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Assessment of N_2O emissions from a fertilised vegetable cropping soil under different plant residue management strategies using ^{15}N tracing techniques

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Abstract

Combined application of plant residues and N fertilisers strongly affect soil mineral N dynamics and N_2O 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 ^{15}N fertiliser on N dynamics and N_2O 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 N_2O emissions. Application of plant residues, both on the soil surface or into soil, resulted in net N mineralisation and increased cumulative N_2O emission compared with the application of N fertiliser alone. Soil surface mulching of sweet corn decreased total and residue-induced cumulative N_2O emission compared with the incorporation method, while it showed opposite effect on N_2O emissions from cauliflower residue. The application of DMPP with sweet corn residue reduced total, residue- and fertiliser-induced N_2O emissions; however its application with cauliflower residue did not show any mitigating effect on the N_2O emissions. The residue application methods and the use of DMPP did not significantly affect ^{15}N recovery by the basil plants. In contrast, soil incorporation of these residues doubled the microbial immobilisation of applied ^{15}N into soil organic matter. Linear regression analysis of N_2O emission during the experimental period indicated that in the treatments without DMPP application, soil NO_3^--N concentration was the most important factor in controlling the magnitude of N_2O emissions, while the application of DMPP changed the dominant regulating factor from NO_3^--N to NH_4^+-N concentration.

Keywords:

Crop residue management, Nitrification inhibitor, 3,4-Dimethylpyrazol phosphate (DMPP), Nitrous oxide (N_2O), ^{15}N tracing

1. Introduction

Agricultural lands are the major source of anthropogenic N_2O, 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 N_2O emissions occur through microbial nitrification and denitrification (Pang et al., 2009;

Rezaei Rashti et al., 2015; Scheer et al., 2014). Therefore, reduction in N_2O emissions from these cropping systems could potentially make a significant contribution to the mitigation of global anthropogenic N_2O emissions.

Agricultural activities provide nearly 4 billion metric tons of plant residues per year at the global scale (Lal, 2005). Returning of these residues to cropping lands can sustain soil organic matter and enhance soil fertility by increasing microbial activity and nutrient availability (Ma et al., 2010; Smith et al., 1993) as well as reducing water loss and limiting weed growth. It has been reported in previous studies that residue application may increase or decrease N_2O emission depending on the quantity and quality (nutrient content, biochemical composition and physical features) of the applied residues (Baggs et al., 2003; Chen et al., 2013; Garcia-Ruiz and Baggs, 2007; Rezaei Rashti et al., 2016), while denitrification is considered as the primary source of N_2O emissions in plant residue amended soils (Kong et al., 2017; Li et al., 2016). Vegetable residues can release up to 150 kg N ha^{-1} through mineralization (De Neve and Hofman, 1998), and different management practices of the harvested vegetable residues (such as soil incorporation or surface mulching) may affect N_2O emissions differently in these cropping systems. Generally, plant residues applied as a surface mulch have a slower decomposition rate than soil incorporated residues due to the greater fluctuations in soil moisture content and temperature, lower availability of soil nutrients and limited contact of applied residues with soil in this application method (Schomberg et al., 1994; Thonnissen et al., 2000). Huang et al. (2004) and Zhu et al. (2013) reported that decomposition of soil incorporated plant residues provided more bioavailable carbon and nitrogen sources for soil microbial activities. The enhancement of soil microbial respiration may also facilitate the development of anaerobic micro-sites which favour the denitrification process.

Combined application of plant residues and chemical N fertilisers has been reported to be beneficial in increasing N use efficiency of applied fertilisers (Mohammad et al., 2012). This may occur through enhancing the microbial immobilisation of applied mineral N, in the early days after application, and synchronising soil N dynamics with N demands of the cultivated crop. However, studies by Garcia-Ruiz and Baggs (2007) and Gentile et al. (2008) indicated that soil incorporation of plant residues in combination with N fertilisers may increase N_2O emissions. Carmo et al. (2013) and Wang et al. (2016) also reported increases in N_2O emissions after surface application of sugarcane residue. It has been suggested that mineral N application may increase the decomposition rate of labile carbon compounds in applied plant residues (Jiang et al., 2015). The increase of soluble organic carbon in the presence of high levels of soil mineral N would consequently increase N_2O 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 Ciocalteau 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 ^{13}C-cross-poralization 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 ^{13}C 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).

Table 1: Chemical and biochemical composition of the plant materials determined by chemical 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

2.2. Glasshouse preparation and experimental design

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

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

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

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

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

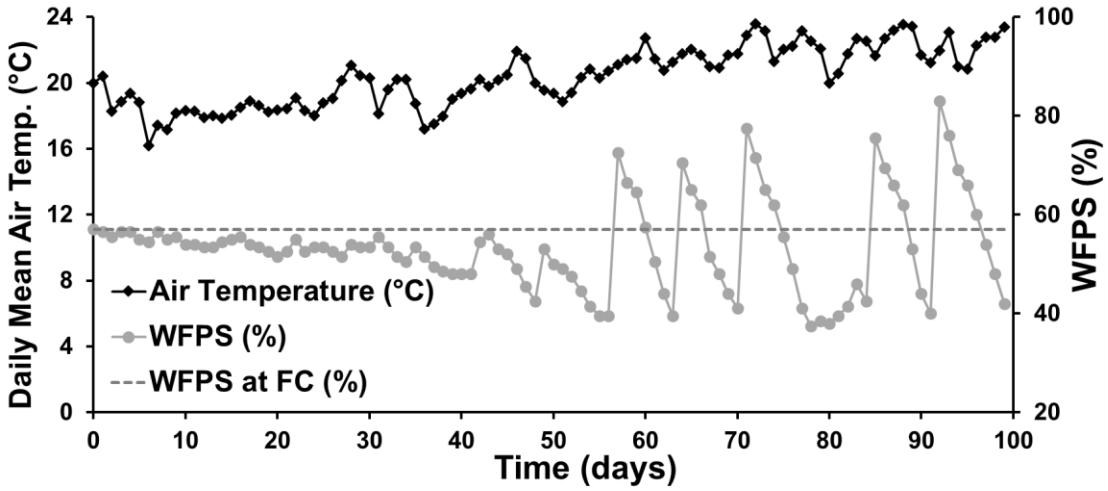

Fig.1. Daily air temperature of the glasshouse, field capacity (FC) of the experimental soil (dash line) and soil moisture fluctuation in water-filled pore space (WFPS) during the study period.

2.3. Measurement of N_2O emissions

Gas sampling was undertaken every 1 to 4 days depending on soil moisture conditions and the expected levels of N_2O emissions. Gas samples were collected from the chambers' headspaces, in four replicates, one hour after closure using a 25 mL gas-tight syringe and immediately transferred to pre-evacuated 12 mL glass vials (Exetainer, Labco Ltd, High Wycombe, UK). The gas samples were analysed for N_2O 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 N_2O emissions were calculated according to the ^{15}N 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 N_2O emissions were estimated from total N_2O 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 (NO_3^- and NH_4^+), water soluble organic C (WSOC) and microbial biomass C (MBC) on all sampling days. The NO_3^- and NH_4^+ 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 Quaatro). 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 ^{15}N abundance analysis of soil, plant and extracted solution

The ^{15}N abundance of NH_4^+ and NO_3^- 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 $KHSO_4$) was hung inside the container using a stainless steel wire. The container was closed immediately and placed at

231 room temperature for 6 days to complete the diffusion of $^{15}N\text{-}NH_4^+$ onto the filter paper. After taking
232 the filter paper out, the container was left open overnight. At this stage, after addition of 0.4 g
233 Devarda's alloy powder, a new acidified filter paper was hung inside the container for another 6 days
234 to complete the diffusion of $^{15}N\text{-}NO_3^-$. The dried filter paper was then pushed off the wire into a
235 widened 5×8 mm Sn capsule and kept in a microtiter plate before direct combustion. To prepare
236 plant and soil samples for ^{15}N abundance analysis at the end of the experiment, harvested basil plants
237 and the soil samples after KCl extraction and repeated washing with 0.01 M $CaCl_2$ solution, were
238 dried at 60°C for two days, finely ground (< 150μm) and transferred to 5×8 mm Sn capsules. The
239 ^{15}N abundance in the filter papers, soil and plant tissue samples, were determined with direct
240 combustion using an Isotope Ratio Mass Spectrometer (Sercon Hydra 20-22, Sercon Europa EA-
241 GSL).

242

243 **2.6. Statistical analysis**

244 All data were statistically analysed by univariate analysis of variance using the IBM SPSS Statistics
245 23 software package. The differences at $P \leq 0.05$ between treatments using LSD test were considered
246 statistically significant and variables were tested for normality of distribution using Kolmogrov-
247 Smirnov test. Stepwise multiple linear regression analysis was used to identify relationships between
248 daily N_2O emission and different properties of the treatments.

249

250 **3. Results**

251 **3.1. Soil mineral N dynamics**

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

The application of DMPP to plant residues in CFD and SCD treatments significantly ($P < 0.05$) reduced soil NO_3^- and fertiliser-induced $^{15}NO_3^-$ concentrations in the first two weeks of the experiment, compared with other plant material amended treatments. Although the Urea only treatment showed high $^{15}NO_3^-$ concentration at the start of the experiment, after 40 days all treatments (except SCD) showed significantly ($P < 0.05$) higher $^{15}NO_3^-$ concentration than the Urea only treatment. The amendment of DMPP significantly ($P < 0.01$) increased soil NH_4^+ and $^{15}NH_4^+$ concentrations in the early days of the study when compared with other treatments. The similar concentration of fertiliser $^{15}NH_4^+$ in the presence of cauliflower and sweet corn residues implies that the residue type did not have a significant effect on DMPP efficiency in inhibiting the nitrification of applied N fertiliser.

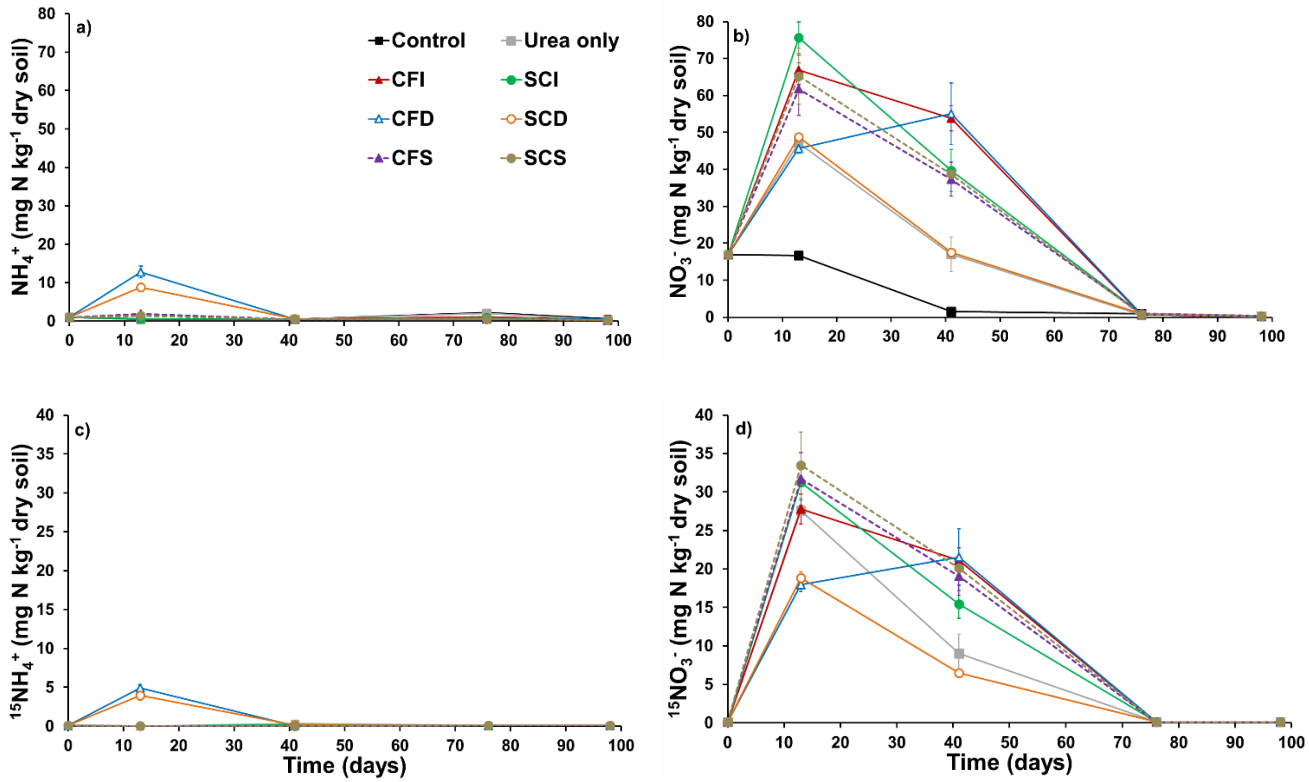

Fig.2. Soil mineral N (a and b) and fertiliser-derived mineral ^{15}N (c and d) concentrations. Vertical bars are standard error of three replicates. CFI = Incorporated cauliflower residue + ^{15}N-urea; SCI = Incorporated sweet corn residue + ^{15}N-urea; CFD = Incorporated cauliflower residue + DMPP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

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.

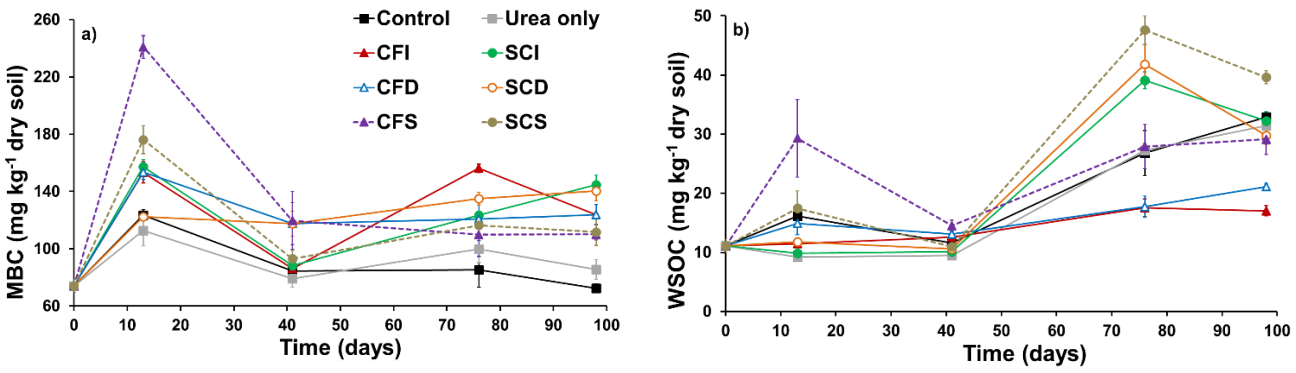

Fig.3. MBC (a) and WSOC (b) concentrations during the experimental period. Vertical bars are standard error of three replicates. CFI = Incorporated cauliflower residue + ^{15}N-urea; SCI = Incorporated sweet corn residue + ^{15}N-urea; CFD = Incorporated cauliflower residue + DMPP + ^{15}N-

301 urea; SCD = Incorporated sweet corn residue + DMPP + ^{15}N-urea; CFS = surface applied cauliflower
302 residue + ^{15}N-urea; SCS = surface applied sweet corn residue + ^{15}N-urea.
303

304 **3.3. Daily and cumulative N_2O emissions**

305 The N_2O emissions from all treatments, except the control, reached their highest rate within the first
306 week of the experiment (Fig. 4). The maximum daily N_2O emissions for cauliflower and sweet corn
307 residue applications were observed in the CFD (121.2 μg N_2O-N kg^{-1} d^{-1}) and SCI (42.5 μg N_2O-N
308 kg^{-1} d^{-1}) treatments, respectively. Generally, in all treatments except CFS, no substantial emissions
309 occurred after the first two weeks of the experiment. Therefore, moisture fluctuations in the second
310 part of the study only significantly ($P< 0.05$) increased N_2O emissions from CFS treatment.

311 Plant residue application in all treatments, except the SCS, significantly ($P< 0.05$) increased
312 cumulative N_2O emissions when compared with the Urea only treatment (Table 2). In treatments
313 with sweet corn residue, the highest cumulative N_2O emission was observed in the soil incorporated
314 condition (SCI), while the lowest N_2O emission occurred in the surface mulching of the residue
315 (SCS). The DMPP amendment to sweet corn residue (SCD) significantly ($P< 0.05$) reduced both
316 total and residue-induced N_2O emissions compared with its incorporation only method, but was not
317 able to successfully reduce N_2O emissions to levels lower than the sweet corn surface mulching and
318 Urea only treatments. In contrast, soil incorporation of cauliflower residue (CFI) resulted in the
319 lowest cumulative N_2O emission compared with CFD and CFS treatments. However, the difference
320 between cumulative N_2O emissions in CFI and CFS treatments was not statistically significant in the
321 first phase of the experiment (41 days). The application of DMPP to cauliflower residue (CFD)
322 significantly ($P< 0.05$) increased both total and residue-induced cumulative N_2O emissions compared
323 with CFI treatment.

324 The application of cauliflower and sweet corn residues to the investigated vegetable soil had
325 different effects on fertiliser-induced N_2O emissions (Fig. 4d). Soil incorporation and surface
326 mulching of cauliflower residue showed a similar effect on fertiliser-induced N_2O emission, and both
327 treatments emitted significantly ($P< 0.05$) lower fertiliser-induced N_2O than the CFD treatment. In
328 contrast, incorporation of sweet corn residue resulted in higher fertiliser-induced N_2O emission
329 compared with the SCS and SCD treatments. The surface mulching treatment and DMPP amendment
330 to sweet corn residue showed a similar pattern of fertiliser-induced N_2O emission to the Urea only
331 treatment. Thus, spraying DMPP onto the plant residues before their incorporation to soil did not
332 have a consistent effect on fertiliser-induced N_2O emission. Although DMPP amendment

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

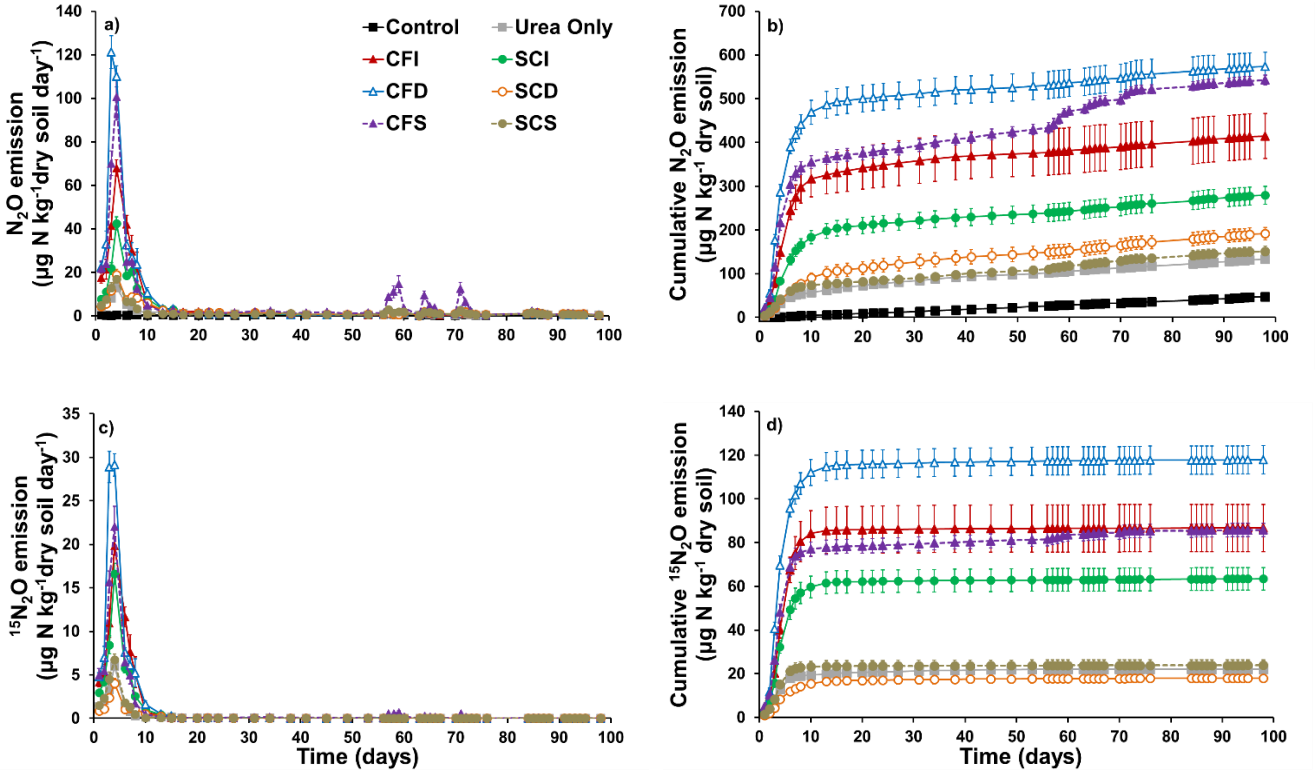

Fig.4. Daily (a and c) and cumulative (b and d) N_2O emission from different treatments and the contribution of ^{15}N fertiliser during the experimental period. Vertical bars are standard error of four replicates. CFI = Incorporated cauliflower residue + ^{15}N-urea; SCI = Incorporated sweet corn residue + ^{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.

Table 2: Cumulative N_2O emissions (μg N_2O-N kg^{-1} dry soil) of treatments during different periods 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)

* The different letters in parentheses within a column indicate significant differences between the treatments ($P< 0.05$); ** Not determined. 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.

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

Linear regression analysis using soil moisture content (WFPS), air temperature, WSOC, MBC, NO_3^--N and/or NH_4^+-N concentrations as independent variables accounted for 45-99% of the variability in daily N_2O emissions across different treatments (Table 3). The results indicated that daily variations in N_2O emissions from the control treatment were negatively correlated with soil WSOC concentration. In Urea only and residue applications without DMPP, soil NO_3^--N concentration was

364 the most important factor controlling the magnitude of N_2O emissions. The application of DMPP in
365 CFD and SCD treatments changed the dominant regulating factor of N_2O emissions from soil NO_3^--
366 N to NH_4^+-N concentrations.

367

368 Table 3: Regression equations between daily N_2O emission and treatment properties (n=12)

Treatment	Equation	R^2	P
Control	$Y^* = 0.65 – 0.01\ (WSOC)^{**}$	0.51	≤ 0.01
Urea Only	$Y = 0.34 + 0.04\ (NO_3^-)$	0.82	≤ 0.01
CFI	$Y = -3.32 + 0.01\ (NO_3^-) + 0.08\ (WFPS)$	0.92	≤ 0.01
CFD	$Y = 0.56 + 0.25\ (NH_4^+)$	0.87	≤ 0.01
CFS	$Y = 0.58 + 0.02\ (NO_3^-)$	0.87	≤ 0.01
SCI	$Y = 0.29 + 0.04\ (NO_3^-)$	0.51	≤ 0.01
SCD	$Y = -6.63 + 0.07\ (NH_4^+) + 0.15\ (WFPS)$	0.99	≤ 0.01
SCS	$Y = 0.46 + 0.01\ (NO_3^-)$	0.45	≤ 0.05

369 * Daily N_2O emission (μg N_2O-N kg^{-1} dry soil d^{-1}); ** NO_3^- (mg NO_3^--N kg^{-1} dry soil); NH_4^+ (mg
370 NH_4^+-N kg^{-1} dry soil); WSOC (mg C kg^{-1} dry soil); MBC (mg C kg^{-1} dry soil). CFI = Incorporated
371 cauliflower residue + ^{15}N-urea; SCI = Incorporated sweet corn residue + ^{15}N-urea; CFD =
372 Incorporated cauliflower residue + DMPP + ^{15}N-urea; SCD = Incorporated sweet corn residue +
373 DMPP + ^{15}N-urea; CFS = surface applied cauliflower residue + ^{15}N-urea; SCS = surface applied
374 sweet corn residue + ^{15}N-urea.

375

376 **3.5. The ^{15}N fertiliser recovery in soil and plants**

377 The application of crop residues by soil incorporation or surface mulching methods generally
378 decreased ^{15}N recovery by the growing plants (Table 4) compared with the Urea only treatment,
379 although the differences were not always statistically significant. The DMPP amendment to CFD and
380 SCD treatments showed no significant (P> 0.05) effect on ^{15}N recovery by basil plants at the end of
381 the study, compared to the incorporation without DMPP or surface mulching treatments. In contrast,
382 soil incorporation of plant residues almost doubled ^{15}N recovery in soil organic N and the application
383 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 N_2O 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 N_2O 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 N_2O 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 N_2O 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 N_2O emission from the wet soil. Due to the relatively low contribution of ^{15}N fertiliser in the emitted N_2O after increasing soil moisture contents, the increased N_2O 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 N_2O 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 N_2O 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 N_2O emission and N recovery

The applied plant residues resulted in different N_2O 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 N_2O emissions during the first 40 days of the experiment, which is consistent with the findings of Nett et al. (2015) who reported similar cumulative N_2O 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 N_2O 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 N_2O emissions, this application method enhanced the residue-induced N_2O 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 N_2O emissions, compared with its soil incorporation, in the first 40 days of the experiment; however its effect on the plant residue-induced and total N_2O emissions was small and insignificant afterwards. The lower N_2O 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 N_2O 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 N_2O 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 N_2O 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 N_2O 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 N_2O emission

The amendment of DMPP successfully reduced total and fertiliser-derived NO_3^- 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 NO_3^- 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 N_2O 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 NH_4^+ concentration after soil incorporation of DMPP coated fertiliser at 20 °C and 30 °C, respectively. The performance of DMPP in reducing N_2O 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 N_2O emissions. Although DMPP significantly ($P< 0.05$) reduced N_2O emissions in the SCD treatment during high emission peaks, its application significantly ($P< 0.05$) increased N_2O emissions in the CFD treatment. The increasing effect of DMPP amendment on N_2O 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 N_2O/N_2 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 N_2O 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 N_2O 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 N_2O emission compared with the Urea only treatment. The low N content sweet corn residue also increased N_2O emission when incorporated with N fertiliser, while it significantly mitigated N_2O emission when mulched on the surface. The amendment of DMPP did not show a consistent effect on N_2O emissions, suggesting that the high efficacy of DMPP for inhibiting the nitrification process does not always coincide with reduction in N_2O emissions. Application of DMPP with sweet corn residue decreased N_2O emission, but significantly increased N_2O emission when applied with cauliflower residue. However, higher concentrations of DMPP may be more efficient in reducing plant residue- and fertiliser-induced N_2O 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.

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