300 urea application from 200 kg N ha⁻¹ to 100 kg N ha⁻¹ did not have any significant negative impact on
 301 lettuce yield.

302 Table 3: Yield components of lettuce in response to the different N treatments

Treatments	TFY (t ha ⁻¹)	TDY (t ha ⁻¹)	FHY (t ha ⁻¹)	HDMY (t ha ⁻¹)	HDM (%)	FWY (t ha ⁻¹)	WDMY (t ha ⁻¹)	WDM (%)
Control	34.8 (a) ^a	1.8 (a)	26.7 (a)	1.4 (a)	5.0 (a)	8.1 (a)	0.4 (a)	5.5 (a)
U100	77.0 (b)	3.1 (b)	64.7 (b)	2.5 (b)	3.9 (b)	12.3 (ab)	0.6 (a)	4.9 (a)
U200	81.5 (b)	3.2 (b)	67.7 (b)	2.6 (b)	3.8 (b)	13.8 (b)	0.6 (a)	4.8 (a)
N100	80.9 (b)	3.3 (b)	68.5 (b)	2.7 (b)	3.9 (b)	12.4 (ab)	0.6 (a)	5.2 (a)

303 ^a The different letters in parentheses within a column indicate significant differences between the treatments (P< 0.05)
 304 Abbreviations: **TFY**, total fresh yield; **TDY**, total dry yield; **FHY**, fresh head yield; **HDMY**, head dry matter yield;
 305 **HDM**, head dry matter; **FWY**, fresh wrapper yield; **WDMY**, wrapper dry matter yield; **WDM**, wrapper dry matter;
 306 **Control**, no fertiliser; **U100**, 100 kg N ha⁻¹ as urea; **U200**, 200 kg N ha⁻¹ as urea; **N100**, 100 kg N ha⁻¹ mainly as nitrate-
 307 based fertilisers.

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311 *Nitrogen balance*

312 Mineral N concentrations in the soil profiles at the start and the end of the experiment are shown in
313 Table 4. The post-harvest mineral N concentrations in the soil profiles showed no significant increase
314 in the 100 kg N ha⁻¹ treatments (U100 and N100) compared with the Control, but slight increases in
315 NO₃⁻ concentrations in the U200 treatment (P< 0.05) were observed. In this latter treatment there was
316 a movement of NO₃⁻-N to the 60-90 cm layer, indicating N leaching out of the lettuce rooting depth
317 in the presence of excessive available mineral N. The similar mineral N concentrations in the U100
318 and N100 treatments down to 90 cm confirmed that fertiliser type did not appear to have any
319 significant effect on mineral N leaching in this controlled irrigation system.

320 Assuming N uptake in the above-ground biomass accounted for 88% of the total crop N uptake
321 (Jackson *et al.* 1994; Gallardo *et al.* 1996), the amount of N in the below-ground biomass was
322 estimated to be 5.3, 14.0, 15.5 and 14.1 kg N ha⁻¹ for the Control, U100, U200 and N100 treatments,
323 respectively. The value of calculated N balance for the Control treatment (Table 5) was positive, with
324 (N_{crop} + N_{min harvest}) exceeding (N_{min planting} + N_{fer} + N_{irri}) by 18.7 kg N ha⁻¹. This extra amount of N
325 was attributed to the mineralisation of soil organic N. The crop N uptake was significantly enhanced
326 by N fertiliser application and was higher for the U200 treatment than the U100 treatment but was
327 not significantly different between different fertiliser types applied at 100 kg N ha⁻¹. The N balances
328 for the fertilised treatments were all negative and the highest negative N balance in the U200
329 treatment showed a substantially greater N loss compared to other fertilised treatments.

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Table 4: Mineral nitrogen concentration (mg kg⁻¹) in different soil layers at the start and end of the lettuce growing season.

Soil Depth (cm)	NH ₄ ⁺					NO ₃ ⁻				
	Start	End				Start	End			
		Control	U100	U200	N100		Control	U100	U200	N100
0-10	0.9(a) ^a	0.7(a)	0.5(a)	1.3(a)	0.6(a)	4.9(a)	1.0(a)	2.5(a)	4.0(a)	3.0(a)
10-30	0.6(a)	0.3(a)	0.8(a)	0.2(a)	0.3(a)	7.5(a)	0.8(b)	1.1(bc)	2.3(c)	1.1(bc)
30-60	1.1(a)	0.5(a)	0.4(a)	0.5(a)	0.5(a)	0.6(a)	0.6(a)	1.0(a)	2.0(a)	0.9(a)
60-90	1.2(a)	0.9(a)	0.9(a)	1.2(a)	0.9(a)	0.2(a)	0.7(a)	1.4(b)	1.8(b)	1.3(b)

^a The different letters in parentheses within a row (separate for NH₄⁺-N and NO₃⁻-N) indicate significant differences between the treatments (P< 0.05). Control = no fertiliser; U100 = 100 kg N ha⁻¹ as urea; U200 = 200 kg N ha⁻¹ as urea; N100 = 100 kg N ha⁻¹ mainly as nitrate-based fertilisers

Table 5: Effect of different N treatments on N balance in the crop-soil system (kg N ha⁻¹).

Treatments	Soil 0-90 cm N _{min} transplanting	N fertiliser	N irrigation	crop N uptake	Soil 0-90 cm N _{min} harvest	N balance ^a
Control ^b	41.5	0	1.2	44.4(a) ^c	17.0(a)	18.7(a)
U100	41.5	100	1.2	116.6(b)	25.0(a)	-1.1(b)
U200	41.5	200	1.2	129.5(c)	36.7(b)	-76.5(c)
N100	41.5	100	1.2	117.2(b)	23.9(a)	-1.6(b)

^a N_{balance} = (N_{crop} + N_{min} harvest) - (N_{min} planting + N_{fer} + N_{irri}); ^b Control = no fertiliser; U100 = 100 kg N ha⁻¹ as urea; U200 = 200 kg N ha⁻¹ as urea; N100 = 100 kg N ha⁻¹ mainly as nitrate-based fertilisers; ^c The different letters in parentheses within a column indicate significant differences between the treatments (P< 0.05)

Discussion

Methane emissions

Unlike the finding by Jia et al. (2012) that vegetable cropping soils normally show high CH₄ emissions due to more frequent irrigation compared to other crops, the results of the current experiment in winter lettuce production on a Vertosol showed minor net CH₄ emission in a low-intensity, high-frequency irrigation system. Low CH₄ emissions are generally observed under high soil temperature and relatively low soil moisture conditions (Ambus and Christensen 1995; Dobbie and Smith 1996; Ruser *et al.* 1998). Previous studies on aerated soils also indicated that CH₄

diffusion into the soil profile is one of the main limiting factors controlling CH₄ emission rates (Striegl 1993; Ball *et al.* 1997).

The N fertiliser type and application rate did not significantly increase CH₄ emission compared to the control treatment. These results are in agreement with findings of Ruser *et al.* (1998) and Wang *et al.* (2011) who reported that application of N fertilisers did not change CH₄ emission rates in soils under long-term experiments.

Factors regulating N₂O emissions

It is essential to identify and understand the factors regulating agricultural soil N₂O emissions so that improved mitigation techniques can be developed. Vegetable production systems are especially important for agricultural N₂O mitigation as they are usually under multiple planting-harvest cycles during the year and receive high N application rates. Any factor that affects the soil N cycle is likely to alter N₂O emission (Xiong *et al.* 2006).

The present study showed that almost two thirds of the recorded N₂O emission peaks occurred after an increase in daily soil temperature (Fig. 1b and 3a). The only exceptions were two peaks (8/06/2012 and 22/06/2012) just after fertiliser topdressings where high emissions were observed without a significant increase in soil temperature. Soil temperature is one of the main controlling parameters of N₂O emission from fertilised agricultural soils when N₂O production in the soil is not limited by other factors such as WFPS and soil available N (Dobbie *et al.* 1999). Soil temperature influences N₂O emissions by regulating soil microbial activity, such as nitrification and denitrification. Ding *et al.* (2007b) reported a positive correlation between N₂O emission and soil temperatures up to 25°C in laboratory incubation at a certain WFPS condition favourable for nitrification and denitrification processes. Diao *et al.* (2013) indicated that N₂O emission from

376 vegetable fields was associated with both N fertilisation and soil temperature under appropriate soil
377 moisture regimes (50-70% WFPS).

378 Soil moisture affects N₂O emissions mainly by controlling the dominance of aerobic and anaerobic
379 conditions during the growing season. Many investigations report that high N₂O fluxes are mainly
380 related to the denitrification process (Davidson 1991; Thornton *et al.* 1998), which is generally
381 limited when soil moisture is below field capacity (De Klein and Van Logtestijn 1996). The frequent
382 and low intensity irrigation scheme used in this study should have minimised anaerobic soil
383 conditions (WFPS = 57%) and therefore probably avoided high N₂O spikes from denitrification.
384 These results are similar to the observations of Davidson (1991) and Russow *et al.* (2000) who
385 reported nitrification as the main process of N₂O production at soil moistures below 60% WFPS.

386 Ludwig *et al.* (2001) reported that at favourable soil temperatures and moistures for microbial
387 activity, soil mineral N concentration is a regulatory factor of nitrification and denitrification rates.
388 Dobbie and Smith (2003) and Vilain *et al.* (2010) also demonstrated the intensity of nitrification and
389 denitrification processes as a function of soil NH₄⁺-N and NO₃⁻-N concentrations. In the current
390 study under predominantly aerobic soil conditions, NH₄⁺-N rather than NO₃⁻-N concentrations
391 regulated N₂O emissions through the nitrification process (Eq 5, 6 and 7). In addition, the application
392 of N fertilisers was split so that supply of mineral N more closely matched the crop demand at
393 different stages of the growing season. This avoided accumulation of large amounts of mineral N,
394 and thus would have restricted N₂O emissions (Sehy *et al.* 2003; Tan *et al.* 2009).

395

396 *Nitrous oxide emission factors and fertiliser nitrogen use efficiency under different management*
397 *practices*

398 Nitrous oxide emissions from cropping land can be derived from fertiliser N and other sources
399 (mainly background soil N). The N₂O emission factors of urea N (0.12% for U100 and 0.09% for

U200) derived from this study were substantially lower than the default 1.0% emission factor used by the IPCC (IPCC 2006). The reason could be related to the low temperature during the late autumn/winter seasons and the N/irrigation management practices as discussed below. As both cumulative N₂O emission and fertiliser N would increase in a multi-season vegetable crop system, we considered that the length of measurement period should not be a major factor leading to the low emission factors.

The U100 treatment resulted in lower total N₂O emissions than the U200 treatment, but slightly increased the direct emission factor of the fertiliser N. Meta- analyses by Kim et al. (2013) and Shcherbak et al. (2014) suggested a general trend of increasing N₂O emissions in response to increasing N inputs, while the emission factors may remain constant, increase or decrease with changing N input for most crop types. Ding et al. (2007a) reported that an increase in the N₂O emission factor with increasing N fertiliser application only occurs when soil conditions are favourable for N₂O production.

The N balances for the fertilised treatments in this experiment were all negative; thus N losses occurred. When the 18.7 kg N ha⁻¹ recovered from soil N mineralisation in the Control treatment was accounted for in the N supply, the U100, U200 and N100 treatments showed a net N loss of 20, 95 and 20 kg N ha⁻¹, respectively. The fertiliser N recovery for the U100, U200 and N100 treatments was 80%, 52% and 80%, respectively. Although not quantified in this study, the unrecovered N might have been lost through NH₃ volatilisation, NO₃⁻ leaching to deeper soil layers (> 90 cm) as well as denitrification. The higher N loss in U200 (48% of applied N) along with no positive effect of this treatment on lettuce yield (compared to U100) suggested that application of 200 kg urea-N ha⁻¹ was inefficient in terms of both lettuce yield and N use, whilst significantly increasing N₂O emissions. This result supports previous findings that optimising N fertiliser application rates provide an efficient way of reducing N₂O emissions (Del Grosso *et al.* 2009).

One of the findings in this study was the significant relationship between N₂O emissions and soil NH₄⁺ concentrations as discussed above. These results indicate that, if excessive wet conditions can be avoided by using moderate irrigation, one of the strategies for reducing N₂O emissions from intensively cropped vegetable fields in this region is to replace NH₄⁺-based fertilisers with NO₃⁻-based N fertilisers during the low rainfall seasons. However, this strategy may not apply in wet seasons when N₂O emissions might be mainly produced through denitrification (Wang *et al.* 2011). When NO₃⁻-based N fertilisers are used, it is important to use low-intensity irrigation techniques to minimise risks of denitrification under wet conditions.

Nitrophoska Blue Special (12.0% N, 5.2% P and 14.1% K; trade name Incitec Pivot Ltd.) was used for the first fertiliser application for the N100 treatment; it is considered to be a multi-nutrient fertiliser suitable for pre-planting application (to avoid direct contact with establishing seedlings) in intensive crop production systems such as vegetable fields. However, its application in the N100 treatment did not result in significant improvement in lettuce yield compared to U100. Thus, the phosphorus and potassium contained in this fertiliser provided little benefit to crop growth because of the high Colwell-P and exchangeable K contents in this soil (Table 1). The presence of 6.5% NH₄⁺-N in this fertiliser might have contributed to the higher N₂O emissions than those from the control, which were observed following the application of Nitrophoska but not Ca(NO₃)₂ and KNO₃. The results suggested that substitution of purely NO₃⁻-based fertilisers for Nitrophoska may further reduce N₂O emissions whilst maintaining the yield and thus warrant more studies.

When choosing the best fertiliser management practices for N₂O emission mitigation in a specific region, both N₂O emissions and economic returns should be considered. In this study, the use of NO₃⁻-based N fertiliser combined with low intensity irrigation resulted in a considerable reduction in N₂O emission compared to the urea treatments. However, the higher cost of NO₃⁻-based fertiliser than urea (nearly three times more expensive per kg N) and similar crop yields and crop N uptakes under N100 and U100 treatments may impede implementation of this management option.

449

450 **Conclusions**

451 Nitrous oxide emissions were low in these fertilised and sparingly irrigated subtropical vegetable
452 cropping regimes in north east Australia during the autumn/winter seasons, while CH₄ emissions
453 were negligible. Regression analysis indicated that the magnitude of N₂O emissions was mainly
454 regulated by soil temperature and NH₄⁺ concentration when soil moisture was maintained around
455 field capacity. Reducing irrigation intensity to keep soil WFPS at levels near to field capacity
456 successfully controlled N₂O production in soil by decreasing anaerobic conditions favourable for
457 denitrification. Substitution of NO₃⁻-based fertilisers for urea combined with low intensity irrigation
458 led to significant reductions in N₂O emissions during the low rainfall season.

459 Split application of N fertilisers should have improved synchrony between soil mineral N availability
460 in fertilised treatments and crop demand, to avoid large mineral N accumulation and thus reduce the
461 risk of N losses and high N₂O emissions during the growing season. Reducing N fertiliser application
462 from 200 kg N ha⁻¹ to 100 kg N ha⁻¹ significantly reduced the total N₂O emission and did not
463 decrease crop yield. Overall, using NO₃⁻-based fertiliser in combination with split application and
464 efficient irrigation provides an effective N₂O mitigation strategy without losing yield for vegetable
465 production during the low rainfall season.

466

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Fig.1. Rainfall, irrigation and soil moisture (WFPS) at 7-13 cm depth (a) and daily mean air and soil temperature at 5-7 cm depth (b) during the lettuce growing season.

Fig.2. Soil $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations in the 0-10 cm (a and c) and 10-30 cm (b and d) depth intervals in all treatments. The black arrows indicate the timing of fertiliser application. Control = no fertiliser; U100 = 100 kg N ha⁻¹ as urea; U200 = 200 kg N ha⁻¹ as urea; N100 = 100 kg N ha⁻¹ mainly as nitrate-based fertilisers

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649 Fig.3. Daily (a) and cumulative (b) N₂O emissions from different fertiliser treatments in the lettuce
650 field. The black arrows indicate the timing of fertiliser application. Control = no fertiliser; U100 =
651 100 kg N ha⁻¹ as urea; U200 = 200 kg N ha⁻¹ as urea; N100 = 100 kg N ha⁻¹ mainly as nitrate-based
652 fertilisers.

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666 Fig.4. Daily (a) and cumulative (b) CH₄ emissions from different fertiliser treatments in the lettuce
667 field. Control = no fertiliser; U100 = 100 kg N ha⁻¹ as urea; U200 = 200 kg N ha⁻¹ as urea; N100 =
668 100 kg N ha⁻¹ mainly as nitrate-based fertilisers.

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683 Table 1: Initial physico-chemical characteristics of the experimental soil

Soil Depth (cm)	Bulk Density (g cm ⁻³)	pH (1:5 water)	Clay (%)	Silt (%)	Sand (%)	CEC (cmol kg ⁻¹)	Exchange -able ^a K (mg kg ⁻¹)	Colwell ^b P (mg kg ⁻¹)	Total OC (%)	Total N (%)
0-10	1.17	7.69	41	24	35	37.9	312	136	1.54	0.11
10-30	1.32	7.79	43	23	34	37.2	207	115	1.53	0.11
30-60	1.41	7.77	55	15	30	41.2	-----	-----	-----	-----
60-90	1.51	7.84	47	15	38	40.1	-----	-----	-----	-----

684 ^a Exchangeable K extracted with 1M NH₄Cl at pH = 7 ; ^b Colwell P with 0.5M NaHCO₃ ; ^c Total organic carbon was determined by
685 pre-treatment of soil samples with sulphurous acid (H₂SO₃) and then dry combustion using a LECO CN analyser (TruMac NO. 830-
686 300-400).

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Table 2: Date, type and application rates (kg N ha⁻¹) of N fertilisers

Treatment	Application Date			Total N Rate (kg ha ⁻¹)
	9/05/2012	4/06/2012	18/06/2012	
Control	0	0	0	0
U100	50 kg N as urea	25 kg N as urea	25 kg N as urea	100
U200	50 kg N as urea	50 kg N as urea	100 kg N as urea	200
N100	27 kg NH ₄ -N + 23 kg NO ₃ -N as Nitrophoska ^a	25 kg N as Ca(NO ₃) ₂	25 kg N as KNO ₃	100

^a Nitrophoska Blue Special: Incitec Pivot, Australia; containing 6.5% NH₄⁺-N and 5.5% NO₃⁻-N

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Table 3: Yield components of lettuce in response to the different N treatments

Treatments	TFY (t ha ⁻¹)	TDY (t ha ⁻¹)	FHY (t ha ⁻¹)	HDMY (t ha ⁻¹)	HDM (%)	FWY (t ha ⁻¹)	WDMY (t ha ⁻¹)	WDM (%)
Control	34.8 (a) ^a	1.8 (a)	26.7 (a)	1.4 (a)	5.0 (a)	8.1 (a)	0.4 (a)	5.5 (a)
U100	77.0 (b)	3.1 (b)	64.7 (b)	2.5 (b)	3.9 (b)	12.3 (ab)	0.6 (a)	4.9 (a)
U200	81.5 (b)	3.2 (b)	67.7 (b)	2.6 (b)	3.8 (b)	13.8 (b)	0.6 (a)	4.8 (a)
N100	80.9 (b)	3.3 (b)	68.5 (b)	2.7 (b)	3.9 (b)	12.4 (ab)	0.6 (a)	5.2 (a)

^a The different letters in parentheses within a column indicate significant differences between the treatments ($P < 0.05$)
Abbreviations: **TFY**, total fresh yield; **TDY**, total dry yield; **FHY**, fresh head yield; **HDMY**, head dry matter yield;
HDM, head dry matter; **FWY**, fresh wrapper yield; **WDMY**, wrapper dry matter yield; **WDM**, wrapper dry matter;
Control, no fertiliser; **U100**, 100 kg N ha⁻¹ as urea; **U200**, 200 kg N ha⁻¹ as urea; **N100**, 100 kg N ha⁻¹ mainly as nitrate-
based fertilisers.

768 Table 4: Mineral nitrogen concentration (mg kg⁻¹) in different soil layers at the start and end of the
769 lettuce growing season.

Soil Depth (cm)	NH ₄ ⁺					NO ₃ ⁻				
	Start	End				Start	End			
		Control	U100	U200	N100		Control	U100	U200	N100
0-10	0.9(a) ^a	0.7(a)	0.5(a)	1.3(a)	0.6(a)	4.9(a)	1.0(a)	2.5(a)	4.0(a)	3.0(a)
10-30	0.6(a)	0.3(a)	0.8(a)	0.2(a)	0.3(a)	7.5(a)	0.8(b)	1.1(bc)	2.3(c)	1.1(bc)
30-60	1.1(a)	0.5(a)	0.4(a)	0.5(a)	0.5(a)	0.6(a)	0.6(a)	1.0(a)	2.0(a)	0.9(a)
60-90	1.2(a)	0.9(a)	0.9(a)	1.2(a)	0.9(a)	0.2(a)	0.7(a)	1.4(b)	1.8(b)	1.3(b)

770 ^a The different letters in parentheses within a row (separate for NH₄⁺-N and NO₃⁻-N) indicate significant differences
771 between the treatments (P< 0.05). Control = no fertiliser; U100 = 100 kg N ha⁻¹ as urea; U200 = 200 kg N ha⁻¹ as urea;
772 N100 = 100 kg N ha⁻¹ mainly as nitrate-based fertilisers

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791 Table 5: Effect of different N treatments on N balance in the crop-soil system (kg N ha⁻¹).

Treatments	Soil 0-90 cm N _{min} transplanting	N fertiliser	N irrigation	crop N uptake	Soil 0-90 cm N _{min} harvest	N balance ^a
Control ^b	41.5	0	1.2	44.4(a) ^c	17.0(a)	18.7(a)
U100	41.5	100	1.2	116.6(b)	25.0(a)	-1.1(b)
U200	41.5	200	1.2	129.5(c)	36.7(b)	-76.5(c)
N100	41.5	100	1.2	117.2(b)	23.9(a)	-1.6(b)

792 ^a N_{balance} = (N_{crop} + N_{min harvest}) - (N_{min planting} + N_{fer} + N_{irri}); ^b Control = no fertiliser; U100 = 100 kg N ha⁻¹ as urea; U200 =
793 200 kg N ha⁻¹ as urea; N100 = 100 kg N ha⁻¹ mainly as nitrate-based fertilisers; ^c The different letters in parentheses
794 within a column indicate significant differences between the treatments (P< 0.05)
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