Assessment of N₂O emissions from a fertilised vegetable cropping soil under different plant residue management strategies using ¹⁵N tracing techniques

M. Rezaei Rashti^{A,B,D,F}, W.J. Wang^{A,C,F}, C.R. Chen^{B,D}, S.H. Reeves^A, C. Scheer^E

^A Department of Science, Information Technology and Innovation (DSITI), Dutton Park, QLD 4102, Australia

^B Australian Rivers Institute, Griffith University, Nathan, QLD 4111, Australia

^C Environmental Futures Research Institute, Griffith University, Nathan, QLD 4111, Australia

^D Griffith School of Environment, Griffith University, Nathan, QLD 4111, Australia

^E Institute for Future Environments, Queensland University of Technology, QLD 4000, Australia

^F Corresponding authors email: m.rezaeirashti@griffith.edu.au; weijin.wang@qld.gov.au

Abstract

Combined application of plant residues and N fertilisers strongly affect soil mineral N dynamics and N₂O emissions depending on the quality of the plant residues, their application methods and other management strategies. We investigated the effect of combined application of two vegetable plant residues (cauliflower and sweet corn) and ¹⁵N fertiliser on N dynamics and N₂O emission in a glasshouse pot study. The experiment was conducted under two residue management practices (soil incorporation vs surface mulching) over 98 days with growing basil (*Ocimum basilicum*) plants. We also assessed the efficacy of applying the nitrification inhibitor, 3,4-dimethylpyrazole phosphate (DMPP) to the plant residues, for reducing N loss and mitigating N₂O emissions. Application of plant residues, both on the soil surface or into soil, resulted in net N mineralisation and increased cumulative N₂O emission compared with the application of N fertiliser alone. Soil surface mulching of sweet corn decreased total and residue-induced cumulative N₂O emission compared with the incorporation method, while it showed opposite effect on N₂O emissions from cauliflower residue. The application of DMPP with sweet corn residue reduced total, residue- and fertiliser-induced N₂O emissions; however its application with cauliflower residue did not show any mitigating effect on the N₂O emissions. The residue application methods and the use of DMPP did not significantly affect ¹⁵N recovery by the basil plants. In contrast, soil incorporation of these residues doubled the microbial immobilisation of applied ¹⁵N into soil organic matter. Linear regression analysis of N₂O emission during the experimental period indicated that in the treatments without DMPP application, soil NO₃⁻-N concentration was the most important factor in controlling the magnitude of N₂O emissions, while the application of DMPP changed the dominant regulating factor from NO₃⁻-N to NH₄⁺-N concentration.

Keywords:

Crop residue management, Nitrification inhibitor, 3,4-Dimethylpyrazol phosphate (DMPP), Nitrous oxide (N₂O), ¹⁵N tracing

1. Introduction

Agricultural lands are the major source of anthropogenic N₂O, which has a significant role in global warming and destruction of the ozone layer (IPCC, 2013). Intensive vegetable cropping systems are generally characterised by heavy fertiliser N applications to maintain productivity, and consequently high N₂O emissions occur through microbial nitrification and denitrification (Pang et al., 2009;

57 Rezaei Rashti et al., 2015; Scheer et al., 2014). Therefore, reduction in N₂O emissions from these
58 cropping systems could potentially make a significant contribution to the mitigation of global
59 anthropogenic N₂O emissions.

60 Agricultural activities provide nearly 4 billion metric tons of plant residues per year at the global
61 scale (Lal, 2005). Returning of these residues to cropping lands can sustain soil organic matter and
62 enhance soil fertility by increasing microbial activity and nutrient availability (Ma et al., 2010; Smith
63 et al., 1993) as well as reducing water loss and limiting weed growth. It has been reported in
64 previous studies that residue application may increase or decrease N₂O emission depending on the
65 quantity and quality (nutrient content, biochemical composition and physical features) of the applied
66 residues (Baggs et al., 2003; Chen et al., 2013; Garcia-Ruiz and Baggs, 2007; Rezaei Rashti et al.,
67 2016), while denitrification is considered as the primary source of N₂O emissions in plant residue
68 amended soils (Kong et al., 2017; Li et al., 2016). Vegetable residues can release up to 150 kg N ha⁻¹
69 through mineralization (De Neve and Hofman, 1998), and different management practices of the
70 harvested vegetable residues (such as soil incorporation or surface mulching) may affect N₂O
71 emissions differently in these cropping systems. Generally, plant residues applied as a surface mulch
72 have a slower decomposition rate than soil incorporated residues due to the greater fluctuations in
73 soil moisture content and temperature, lower availability of soil nutrients and limited contact of
74 applied residues with soil in this application method (Schomberg et al., 1994; Thonissen et al.,
75 2000). Huang et al. (2004) and Zhu et al. (2013) reported that decomposition of soil incorporated
76 plant residues provided more bioavailable carbon and nitrogen sources for soil microbial activities.
77 The enhancement of soil microbial respiration may also facilitate the development of anaerobic
78 micro-sites which favour the denitrification process.

79 Combined application of plant residues and chemical N fertilisers has been reported to be beneficial
80 in increasing N use efficiency of applied fertilisers (Mohammad et al., 2012). This may occur
81 through enhancing the microbial immobilisation of applied mineral N, in the early days after
82 application, and synchronising soil N dynamics with N demands of the cultivated crop. However,
83 studies by Garcia-Ruiz and Baggs (2007) and Gentile et al. (2008) indicated that soil incorporation of
84 plant residues in combination with N fertilisers may increase N₂O emissions. Carmo et al. (2013) and
85 Wang et al. (2016) also reported increases in N₂O emissions after surface application of sugarcane
86 residue. It has been suggested that mineral N application may increase the decomposition rate of
87 labile carbon compounds in applied plant residues (Jiang et al., 2015). The increase of soluble
88 organic carbon in the presence of high levels of soil mineral N would consequently increase N₂O
89 emissions by stimulating the denitrification process (Paul and Beauchamp, 1989, Lan et al., 2017).

In order to reduce N losses and increase fertiliser N use efficiency, nitrification inhibitors have been introduced to agricultural soils (Boeckx et al., 2005; Di and Cameron, 2003; Pereira et al., 2010). Nitrification inhibitors can delay the conversion of NH_4^+ to NO_3^- and provide more opportunities for plant uptake and microbial immobilization of NH_4^+ within the soil profile. The inhibition of O_2 consumption by the nitrification process may also improve soil O_2 status and reduce N_2O loss through denitrification (Zhu et al., 2015). The 3,4-dimethylpyrazole phosphate (DMPP) is one of the most popular forms of such inhibitors, which has been widely used over the past years (Hatch et al., 2005; Zerulla et al., 2001). This nitrification inhibitor is effective at low application rates of 0.5-1.5 kg ha^{-1} . DMPP has a low water solubility, a slow degradation rate and can reduce the risk of NO_3^- leaching and N_2O emission (Li et al., 2009; Menendez et al., 2006; Zerulla et al., 2001). Menendez et al. (2012) reported that the addition of DMPP to mineral N fertilisers can significantly reduce N_2O emissions, but the efficacy of this nitrification inhibitor strongly depends on the environmental conditions. The effect of DMPP on reducing N_2O emissions from fertilised vegetable fields has been investigated recently by Pfab et al. (2012) and Scheer et al. (2014), but to our knowledge no published data are currently available on the mitigating effects of DMPP in combined application of vegetable residues and N fertilisers.

The main objectives of the present study were to: (1) monitor the dynamics of soil mineral N and N_2O emissions following the application of two contrasting vegetable residues with ^{15}N -labelled fertiliser, in the presence of growing plants; (2) evaluate the effect of different plant residue management strategies (incorporation vs. surface mulching) on N_2O emission and N use efficiency of the applied N sources; (3) determine the effects of DMPP application on reducing N losses and N_2O emissions following plant residue application; (4) assess the effect of moisture fluctuations on soil N dynamics and N_2O production from combined applications of vegetable residues and ^{15}N -labelled fertiliser. The underlying hypotheses were: (a) Vegetable residue quality and its application method would affect soil N dynamics and N_2O emissions; (b) DMPP application onto vegetable residues before incorporation into soil may increase N use efficiency and consequently reduce N_2O emissions.

2. Materials and Methods

2.1. Plant materials and biochemical analysis

Two common crop residues in Australian sub-tropical vegetable cropping systems with substantial differences in N content and chemical/biochemical characteristics, namely cauliflower (*Brassica oleracea* ver. *botrytis* L.) and sweet corn (*Zea mays* L.), were selected. The plant residues were dried at 60°C for two days and then cut into 2 cm pieces for application to pots and ground to <1 mm for chemical and biochemical analyses (Table 1). Total carbon (TC) and nitrogen (TN) contents of plant materials were determined by dry combustion using a LECO CN analyser (TruMac NO. 830-300-400, USA). Lignin and cellulose contents were determined sequentially with the acid detergent pre-treatment method (Wang et al., 2004). Total polyphenol contents in residue samples were determined using 50% methanol extractant followed by the Folin Ciocalteu colorimetric method calibrated with gallic acid (Waterman and Mole, 1994). The results are reported on an oven-dry weight basis.

The chemistry of each plant material was also assessed with solid state ¹³C-cross-polarization magic angle spinning (CPMAS) nuclear magnetic resonance (NMR) spectroscopy using a Varian Unity Inova 400 spectrometer (Varian Inc., Palo Alto, CA) operating at a frequency of 100.6 MHz. A measured mass of each plant material (250 mg) was packed into a silicon nitride rotor (7 mm OD) and spun at 5 kHz at the magic angle. A standard cross-polarization pulse sequence was applied with single contact times of 2 ms, an acquisition time of 14 ms, and a recycle delay of 2.5 s. Chemical shift values were referenced externally to hexamethylbenzene at 132.1 ppm, equivalent to tetramethylsilane at 0 ppm. The ¹³C CPMAS NMR spectra were divided into seven major chemical shift regions: alkyl C (-50 to 45 ppm), N-alkyl/methoxyl C (45 to 60 ppm), O-alkyl C (60 to 95 ppm), di-O-alkyl C (95 to 110 ppm), aryl C (110 to 145 ppm), O-aryl C (145 to 165 ppm) and carbonyl C (165 to 210 ppm). The relative intensities for each region were determined by integration using the NMR software package MestReNova (Version 8.1.4, Mestrelab Research S.L., 2013).

152 Table 1: Chemical and biochemical composition of the plant materials determined by chemical
153 methods and NMR spectroscopy

Plant Materials	Chemical analysis (mg g ⁻¹ plant material)						¹³ C NMR analysis (mg g ⁻¹ plant material)						
	TC	TN	C/N	Lignin	Cellu- lose	Poly- phenol	Alkyl	N-alkyl/ methoxyl	O- alkyl	di-O- alkyl	Aryl	O- Aryl	carbo- nyl
Cauliflower	379	34	11	36	136	7	98	46	144	24	17	8	42
Sweet corn	405	15	27	38	258	8	58	43	176	46	27	15	40

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155 2.2. Glasshouse preparation and experimental design

156 The fresh soil used in this study was collected from the top 20 cm of a cultivated vegetable field at
157 Gatton Horticultural Research Station (27° 32' S, 152° 19' E) in the Lockyer Valley, Queensland,
158 Australia. The soil is classified as a Vertosol (Isbell, 2002) and comprised 35% sand, 24% silt and
159 41% clay with an initial pH of 7.7 (1:5 water) and a water holding capacity of 530 g kg⁻¹. Total
160 organic carbon (OC) and N were 15.4 and 1.1 g kg⁻¹, respectively.

161 The chamber units specially designed for this experiment consisted of two identical cylindrical
162 polyethylene chambers (15 cm diameter and 22 cm height). The lower part was designed as a water-
163 tight pot and the detachable upper part was designed as a gas sampling chamber. The two parts could
164 be connected together using an elastic rubber band (10 cm wide) and high vacuum silicon grease was
165 applied to ensure an airtight seal between them during gas sampling.

166 The study was conducted using eight treatments (4.5 kg fresh soil, equivalent of 3.9 kg dry-mass per
167 pot, in seven replicates) namely: (1) Control: no added N fertiliser or plant residues; (2) Urea only:
168 50 mg N kg⁻¹ soil, equivalent to 120 kg N ha⁻¹ as 10.3 atom% ¹⁵N-urea; (3) CFI: incorporated
169 cauliflower residue (2.08 g kg⁻¹ soil, equivalent to 5 tonnes ha⁻¹ residue in the top 20 cm of soil) +
170 ¹⁵N-urea; (4) SCI: incorporated sweet corn residue (2.08 g kg⁻¹ soil, equivalent to 5 tonnes ha⁻¹
171 residue in the top 20 cm of soil) + ¹⁵N-urea; (5) CFD: incorporated cauliflower residue + DMPP (0.7
172 mg active ingredient (a.i.) kg⁻¹ soil, equivalent to 1.67 kg a.i. ha⁻¹; Incitec Pivot Ltd, Australia) + ¹⁵N-
173 urea; (6) SCD: incorporated sweet corn residue + DMPP + ¹⁵N-urea; (7) CFS: surface applied
174 cauliflower residue + incorporated ¹⁵N-urea; and (8) SCS: surface applied sweet corn residue +
175 incorporated ¹⁵N-urea. The ¹⁵N-labelled urea was evenly mixed through the soil in the fertilised
176 treatments prior to residue application. Plant residues in CFD and SCD treatments were moistened

177 with diluted liquid DMPP in sealed plastic bags for one hour and then evenly mixed with fresh soil.
 178 The soil was packed into the lower part of the chamber unit by pushing gently to a bulk density of
 179 1.2 g cm^{-3} (field bulk density). Seeds of Genovese basil (*Ocimum basilicum*) were sown after
 180 preparation of the pots. Following germination, four plants were left in each pot and plants were
 181 harvested after 98 days. The pots were moistened to 57% water-filled pore space (WFPS) with
 182 distilled water and kept close to this moisture level for the first six weeks of the study (Fig. 1). After
 183 this period (from day 42), the moisture levels in the pots were managed according to the designated
 184 fluctuating regime (40-85% WFPS) for the rest of the experiment.

185 Daily air temperature inside the glasshouse was measured using a temperature data logger (Tinytag
 186 Plus 2 TGP-4020) during the experimental period (Fig. 1). The WFPS was measured using the soil
 187 bulk density and volumetric soil moisture content of the treatments. The moisture content of each pot
 188 was adjusted by adding distilled water according to its weight loss every three days, until the end of
 189 the study.

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191

192 Fig.1. Daily air temperature of the glasshouse, field capacity (FC) of the experimental soil (dash line)
 193 and soil moisture fluctuation in water-filled pore space (WFPS) during the study period.

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195 2.3. Measurement of N₂O emissions

196 Gas sampling was undertaken every 1 to 4 days depending on soil moisture conditions and the
 197 expected levels of N₂O emissions. Gas samples were collected from the chambers' headspaces, in
 198 four replicates, one hour after closure using a 25 mL gas-tight syringe and immediately transferred to
 199 pre-evacuated 12 mL glass vials (Exetainer, Labco Ltd, High Wycombe, UK). The gas samples were
 200 analysed for N₂O concentration using a gas chromatograph (Varian CP-3800, Varian Inc.,

Middleburgh, the Netherlands) as described by Wang et al. (2011). Linearity tests on gas concentration increases were performed on a subset of sampling occasions during the study for all treatments by taking samples after the closure of chambers, every 30 min for 2 hours. Nitrous oxide emissions showed a linear trend over the first hour of the measurement period. The emissions for days without gas sampling were estimated using the arithmetic mean of the measurements on the two closest days. The cumulative emissions were calculated by summing the daily emissions.

Fertiliser-induced N₂O emissions were calculated according to the ¹⁵N abundance in the gas samples, which was detected using an automated isotope ratio mass spectrometer (IRMS) (SERCON, 20-20, UK) linked to a Sercon Cryoprep trace gas preparation system. Plant residue-induced N₂O emissions were estimated from total N₂O emissions by subtracting the emissions from the control and fertiliser-induced emissions of each treatment.

2.4. Soil sampling and biochemical analysis

Soil samples were collected at 13, 41, 76 and 98 days after the commencement of the experiment. Three replicates of each treatment were non-destructively sampled, using polyethylene columns of 2 cm diameter, for soil mineral N (NO₃⁻ and NH₄⁺), water soluble organic C (WSOC) and microbial biomass C (MBC) on all sampling days. The NO₃⁻ and NH₄⁺ concentrations were determined by colorimetric techniques (Rayment and Lyons, 2011) after extracting the fresh soil samples with 2 M KCl at 1:5 ratio of soil to extractant, using a continuous segmented flow auto-analyser (SEAL Analytical Quattro). The WSOC was extracted with distilled water at 1:2 ratio of soil to water and determined using the colorimetric method described by Burford and Bremner (1975). The MBC was determined using the chloroform fumigation-extraction method (Vance et al., 1987).

2.5. The ¹⁵N abundance analysis of soil, plant and extracted solution

The ¹⁵N abundance of NH₄⁺ and NO₃⁻ in the KCl extracts of soil was measured using a diffusion technique followed by direct combustion spectrometry as described by Stark and Hart (1996) and Bedard-Haughn et al. (2004). Briefly, a 5.00 to 40.00 mL aliquot (equal to 100 µg N) of 2 M KCl extract was pipetted into a specimen container. After the addition of 0.2 g MgO powder, an acidified filter paper (7 mm diameter Whatman No. 3 disc acidified with 10 µL of 2.5 M KHSO₄) was hung inside the container using a stainless steel wire. The container was closed immediately and placed at

room temperature for 6 days to complete the diffusion of $^{15}\text{N-NH}_4^+$ onto the filter paper. After taking the filter paper out, the container was left open overnight. At this stage, after addition of 0.4 g Devarda's alloy powder, a new acidified filter paper was hung inside the container for another 6 days to complete the diffusion of $^{15}\text{N-NO}_3^-$. The dried filter paper was then pushed off the wire into a widened 5×8 mm Sn capsule and kept in a microtiter plate before direct combustion. To prepare plant and soil samples for ^{15}N abundance analysis at the end of the experiment, harvested basil plants and the soil samples after KCl extraction and repeated washing with 0.01 M CaCl_2 solution, were dried at 60°C for two days, finely ground (< 150µm) and transferred to 5×8 mm Sn capsules. The ^{15}N abundance in the filter papers, soil and plant tissue samples, were determined with direct combustion using an Isotope Ratio Mass Spectrometer (Sercon Hydra 20-22, Sercon Europa EA-GSL).

2.6. Statistical analysis

All data were statistically analysed by univariate analysis of variance using the IBM SPSS Statistics 23 software package. The differences at $P \leq 0.05$ between treatments using LSD test were considered statistically significant and variables were tested for normality of distribution using Kolmogorov-Smirnov test. Stepwise multiple linear regression analysis was used to identify relationships between daily N_2O emission and different properties of the treatments.

3. Results

3.1. Soil mineral N dynamics

The combined application of N fertiliser and plant residues in all treatments (except SCD) resulted in significantly ($P < 0.05$) higher mineral N ($\text{NH}_4^+ + \text{NO}_3^-$) contents compared with the Urea only treatment in the first 41 days of the experiment (Fig. 2). Generally, soil NO_3^- concentration increased shortly after N fertiliser and residue applications, with the highest concentration observed around two weeks after the start of experiment, and then gradually decreased throughout the plant growing period. There were no significant differences in soil NH_4^+ and NO_3^- concentrations between different residues (sweet corn vs cauliflower). However, surface mulching of both plant residues in the CFS and SCS treatments significantly ($P < 0.05$) decreased NO_3^- concentration when compared with incorporated plant materials in the CFI and SCI treatments in the early stage of decomposition.

261 The application of DMPP to plant residues in CFD and SCD treatments significantly ($P < 0.05$)
 262 reduced soil NO_3^- and fertiliser-induced $^{15}\text{NO}_3^-$ concentrations in the first two weeks of the
 263 experiment, compared with other plant material amended treatments. Although the Urea only
 264 treatment showed high $^{15}\text{NO}_3^-$ concentration at the start of the experiment, after 40 days all
 265 treatments (except SCD) showed significantly ($P < 0.05$) higher $^{15}\text{NO}_3^-$ concentration than the Urea
 266 only treatment. The amendment of DMPP significantly ($P < 0.01$) increased soil NH_4^+ and $^{15}\text{NH}_4^+$
 267 concentrations in the early days of the study when compared with other treatments. The similar
 268 concentration of fertiliser $^{15}\text{NH}_4^+$ in the presence of cauliflower and sweet corn residues implies that
 269 the residue type did not have a significant effect on DMPP efficiency in inhibiting the nitrification of
 270 applied N fertiliser.

271

272 Fig.2. Soil mineral N (a and b) and fertiliser-derived mineral ^{15}N (c and d) concentrations. Vertical
 273 bars are standard error of three replicates. CFI = Incorporated cauliflower residue + ^{15}N -urea; SCI =
 274 Incorporated sweet corn residue + ^{15}N -urea; CFD = Incorporated cauliflower residue + DMPP + ^{15}N -
 275 urea; SCD = Incorporated sweet corn residue + DMPP + ^{15}N -urea; CFS = surface applied cauliflower
 276 residue + ^{15}N -urea; SCS = surface applied sweet corn residue + ^{15}N -urea.

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3.2. Microbial biomass carbon and water soluble organic carbon dynamics

The combined application of N fertiliser and plant residues generally increased MBC concentration compared with the control and Urea only treatments (Fig. 3). The MBC concentrations in cauliflower amended treatments were generally higher than sweet corn treatments during the experimental period, however the differences were not always statistically significant. Surface mulching of residues showed the highest MBC concentrations in the early stage of the study, however the MBC concentrations in CFS and SCS treatments then remained at moderate and relatively steady levels during the rest of the experiment. In contrast, the soil incorporated treatments showed higher MBC concentrations in the second stage of the experiment along with decomposition of applied plant residues.

The surface mulching treatments of both applied residues increased soil WSOC concentration in comparison with the soil incorporated treatments. Generally, the differences in WSOC concentrations between cauliflower and sweet corn treatments were not statistically significant in the early stages of the experiment; however all treatments with sweet corn amendment (SCI, SCD and SCS) had significantly ($P < 0.05$) higher WSOC compared with cauliflower amended treatments (CFI, CFD and CFS) in the second part of the study. The similar dynamic patterns of MBC and WSOC between the Control and Urea only treatments also indicated that application of N fertiliser alone, without amendment of external organic material, did not significantly increase soil microbial activity.

Fig.3. MBC (a) and WSOC (b) concentrations during the experimental period. Vertical bars are standard error of three replicates. CFI = Incorporated cauliflower residue + ¹⁵N-urea; SCI = Incorporated sweet corn residue + ¹⁵N-urea; CFD = Incorporated cauliflower residue + DMPP + ¹⁵N-

urea; SCD = Incorporated sweet corn residue + DMPP + ¹⁵N-urea; CFS = surface applied cauliflower residue + ¹⁵N-urea; SCS = surface applied sweet corn residue + ¹⁵N-urea.

3.3. Daily and cumulative N₂O emissions

The N₂O emissions from all treatments, except the control, reached their highest rate within the first week of the experiment (Fig. 4). The maximum daily N₂O emissions for cauliflower and sweet corn residue applications were observed in the CFD (121.2 µg N₂O-N kg⁻¹ d⁻¹) and SCI (42.5 µg N₂O-N kg⁻¹ d⁻¹) treatments, respectively. Generally, in all treatments except CFS, no substantial emissions occurred after the first two weeks of the experiment. Therefore, moisture fluctuations in the second part of the study only significantly (P < 0.05) increased N₂O emissions from CFS treatment.

Plant residue application in all treatments, except the SCS, significantly (P < 0.05) increased cumulative N₂O emissions when compared with the Urea only treatment (Table 2). In treatments with sweet corn residue, the highest cumulative N₂O emission was observed in the soil incorporated condition (SCI), while the lowest N₂O emission occurred in the surface mulching of the residue (SCS). The DMPP amendment to sweet corn residue (SCD) significantly (P < 0.05) reduced both total and residue-induced N₂O emissions compared with its incorporation only method, but was not able to successfully reduce N₂O emissions to levels lower than the sweet corn surface mulching and Urea only treatments. In contrast, soil incorporation of cauliflower residue (CFI) resulted in the lowest cumulative N₂O emission compared with CFD and CFS treatments. However, the difference between cumulative N₂O emissions in CFI and CFS treatments was not statistically significant in the first phase of the experiment (41 days). The application of DMPP to cauliflower residue (CFD) significantly (P < 0.05) increased both total and residue-induced cumulative N₂O emissions compared with CFI treatment.

The application of cauliflower and sweet corn residues to the investigated vegetable soil had different effects on fertiliser-induced N₂O emissions (Fig. 4d). Soil incorporation and surface mulching of cauliflower residue showed a similar effect on fertiliser-induced N₂O emission, and both treatments emitted significantly (P < 0.05) lower fertiliser-induced N₂O than the CFD treatment. In contrast, incorporation of sweet corn residue resulted in higher fertiliser-induced N₂O emission compared with the SCS and SCD treatments. The surface mulching treatment and DMPP amendment to sweet corn residue showed a similar pattern of fertiliser-induced N₂O emission to the Urea only treatment. Thus, spraying DMPP onto the plant residues before their incorporation to soil did not have a consistent effect on fertiliser-induced N₂O emission. Although DMPP amendment

333 significantly ($P < 0.05$) reduced fertiliser-induced N_2O emission in incorporated sweet corn residue
 334 treatment, to the levels close to the Urea only treatment, its application greatly boosted fertiliser-
 335 induced N_2O emission in cauliflower residue incorporated vegetable soil.

336

337 Fig.4. Daily (a and c) and cumulative (b and d) N_2O emission from different treatments and the
 338 contribution of ¹⁵N fertiliser during the experimental period. Vertical bars are standard error of four
 339 replicates. CFI = Incorporated cauliflower residue + ¹⁵N-urea; SCI = Incorporated sweet corn residue
 340 + ¹⁵N-urea; CFD = Incorporated cauliflower residue + DMPP + ¹⁵N-urea; SCD = Incorporated sweet
 341 corn residue + DMPP + ¹⁵N-urea; CFS = surface applied cauliflower residue + ¹⁵N-urea; SCS =
 342 surface applied sweet corn residue + ¹⁵N-urea.

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350 Table 2: Cumulative N₂O emissions ($\mu\text{g N}_2\text{O-N kg}^{-1}$ dry soil) of treatments during different periods
 351 of the experiment

Treatment	Time periods								
	0 - 41 days			42 - 98 days			0 - 98 days		
	Total	Fertiliser	Crop residue	Total	Fertiliser	Crop residue	Total	Fertiliser	Crop residue
Control	18.8(f)*	-----**	-----	28.9(c)	-----	-----	47.7(f)	-----	-----
Urea Only	94.0(e)	21.7(d)	-----	39.2(b)	0.4(b)	-----	133.2(e)	22.1(d)	-----
CFI	369.6(b)	86.3 (b)	211.0(b)	45.1(b)	0.4(b)	6.0(b)	414.7(b)	86.7(b)	217.0(b)
CFD	521.2(a)	116.9(a)	332.0 (a)	52.4(b)	1.0(b)	12.7(b)	573.6(a)	117.9(a)	344.7(a)
CFS	411.5 (b)	80.4(b)	258.8 (b)	131.1(a)	5.4(a)	87.0(a)	542.6(a)	85.8(b)	345.8(a)
SCI	229.7 (c)	62.7(c)	94.8(c)	49.7(b)	0.8(b)	10.2(b)	279.5(c)	63.4(c)	105.0(c)
SCD	138.4 (d)	17.5 (d)	48.6(d)	53.1(b)	0.5(b)	13.8(b)	191.5(d)	18.0(d)	62.4(d)
SCS	100.6 (e)	23.7(d)	4.6 (e)	50.9(b)	0.2(b)	11.9(b)	151.5(e)	23.9(d)	16.5(e)

352 * The different letters in parentheses within a column indicate significant differences between the
 353 treatments ($P < 0.05$); ** Not determined. CFI = Incorporated cauliflower residue + ¹⁵N-urea; SCI =
 354 Incorporated sweet corn residue + ¹⁵N-urea; CFD = Incorporated cauliflower residue + DMPP + ¹⁵N-
 355 urea; SCD = Incorporated sweet corn residue + DMPP + ¹⁵N-urea; CFS = surface applied cauliflower
 356 residue + ¹⁵N-urea; SCS = surface applied sweet corn residue + ¹⁵N-urea.

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358 3.4. Nitrous oxide emission in relation to soil mineral N, WSOC and MBC

359 Linear regression analysis using soil moisture content (WFPS), air temperature, WSOC, MBC, NO₃⁻-
 360 N and/or NH₄⁺-N concentrations as independent variables accounted for 45-99% of the variability in
 361 daily N₂O emissions across different treatments (Table 3). The results indicated that daily variations
 362 in N₂O emissions from the control treatment were negatively correlated with soil WSOC
 363 concentration. In Urea only and residue applications without DMPP, soil NO₃⁻-N concentration was

the most important factor controlling the magnitude of N₂O emissions. The application of DMPP in CFD and SCD treatments changed the dominant regulating factor of N₂O emissions from soil NO₃⁻-N to NH₄⁺-N concentrations.

Table 3: Regression equations between daily N₂O emission and treatment properties (n=12)

Treatment	Equation	R ²	P
Control	$Y^* = 0.65 - 0.01 (\text{WSOC})^{**}$	0.51	≤ 0.01
Urea Only	$Y = 0.34 + 0.04 (\text{NO}_3^-)$	0.82	≤ 0.01
CFI	$Y = -3.32 + 0.01 (\text{NO}_3^-) + 0.08 (\text{WFPS})$	0.92	≤ 0.01
CFD	$Y = 0.56 + 0.25 (\text{NH}_4^+)$	0.87	≤ 0.01
CFS	$Y = 0.58 + 0.02 (\text{NO}_3^-)$	0.87	≤ 0.01
SCI	$Y = 0.29 + 0.04 (\text{NO}_3^-)$	0.51	≤ 0.01
SCD	$Y = -6.63 + 0.07 (\text{NH}_4^+) + 0.15 (\text{WFPS})$	0.99	≤ 0.01
SCS	$Y = 0.46 + 0.01 (\text{NO}_3^-)$	0.45	≤ 0.05

* Daily N₂O emission (μg N₂O-N kg⁻¹ dry soil d⁻¹); ** NO₃⁻ (mg NO₃⁻-N kg⁻¹ dry soil); NH₄⁺ (mg NH₄⁺-N kg⁻¹ dry soil); WSOC (mg C kg⁻¹ dry soil); MBC (mg C kg⁻¹ dry soil). CFI = Incorporated cauliflower residue + ¹⁵N-urea; SCI = Incorporated sweet corn residue + ¹⁵N-urea; CFD = Incorporated cauliflower residue + DMPP + ¹⁵N-urea; SCD = Incorporated sweet corn residue + DMPP + ¹⁵N-urea; CFS = surface applied cauliflower residue + ¹⁵N-urea; SCS = surface applied sweet corn residue + ¹⁵N-urea.

3.5. The ¹⁵N fertiliser recovery in soil and plants

The application of crop residues by soil incorporation or surface mulching methods generally decreased ¹⁵N recovery by the growing plants (Table 4) compared with the Urea only treatment, although the differences were not always statistically significant. The DMPP amendment to CFD and SCD treatments showed no significant (P > 0.05) effect on ¹⁵N recovery by basil plants at the end of the study, compared to the incorporation without DMPP or surface mulching treatments. In contrast, soil incorporation of plant residues almost doubled ¹⁵N recovery in soil organic N and the application of DMPP further promoted this process by around 5% for both applied residues. In addition, surface

mulching of residues also significantly ($P < 0.05$) increased ^{15}N recovery in soil compared with Urea only treatment.

Plant residue application generally increased fertiliser-induced N_2O emissions (except in SCS and SCD) compared with the Urea only treatment. The results also indicated that DMPP application only reduced fertiliser-induced N_2O emissions when applied to sweet corn residue, while its application to cauliflower residue showed the highest fertiliser-induced N_2O emissions among all applied treatments. Surface mulching of plant residues did not have a significant effect on gaseous ^{15}N losses compared with the Urea only treatment, while soil incorporation of sweet corn and cauliflower residues significantly ($P < 0.05$) reduced total gaseous ^{15}N losses during the study period. The treatments with DMPP amendment also further reduced N fertiliser loss by around 3% and 12% compared with plant residue incorporation without DMPP and surface mulching treatments, respectively.

Table 4: The ^{15}N fertiliser fate in soil, air and plant tissues

Treatment	Fertiliser N uptake by plant (%)	Fertiliser N residue in soil (%)	Fertiliser N loss as N_2O (%)	Non- N_2O fertiliser N loss (%) [*]	Total ^{15}N (%)
Urea Only	60.06 (a)	14.64 (e)	0.04 (d)	25.26 (a)	100
CFI	56.32 (ab)	27.20 (c)	0.17 (b)	16.31 (bc)	100
CFD	51.92 (b)	32.88 (a)	0.23 (a)	14.97 (bc)	100
CFS	57.03 (ab)	18.22 (d)	0.17 (b)	24.58 (a)	100
SCI	54.04 (b)	26.82 (c)	0.12 (c)	19.02 (b)	100
SCD	55.77 (ab)	30.49 (b)	0.04 (d)	13.70 (c)	100
SCS	52.94 (b)	18.52 (d)	0.05 (d)	28.49 (a)	100

^{*} The reported values were not measured directly but estimated by differences between the total amount added and the other measured sources. CFI = Incorporated cauliflower residue + ^{15}N -urea; SCI = Incorporated sweet corn residue + ^{15}N -urea; CFD = Incorporated cauliflower residue + DMPP + ^{15}N -urea; SCD = Incorporated sweet corn residue + DMPP + ^{15}N -urea; CFS = surface applied cauliflower residue + ^{15}N -urea; SCS = surface applied sweet corn residue + ^{15}N -urea.

4. Discussion

4.1. Effect of soil moisture fluctuation on N dynamics and N₂O emission

Many studies have reported that soil moisture content significantly affects the activity of nitrifiers and denitrifiers, by altering the availability of substrates and the rate of exchange of oxygen between soil and the ambient atmosphere (Ludwig et al., 2001; Petersen et al., 2008; Skiba and Smith, 2000). While it was expected that soil moisture fluctuation in the second phase of the current experiment (from day 42 to 98 of the study) would increase mineral N concentration due to stimulating the decomposition process of applied crop residues, such an increase did not materialize in any of the treatments. This observation may be attributed to the balance between N mineralisation and plant N uptake during the late stages of the cropping season. This behaviour may control mineral N bioavailability for microbial activities and consequently regulate N dynamics and N₂O emissions after combined application of mineral N fertiliser and plant residues.

It has been stated by Sposito (1989) that at high soil moisture conditions, the reduction of soil redox potential to optimum levels for the denitrification process and N₂O emissions may only take a few hours. Soil moisture increase/fluctuation in the second phase of the current experiment increased WSOC in all of the treatments but the low concentration and bioavailability of soil mineral N, rather than labile C availability, for microbial activities resulted in insignificant changes in N₂O emission rates (except CFS) in that period of time. The result implies that mineral N limitation, rather than the availability of C sources for microbial activity, probably restricted N₂O emission from the wet soil. Due to the relatively low contribution of ¹⁵N fertiliser in the emitted N₂O after increasing soil moisture contents, the increased N₂O emission in the CFS treatment in this time period would be regulated by the N mineralization process through decomposition of the applied cauliflower residue. The large residue-induced N₂O emission peaks from the CFS, but not SCS, treatment during the late wet and moisture fluctuating period, also imply that surface mulching of high N content plant residues may significantly increase the N₂O emissions after heavy irrigation or rainfall events in the late cropping season. This loss process may take a longer time after application of low N content plant residues and would probably occur in the fallow period after harvest of the cultivated crop.

4.2. Effect of different plant residue application and placement on N₂O emission and N recovery

The applied plant residues resulted in different N₂O emission patterns after their placement in the soil. Different application methods of the high N content cauliflower residue did not show any significant effect on total N₂O emissions during the first 40 days of the experiment, which is consistent with the findings of Nett et al. (2015) who reported similar cumulative N₂O emissions after soil incorporation and surface application of cauliflower residue to a sandy soil. However, surface mulching of cauliflower residue significantly ($P < 0.05$) increased the N₂O emissions, compared with its incorporation method, during the second phase of the study. These observations may reflect the higher activity of soil denitrifying microbial community during decomposition of this plant residue under surface mulching condition and release of more organic C and N compounds in this treatment during the second phase of the experiment. Although surface mulching of cauliflower residue did not increase fertiliser-induced N₂O emissions, this application method enhanced the residue-induced N₂O emissions by 37%, compared with the soil incorporation method, at the end of the experiment. It can be concluded that the combined application of N fertilisers with high N content plant residues, either incorporated or surface mulched, should be avoided during the following cropping season.

In contrast, surface mulching of the low N content sweet corn residue reduced plant residue-induced N₂O emissions, compared with its soil incorporation, in the first 40 days of the experiment; however its effect on the plant residue-induced and total N₂O emissions was small and insignificant afterwards. The lower N₂O emission could be attributed to the effect of this application method on increasing soil WSOC in the early stage of the experiment and potentially increasing the activity of soil microbial community to immobilise more mineral N, which may consequently reduce the risk of large N₂O emissions. Flessa et al. (2002) also suggested that the different decomposition and denitrification behaviours of surface applied plant residues maybe partially regulated by their specific 'indigenous' microflora. By the end of the experiment, the SCS treatment significantly ($P < 0.05$) decreased residue- and fertiliser-induced N₂O emissions by 84% and 62% compared with the SCI treatment, respectively. Therefore, surface mulching of low N content plant residues seems to be a promising method of reducing N₂O emission by minimising their direct contact with applied mineral N fertilisers. Based on these findings, surface mulching of low N content crop residues in combination with mineral N fertiliser applications may be recommended in vegetable fields under intensive irrigation systems, or in high rainfall zones, in order to reduce N₂O emissions.

The findings of this study also suggest that the incorporated plant residues promote immobilisation of available mineral N from applied N fertiliser, which is evident from significantly higher recoveries of applied N fertiliser in the soil organic matter. In contrast, surface mulching of both plant residues reduced the N recovery of the applied N fertiliser in soil and plant tissues by around 9%, compared with their soil incorporation method. Therefore it can be concluded that surface mulching of plant residues significantly ($P < 0.05$) reduced the immobilisation of applied fertiliser N by soil microorganisms.

4.3. Influence of nitrification inhibitor (DMPP) on N dynamics and N₂O emission

The amendment of DMPP successfully reduced total and fertiliser-derived NO₃⁻ with both applied plant residues in the first two weeks of the experiment. After this initial period, DMPP gradually lost its inhibition effect on the nitrification process as evidenced from the significant ($P < 0.05$) increase of the total and fertiliser-derived NO₃⁻ in the cauliflower incorporated treatment between days 13 to 41 of the study. Both applied crop residues showed almost the same magnitudes of total fertiliser- and residue-induced N₂O emissions in this period of time, which is similar to their single incorporation without DMPP application. The short persistence time and low efficacy of DMPP in this experiment could be attributed to its fast biological decomposition (Zerulla et al., 2001) due to the high temperature and low soil water content in the early days of the experiment following its application. This is consistent with the findings of Irigoyen et al. (2003), who reported the half-lives of 18 and 8 days for NH₄⁺ concentration after soil incorporation of DMPP coated fertiliser at 20 °C and 30 °C, respectively. The performance of DMPP in reducing N₂O emission has also been reported to be more efficient in cold and wet conditions (Menendez et al., 2012; Merino et al., 2005).

Despite the significant effect of DMPP in inhibiting the nitrification process, its application did not show a consistent effect on N₂O emissions. Although DMPP significantly ($P < 0.05$) reduced N₂O emissions in the SCD treatment during high emission peaks, its application significantly ($P < 0.05$) increased N₂O emissions in the CFD treatment. The increasing effect of DMPP amendment on N₂O emissions from the cauliflower applied treatment may be attributed to the acidic nature of its saturated solution (ca. pH = 3) which may locally reduce soil pH and consequently increase the N₂O/N₂ production ratio of denitrification (Liu et al., 2010). It may also be partly related to the low efficiency of the DMPP in clay soils due to its positive charge and high sorption capacity to soil clay fractions (Barth et al., 2001) and insufficient concentration of applied DMPP (0.7 mg a.i. kg⁻¹ soil) to this high N content plant residue. The findings of this study suggest that the DMPP's high efficiency

for inhibiting the nitrification process does not always coincide with its efficiency in reducing N₂O emissions from applied plant residues. However, since the effect of DMPP on N mineralisation is still unclear, it is hard to predict its efficacy for reducing N₂O emission from plant residues with different biochemical characteristics.

5. Conclusion

In the present study, the application of high N content cauliflower residue, either incorporated into soil or surface mulched, in combination with N fertiliser increased N₂O emission compared with the Urea only treatment. The low N content sweet corn residue also increased N₂O emission when incorporated with N fertiliser, while it significantly mitigated N₂O emission when mulched on the surface. The amendment of DMPP did not show a consistent effect on N₂O emissions, suggesting that the high efficacy of DMPP for inhibiting the nitrification process does not always coincide with reduction in N₂O emissions. Application of DMPP with sweet corn residue decreased N₂O emission, but significantly increased N₂O emission when applied with cauliflower residue. However, higher concentrations of DMPP may be more efficient in reducing plant residue- and fertiliser-induced N₂O emissions from high N content plant residues. Investigating the effect of plant residues of varying biochemical characteristics and assessing other fertiliser and plant residue management strategies may also improve understanding of these complex systems. The use of DMPP combined with different plant residue application methods needs to be evaluated against its benefits in increasing N use efficiency and crop yield to determine whether the recommended management strategy can be affordably adopted or remains cost-prohibitive.

Acknowledgement

The authors are grateful to Stephen Harper for providing soil and plant materials for this project. We are thankful to Marijke Heenan and colleagues of Chemistry Centre of the Department of Science, Information, Technology and Innovation (DSITI) for their technical support. Special thanks to Phil Moody, Diane Allen and anonymous reviewers for their constructive comments in reviewing an early version of this paper.

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