



Linking chemical and biochemical composition of plant materials to their effects on N₂O emissions from a vegetable soil



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ABSTRACT

The magnitudes of nitrogen (N) mineralisation and nitrous oxide (N₂O) emissions after the application of plant materials strongly depend on their quality. Despite the existence of some studies in this field, little is known about the underlying mechanisms and regulating factors of these processes, particularly for vegetable cropping systems. In this study, ten typical vegetable and/or vegetable farming rotation plant materials were finely ground, incorporated into the soil and incubated at 25 °C under fluctuating moisture conditions of 55–85% water-filled pore space (WFPS) without N (-N) or with N (+N) addition (100 mg N kg⁻¹ soil as urea). The applied plant materials were characterised using solid state ¹³C nuclear magnetic resonance (NMR) spectroscopy and wet-chemical analysis. The dynamics of soil mineral N accumulation and N₂O emissions were monitored over 169 days. Under the -N treatment, plant materials with total N (TN) contents ≥ 27 mg g⁻¹ dry matter produced significantly higher cumulative N₂O emissions than those with TN contents < 27 mg g⁻¹ in the first 105 days of incubation. However, there was no significant difference in cumulative N₂O emissions between these two groups at the end of the experiment due to higher N₂O emissions for plant materials with TN contents < 27 mg g⁻¹ during the later stage of the incubation. Under the +N treatment, application of plant materials consistently increased the cumulative N₂O emissions by the end of the incubation compared with the urea only treatment; although a few plant materials resulted in lower or similar N₂O emissions in the initial 2–4 weeks. During the entire incubation, plant materials with high TN contents generally produced higher cumulative N₂O emissions than others in the +N treatment. Stepwise regression analysis indicated a significant correlation between cumulative N₂O emissions and TN, cellulose, lignin, O-aryl C and carbonyl C contents of the plant materials; TN content was the main regulating factor among all chemical and biochemical indices.

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1. Introduction

Nitrous oxide (N₂O) is one of the major greenhouse gases with potent and long-lived global warming effects (298 times higher

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than carbon dioxide (CO₂) over a time period of 100 years), which effectively absorb infrared radiation (IPCC, 2013). The production of N₂O in agricultural soils is primarily derived through the microbial processes of nitrification and denitrification, which are regulated by multiple factors including soil aeration, the supply of mineral nitrogen (N) and labile organic carbon (C) (Firestone and Davidson, 1989).

Plant materials are used as amendments to agricultural soils to increase organic matter content, improve soil physical properties, and increase the availability of essential nutrients for crop production (Smith et al., 1993; Wang et al., 2004b; Wyland et al., 1995).

This management practice has been extensively examined, since the early 1990s, to assess its impact on soil N₂O emissions (Rees and Ball, 2010). Previous investigations suggested that the incorporation of plant materials (particularly those with high N contents) in soil increases the abundance and activity of soil denitrifying bacteria and consequently this may enhance N₂O emissions through denitrification (Henderson et al., 2010; Huang et al., 2004; Potthoff et al., 2005). In intensively managed vegetable cropping, with high soil mineral N concentrations and frequent irrigation, the beneficial effect of plant material incorporation may be offset by an increase in N₂O emissions (Zhu et al., 2013).

The magnitude of N₂O emissions after plant material application strongly depends on the quantity and quality of the applied residues (Baggs et al., 2000), as well as field management practices and climatic conditions. There are different approaches to predict N availability and N₂O emissions from plant materials. Some researchers rely on total N content and C:N ratio of plant materials (Chen et al., 2013; Li et al., 2013; Velthof et al., 2003) while others assume a significant role for the contents of soluble C and N (Coglee et al., 1989), cellulose (Hadas et al., 2004), lignin (Gentile et al., 2008; Zhu et al., 2013) and the protein binding capacity of extractable polyphenols (Millar and Baggs, 2004; Zhu et al., 2013) in applied plant materials. It has also been suggested that the decomposition of plant materials as well as their C and N mineralisation rates can be interactively influenced by their initial N concentration and organic C components determined by NMR spectroscopy (Cheshire and Chapman, 1996; Almendros et al., 2000). Wang et al. (2004) found that C mineralisation rates of plant materials strongly relate to their carbonyl, aryl and O-aryl C as well as initial N contents. However, the time point of shift between main controlling factors of C mineralisation during the decomposition process varied for each specific plant material depending on the relative sufficiency of biologically available N. Despite all the information available, it is still difficult to predict the fate of N from plant materials that differ substantially in their chemical composition, after amendment to agricultural soils.

The relatively slow mineralisation of N from crop residue decomposition is usually insufficient to meet the N demands of vegetable crops with short growing seasons. The combined application of plant materials with inorganic N fertiliser may be beneficial. This management approach could potentially improve the N use efficiency of the system by optimising the synchronisation of soil N availability and crop demand during the growing season. On the other hand this practice could increase or decrease soil N₂O emissions depending on the chemical characteristics of both N sources and the ratio of inorganic to organic N (García-Ruiz and Baggs, 2007).

Accurate estimation of N mineralisation and N₂O emission following the combined incorporation of plant materials and N fertilisers into soil is essential for establishing efficient mitigation strategies to reduce N₂O emissions. Although beneficial interactive effects of combining these N sources on mitigating N₂O emissions have been observed in previous investigations (García-Ruiz and Baggs, 2007; Gentile et al., 2008), little is known about the underlying mechanisms and regulating factors of these interactions, particularly in vegetable cropping systems. The main objectives of this study were to use a controlled incubation experiment to: (1) investigate the dynamics of soil mineral N, microbial biomass C (MBC), water soluble organic C (WSOC), and N₂O emissions, following the incorporation of 10 different vegetable plant materials; (2) determine the effects of the combined application of plant materials with inorganic N fertiliser on mineral N dynamics and N₂O production; and (3) assess the effect of the main regulating factors of N₂O emission, including the ¹³C NMR functional groups of applied plant materials both individually and in combination with

N fertiliser. The underlying hypothesis was that the effects of vegetable plant materials on N₂O emissions would interactively depend on their chemical and biochemical composition, soil mineral N availability and soil moisture levels.

2. Materials and methods

2.1. Plant materials and biochemical analysis

Ten different types of plant residues (aboveground) were selected for this study, including eight typical vegetable crop residues: namely, zucchini (*Cucurbita pepo* var. *Medullosa* Alef.), capsicum (*Capsicum annuum* L.), broccoli (*Brassica oleracea* var. *Italica* plenck), green bean (*Phaseolus vulgaris* L.), potato (*Solanum tuberosum* L.), carrot (*Daucus carota* subsp. *Sativus* arcang), eggplant (*Solanum melongena* L.) and sweet corn (*Zea mays* L.), along with two common vegetable farming rotation crop residues, sorghum (*Sorghum bicolor* L.) and lablab (*Lablab purpureus* L.). The plant residues had substantial differences in their C:N ratio and biochemical composition (Table 1). All residues were dried at 60 °C for two days and then ground to <1 mm before incubation and biochemical analysis.

Total C (TC) and N (TN) contents of plant materials were determined by dry combustion using a LECO CN analyser (TruMac NO. 830-300-400). Lignin and cellulose contents were determined sequentially using the acid detergent pre-treatment method (Wang et al., 2004a). Residue samples were extracted with an acid detergent solution (20 g cetyltrimethylammonium bromide in 0.5 M sulphuric acid) after being placed in a 100 °C water bath for 1 h and then centrifuged at 2500 rpm for 3 min. The residues were then rinsed with hot water followed by acetone, dried in the oven at 60 °C overnight and weighed to obtain the acid detergent fibre (ADF) content. Lignin content was determined by further extracting residues with 72% sulphuric acid for 3 h and were corrected for ash content. Cellulose content was calculated as the difference between ADF and the sum of lignin + ash contents of samples. Total polyphenol content in residue samples was determined using 50% methanol as the extractant followed by measurement using the Folin Ciocalteu colorimetric method calibrated with gallic acid (Waterman and Mole, 1994). The results were 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) spectra 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. For each sample, 1600 transients were collected and a Lorentzian line broadening function of 20 Hz applied to all spectra. 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 (45–60 ppm), O-alkyl C (60–95 ppm), O₂-alkyl C (95–110 ppm), aryl C (110–145 ppm), O-aryl C (145–165 ppm), and carbonyl C (165–210 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).

2.2. Incubation

Fresh soil with an initial water content of 15% (w/w) was

Table 1
Selected chemical and biochemical composition of the plant materials determined by chemical methods and ^{13}C 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	Cellulose	Polyphenol	Alkyl	N-alkyl/methoxyl	O-alkyl	O ₂ -alkyl	Aryl	O-aryl	Carbonyl
Zucchini	303	36	8	37	164	4	65	25	117	24	16	7	49
Capsicum	395	37	11	52	160	11	85	38	148	31	24	13	56
Broccoli	386	31	12	167	40	7	79	30	156	35	16	8	62
Green Bean	391	27	14	197	69	8	70	34	169	36	22	10	50
Potato	367	20	19	141	86	7	89	32	137	29	22	10	48
Carrot	398	20	20	36	153	11	88	32	158	31	22	10	57
Lablab	430	19	22	246	81	9	60	35	209	46	23	12	45
Sorghum	423	12	35	69	277	6	51	28	215	53	27	14	35
Egg Plant	444	9	48	323	167	9	55	35	226	51	27	15	35
Sweet Corn	450	9	51	49	326	6	49	30	239	57	28	15	32

collected from the 0–10 cm depth of a cultivated vegetable field at the Department of Agriculture and Fisheries Gatton Research Facility ($27^{\circ} 32' 39''$ S, $152^{\circ} 19' 38''$ E), Queensland, Australia. The black Vertisol contained 35% sand, 24% silt and 41% clay with an initial pH (1:5 water) of 7.7 and a water holding capacity of 530 g kg^{-1} . Total organic C and N contents were 15.4 and 1.1 g kg^{-1} , respectively.

The plant materials (1.16 g) were mixed with fresh soil (150 g oven-dry equivalent) and transferred to 250 mL flat end polypropylene jars (each jar had three small holes in the lid to allow air circulation) where the soil was pressed gently to a bulk density of 1.2 g cm^{-3} (field bulk density). Each plant material received two treatments with 20 replicates: (i) +N with 100 mg N kg^{-1} soil as urea solution (120 kg N ha^{-1} equivalent); and (ii) -N with nil N application. The same N applications (+N or -N) without the addition of plant material were used as urea only and control treatments. The incubation was carried out in the dark at $25 \pm 0.5^{\circ}\text{C}$. The soil-plant material mixture was moistened to 55% WFPS with distilled water for -N treatments and with urea solution for +N treatments and incubated at this moisture content for the first 28 days of the experiment. To investigate the effect of a wetting-drying cycle on plant material decomposition and N_2O emission, the soil moisture was raised to 85% WFPS for ten days and then gradually reduced to 55% WFPS by day 50. From day 105, the moisture was raised again to 85% WFPS and maintained until the end of the experiment (Fig. 1) at day 169. The moisture content of each jar was adjusted by adding distilled water according to weight loss every two days throughout the experiment apart from the period between 38 and 50 days.

2.3. Measurement of N_2O fluxes

Gas sampling was undertaken every 2–6 days depending on soil moisture conditions and the expected levels of N_2O emissions. At

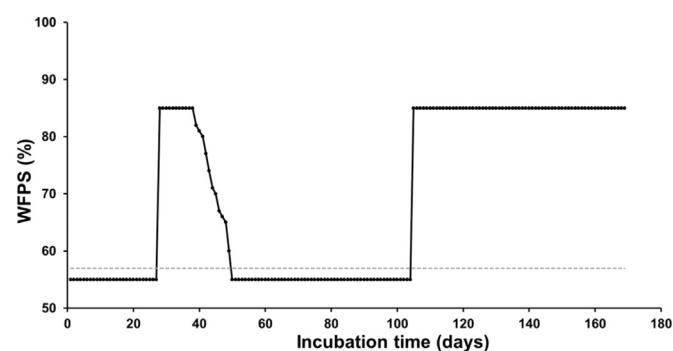


Fig. 1. Field capacity of the experimental soil (dash line) and soil moisture fluctuation during the incubation period.

each gas sampling, four replicates of each treatment were randomly selected and placed into 2 L airtight glass jars that were then flushed with ambient compressed air for one minute before closure. Gas samples were collected from the headspace of the glass jars eight hours 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). 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 all treatments over a subset of sampling times during the incubation. These samples were taken initially after closure of the glass jars and then every 60 mins for 10 hrs. Nitrous oxide emissions showed a linear trend over 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 emission measurements (Wang et al., 2011).

2.4. Soil sampling and analysis

Soil samples were taken at 8, 17, 28, 36, 52, 79, 105, 119 and 169 days after initiation of incubation. Two replicates of each treatment were randomly selected and destructively sampled for soil mineral N (NO_3^- and NH_4^+) on all sampling days, while WSOC was analysed at days 17, 28, 105 and 169 and MBC at days 28, 105 and 169.

Soil mineral N concentrations were determined using colorimetric techniques (Rayment and Lyons, 2011) on fresh soil samples extracted with 2 M KCl at a 1:5 ratio of soil to extractant. The WSOC was determined using a potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) oxidation method described by Burford and Bremner (1975). Briefly, fresh soil samples were extracted with de-ionised water at 1:2 ratio of soil to extractant. The extractant was then filtered through a non-pyrogenic $0.45 \mu\text{m}$ syringe filter. The solution was then digested in a $\text{K}_2\text{Cr}_2\text{O}_7$ and sulfuric acid (H_2SO_4) mixture followed by titration with 33.3 mM ferrous ammonium sulphate, using 1,10-phenanthroline-ferrous sulphate complex solution as an indicator. The MBC was determined using the chloroform fumigation-extraction method (Vance et al., 1987). Both non-fumigated and chloroform fumigated fresh soil samples were extracted with 0.5 M potassium sulphate. The MBC was calculated as the difference in soluble OC (determined with $\text{K}_2\text{Cr}_2\text{O}_7$ digestion) between non-fumigated and chloroform fumigated samples, multiplied by a correction factor of 2.64. All results were expressed on an oven-dry basis.

2.5. Data processing and statistical analysis

The net cumulative N_2O emissions in the current experiment were calculated as the differences in cumulative emissions

between plant material amended treatments and the Control or the Urea only treatment in the -N and +N treatments, respectively. The relative N_2O emission to total N application in the -N treatments was calculated as the difference in emissions between the plant material amended treatments and the Control divided by the TN content of the applied plant material. Since this paper mainly focuses on the effects of chemical composition of applied plant materials on N_2O emissions (not the chemical fertiliser application), the relative N_2O emission was not calculated for the +N treatments.

All data were statistically analysed by univariate analysis of variance using the SPSS (PASW) 19 software package (SPSS Inc., USA). Differences at $P \leq 0.05$ were considered statistically significant and variables were tested for normality of distribution using the Kolmogorov-Smirnov test. Stepwise multiple linear regression analysis was used to identify relationships between N_2O emission and chemical/biochemical composition of plant materials.

3. Results

3.1. Mineral N dynamics

Different plant materials resulted in different mineral N dynamics (Fig. 2). The application of zucchini, capsicum, broccoli and green bean residues showed significant net N mineralization ($P < 0.05$) under both soil N limiting and non-limiting conditions throughout the incubation period. In contrast, sorghum, eggplant and sweet corn residues exhibited a significant net N immobilization ($P < 0.05$) in the first 105 days of both N treatments. However, by the end of the incubation period the difference between these treatments and the control was not significant. The -N treatments of potato, carrot and lablab did not significantly influence mineral N concentration in the first 28 days of incubation, but showed significant N mineralization for the rest of the incubation period. Finally, the +N treatment of potato residue did not affect soil

mineral N concentration in the first 28 days but significant mineral N was released thereafter. Under the same condition, carrot and lablab decreased mineral N concentration in the early stages of decomposition (first 28 days), but significantly increased it by the end of the incubation period (after 105 days). Overall, in both the +N and -N treatments, eggplant and sweet corn residues resulted in net N immobilization/loss, while amendment with other plant materials resulted in net N mineralization. On average, during the 169 days of incubation, total mineral N concentration in the +N treatments increased by 149, 143, 98, 96, 20, 46, 50 and 13 $mg\ kg^{-1}$ in zucchini, capsicum, broccoli, green bean, potato, carrot, lablab and sorghum residues, respectively, but decreased by 8 and 1 $mg\ kg^{-1}$ respectively for eggplant and sweet corn residues compared with the urea only treatment.

3.2. Microbial biomass carbon and water soluble organic carbon dynamics

Plant material amendment significantly ($P < 0.05$) increased MBC compared with the control and urea only treatments but the increases diminished for broccoli in the -N and for capsicum, sorghum and sweet corn in the +N treatments by the end of the incubation (Fig. 3). The MBC decreased significantly ($P < 0.01$) from day 28 onward in all treatments. There were no significant differences in MBC between -N and +N treatments during the incubation. After 169 days of incubation, the MBC was positively correlated ($P < 0.05$) with Aryl C content of the plant materials.

There were no significant changes in WSOC concentrations over time during the incubation for all treatments. Plant material amendments significantly ($P < 0.05$) increased WSOC compared with the control in the first 105 days of the experimental period, except in zucchini -N treatment and in zucchini and sorghum +N treatments (Fig. 3c and d). Fertiliser application significantly ($P < 0.05$) reduced WSOC concentrations in the urea only treatment

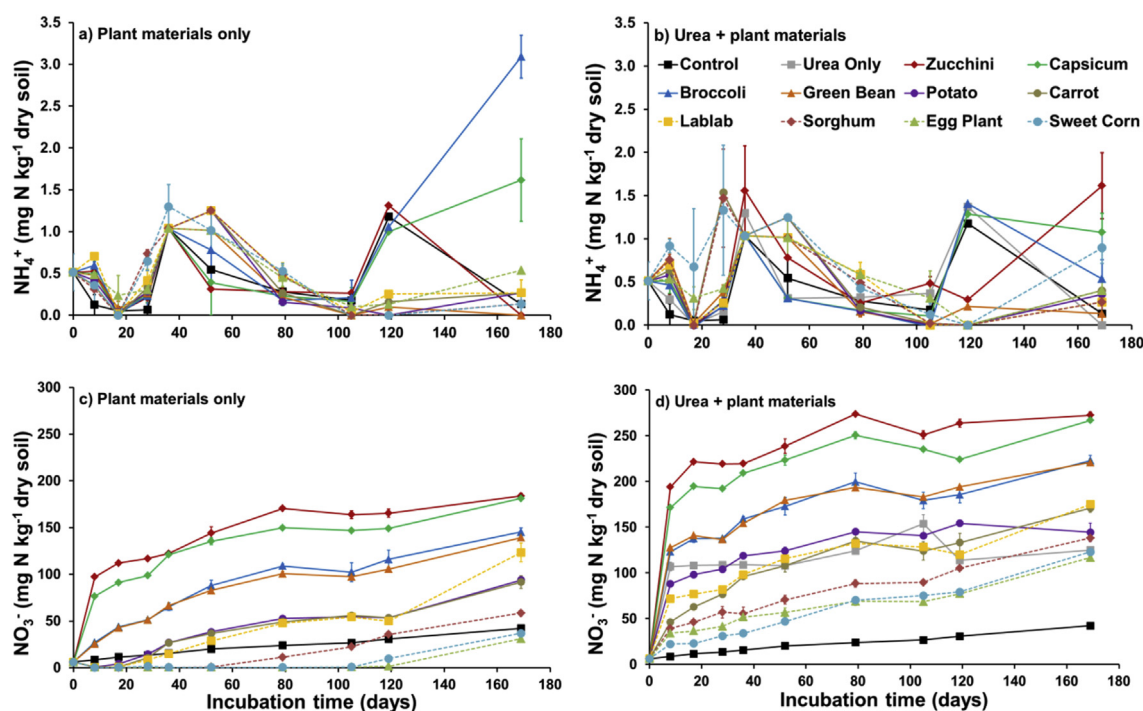


Fig. 2. Mineral N concentrations in soil amended with plant material only (a and c) and plant material with urea (b and d) during the incubation. Vertical bars are standard error of two replicates.

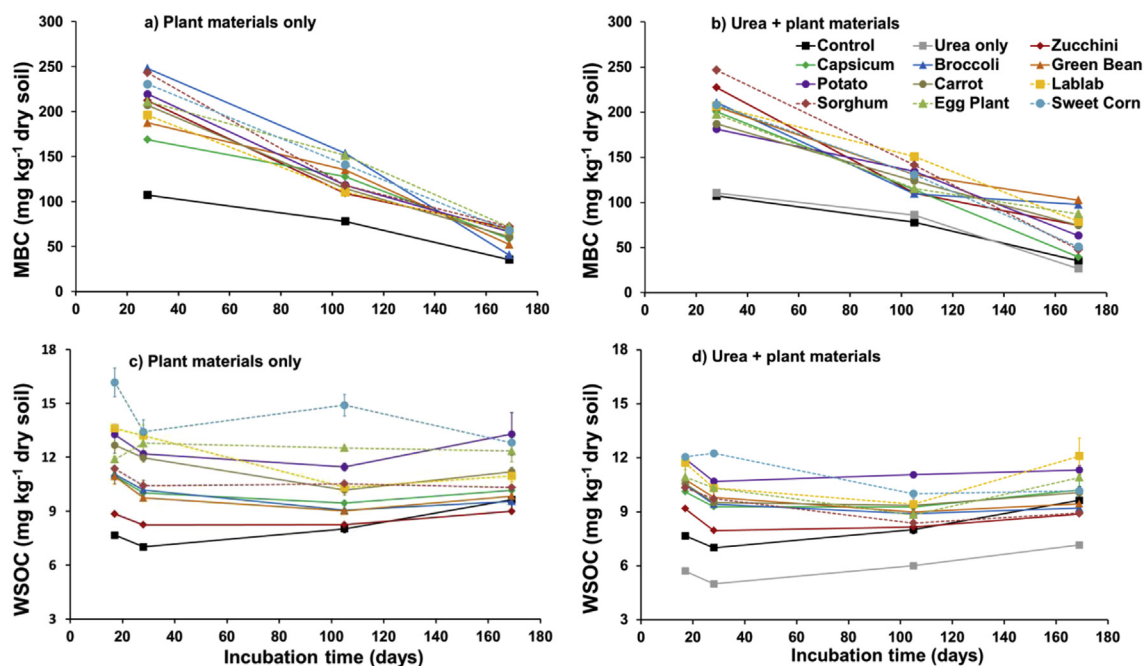


Fig. 3. Microbial biomass C (MBC) and water soluble organic carbon (WSOC) in soil amended with plant material only (a and c) and plant material with urea (b and d) during the incubation. Vertical bars are standard error of two replicates.

compared with the control. The WSOC showed significant ($P < 0.05$) positive correlation with plant material TC and C:N ratios and negative correlation with their carbonyl C content in the first 105 days, but only displayed significant ($P < 0.05$) positive correlation with plant material C:N ratios at the end of incubation.

3.3. Effects of plant material application on N₂O emissions under unfertilised condition

In the -N treatments, the highest daily N₂O emission appeared at different stages for different plant materials (Fig. 4a). While N₂O emissions in the zucchini, capsicum, green bean and lablab treatments reached their highest levels within the first 7 days of incubation, the broccoli, potato and carrot treatments showed maximum N₂O fluxes between days 30–45 following the first increases in soil water content to 85% WFPS, and the sorghum, eggplant and sweet corn treatments reached their highest emissions between 110 and 125 days following the second increases in soil water content to 85% WFPS. The maximum N₂O fluxes in the -N treatments appeared later with decreasing TN content and increasing (Aryl + O-Aryl)/Carbonyl ratios of plant materials.

Applying plant materials increased cumulative N₂O emissions ($P < 0.05$) compared with the control in all -N treatments during the initial 28 days (Fig. 4c and Table 2). The cumulative N₂O emissions also tended to be higher for the plant-amended treatments than the control during the first wet period (28–50 days, except capsicum), the second dry period (50–105 days) and the second wet period (105–169 days, except zucchini) although the differences were not always statistically significant at $P < 0.05$ (Table 2). As a result, addition of plant materials resulted in positive net cumulative N₂O emissions in all treatments (Fig. 4e). During the first 105 days the plant materials with TN contents ≥ 27 mg g⁻¹ dry matter produced higher cumulative N₂O emissions ($P < 0.05$) than other residues. However, during the late stage of the incubation the plant materials with lower TN contents resulted in higher or similar N₂O emissions than those with TN contents ≥ 27 mg g⁻¹. Consequently, at the end of the experiment there was no significant

difference in cumulative N₂O emissions between these two groups of plant materials.

3.4. Effects of plant material application on N₂O emissions under fertilised condition

In the +N treatments, the highest daily N₂O emissions occurred within the first 7 days of incubation for all plant materials except carrot for which N₂O emission peaked at day 30 immediately after the start of the first wet period (Fig. 4b). The application of plant materials plus inorganic N fertiliser (+N) resulted in constantly higher cumulative N₂O emissions than that in the plant material only treatments (-N) and the control during the incubation (Fig. 4c and d). The +N treatments also generally increased cumulative N₂O emissions compared with the urea only treatment during the early weeks (Fig. 4d and f). However, addition of sorghum, eggplant and sweet corn residues had minimal impact and carrot residue addition significantly reduced N₂O emissions ($P < 0.05$) compared with the urea only treatment during this period. At the end of the 169 day incubation, plant materials with high TN contents generally produced higher cumulative N₂O emissions than others in the +N treatment. However, higher cumulative N₂O emissions were observed for the plant materials with low TN contents than those with high TN contents during the wet 105–169 days of the incubation (Table 3).

3.5. Relative N₂O emission to total N application

The maximum N₂O emission per kg of plant material N in the -N treatments was recorded in the sweet corn treatment (95.4 mg N₂O-N kg⁻¹ N added d⁻¹) at day 113 of the experimental period (Fig. 5). In contrast to the widely accepted concept that N₂O emission from plant materials with low N contents should be affected by N limitation, the results of this study showed that plant materials with TN contents ≤ 12 mg g⁻¹ emitted a higher percentage of their N as N₂O compared with other plant materials which contained higher N contents.

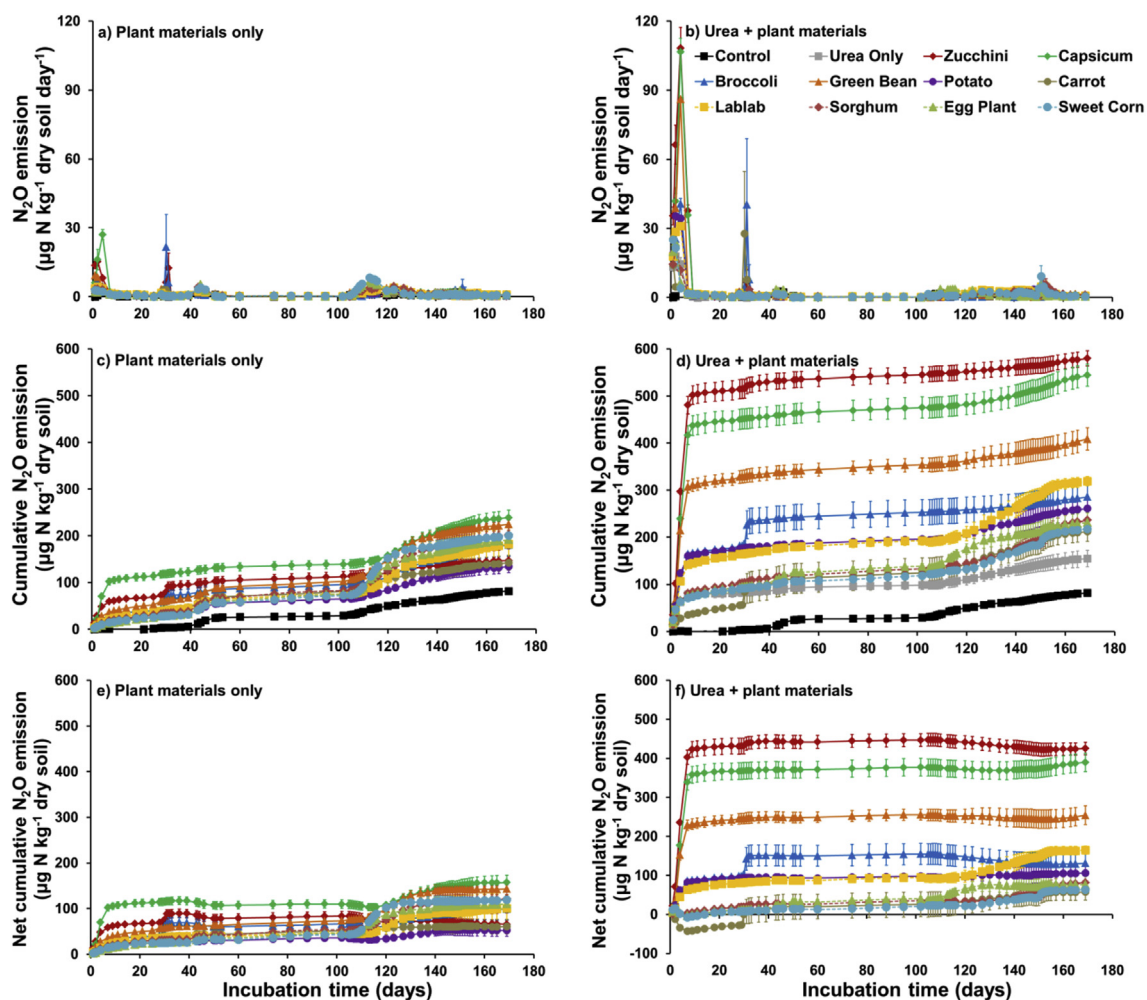


Fig. 4. Daily (a and b), cumulative (c and d) and net cumulative (e and f) N_2O emissions from different treatments during the incubation period. The net cumulative emissions were differences in cumulative emissions between plant material amended treatments and the Control (e) or the Urea only treatment (f). Vertical bars are standard error of four replicates.

Table 2

Cumulative N_2O emissions ($\mu\text{g N}_2\text{O-N kg}^{-1}$ dry soil) from the $-N$ treatments of incorporated vegetable crop residues during different periods of the incubation.

Treatment	Incubation time periods						
	0 - 28 day	28 - 50 day	50 - 105 day	105 - 169 day	0 - 50 day	0 - 105 day	0 - 169 day
Control	3.2 (a) ^a	21.1 (a)	6.4 (a)	51.0 (a)	24.3 (a)	30.7 (a)	81.7 (a)
Zucchini	72.5 (b)	30.0 (ab)	11.3 (ab)	33.5 (a)	102.5 (bc)	113.9 (bc)	147.3 (b)
Capsicum	117.5 (c)	14.0 (a)	8.4 (a)	99.2 (ab)	131.5 (c)	139.9 (c)	239.1 (c)
Broccoli	43.5 (d)	41.1 (b)	12.6 (ab)	83.3 (ab)	84.7 (bd)	97.3 (bd)	180.6 (bc)
Green Bean	59.6 (b)	27.7 (ab)	19.2 (b)	118.4 (b)	87.3 (bd)	106.5 (be)	224.9 (bc)
Potato	29.1 (e)	25.6 (ab)	10.7 (ab)	68.8 (ab)	54.7 (e)	65.4 (d)	134.2 (b)
Carrot	35.7 (de)	32.9 (ab)	13.2 (ab)	60.6 (ab)	68.6 (de)	81.8 (bd)	142.4 (b)
Lablab	39.2 (de)	24.2 (ab)	11.7 (ab)	107.4 (b)	63.3 (de)	75.0 (de)	182.5 (bc)
Sorghum	33.4 (de)	32.5 (ab)	17.4 (b)	119.6 (b)	65.9 (de)	83.3 (bd)	202.9 (bc)
Egg Plant	27.9 (e)	32.7 (ab)	14.2 (ab)	116.9 (b)	60.6 (de)	74.9 (de)	191.7 (bc)
Sweet corn	27.9 (e)	29.4 (ab)	16.5 (b)	126.8 (b)	57.3 (de)	73.8 (de)	200.6 (bc)

^a The different letters in parentheses within a column indicate significant differences between the treatments at $P < 0.05$.

3.6. Nitrous oxide emission in relation to chemical and biochemical composition of plant materials

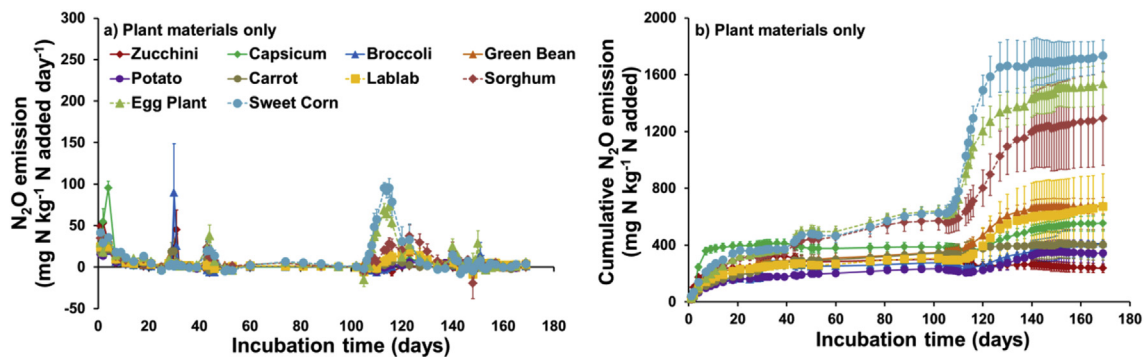
Stepwise linear regression was used to model cumulative N_2O emissions at four different periods of the incubation (28, 50, 105 and 169 days after start) and two different units for cumulative emissions ($\mu\text{g N}_2\text{O-N kg}^{-1}$ dry soil and $\text{mg N}_2\text{O-N kg}^{-1}$ added N),

using parameters related to chemical and biochemical composition of plant materials. The models accounted for 45–97% of the variability in the cumulative N_2O emissions across different units and time frames of incubation.

When calculations were undertaken based on the same amount of plant materials applied (Table 4), the stepwise regressions for $-N$ and $+N$ treatments showed a positive correlation between

Table 3Cumulative N₂O emissions ($\mu\text{g N}_2\text{O-N kg}^{-1}$ dry soil) from the +N treatments of incorporated vegetable crop residues during different periods of the incubation.

Treatment	Incubation time periods						
	0 - 28 day	28 - 50 day	50 - 105 day	105 - 169 day	0 - 50 day	0 - 105 day	0 - 169 day
Urea	83.4 (a) ^a	9.1 (a)	6.5 (a)	55.7 (ab)	92.5 (a)	98.9 (a)	154.6 (a)
Zucchini + N	514.4 (b)	20.0 (ac)	12.1 (b)	33.3 (a)	534.4 (b)	546.5 (b)	579.8 (b)
Capsicum + N	450.6 (b)	12.3 (a)	12.3 (b)	69.1 (ab)	462.8 (bd)	475.2 (bd)	544.2 (b)
Broccoli + N	181.5 (c)	61.3 (b)	10.9 (ab)	32.1 (a)	242.8 (ce)	253.7 (ce)	285.8 (cd)
Green Bean + N	328.1 (d)	12.5 (a)	13.1 (b)	55.2 (ab)	340.6 (de)	353.7 (dc)	408.9 (bc)
Potato + N	175.9 (c)	9.7 (a)	8.7 (ab)	66.7 (ab)	185.6 (cf)	194.3 (ef)	261.0 (de)
Carrot + N	55.3 (e)	56.9 (bc)	11.9 (b)	88.0 (ab)	112.2 (a)	124.2 (a)	212.2 (ad)
Lablab + N	163.9 (c)	15.6 (ac)	11.3 (ab)	128.4 (b)	179.5 (cf)	190.8 (ef)	319.2 (ce)
Sorghum + N	102.2 (a)	16.6 (ac)	15.4 (b)	102.3 (b)	118.7 (a)	134.1 (af)	236.5 (d)
Egg Plant + N	93.7 (a)	31.7 (bc)	11.2 (ab)	94.5 (b)	125.5 (af)	136.7 (af)	231.2 (d)
Sweet corn + N	90.5 (a)	16.2 (ac)	14.9 (b)	96.1 (b)	106.8 (a)	121.7 (a)	217.8 (ad)

^a The different letters in parentheses within a column indicate significant differences between the treatments at $P < 0.05$.**Fig. 5.** Daily (a) and cumulative (b) N₂O-N emission kg⁻¹ added N for different treatments during the incubation period. Vertical bars are standard error of four replicates.

cumulative N₂O emissions and TN of amendments as the main controlling factor of N₂O emissions in these treatments (only first 105 days in -N treatments). Using TN in combination with O-aryl C and carbonyl C of plant materials provided a much better prediction of the cumulative N₂O emissions at different stages of incubation.

The stepwise regression analysis using relative N₂O emission to total N application in -N treatments (Table 5) indicated that the variation in cumulative N₂O emissions was positively related to the

biochemical parameters of applied plant materials including cellulose, lignin, O-aryl C and carbonyl C contents.

3.7. Relationships of N₂O emissions to soil N content, WSOC and MBC

The cumulative N₂O emission was positively correlated ($P < 0.01$) with NO₃-N and total mineral N concentration

Table 4Regression equations between cumulative N₂O emission (according to the same amount of plant material application) and plant material properties during different periods of the incubation.

Treatment	Parameters	Days after start	Equation	R ²
Plant materials	C & N contents + Wet Chemical analysis	28	$Y^a = -0.1 + 2.2 (\text{TN})^b$	0.66**
		50	$Y = 33.0 + 2.0 (\text{TN})$	0.75**
		105	$Y = 50.2 + 1.9 (\text{TN})$	0.70**
		169	- ^c	-
	C & N contents + ¹³ C NMR	28	$Y = -126.4 + 3.9 (\text{TN}) + 7.9 (\text{O-aryl C})$	0.95**
		50	$Y = -55.6 + 3.2 (\text{TN}) + 5.6 (\text{O-aryl C})$	0.94**
		105	$Y = -35.3 + 3.0 (\text{TN}) + 5.4 (\text{O-aryl C})$	0.90**
		169	- ^c	-
Plant materials + Urea	C & N contents + Wet Chemical analysis	28	$Y^d = -158.2 + 13.2 (\text{TN})$	0.73**
		50	$Y = -144.3 + 13.3 (\text{TN})$	0.80**
		105	$Y = -137.4 + 13.2 (\text{TN})$	0.80**
		169	$Y = -68.8 + 11.1 (\text{TN})$	0.72**
	C & N contents + ¹³ C NMR	28	$Y = 212.8 + 22.8 (\text{TN}) - 12.4 (\text{carbonyl C})$	0.97**
		50	$Y = 160.4 + 21.2 (\text{TN}) - 10.2 (\text{carbonyl C})$	0.97**
		105	$Y = 171.5 + 21.3 (\text{TN}) - 10.4 (\text{carbonyl C})$	0.97**
		169	$Y = 244.5 + 19.2 (\text{TN}) - 10.5 (\text{carbonyl C})$	0.95**

Significance levels: * $P < 0.05$, ** $P < 0.01$.^a Cumulative N₂O emission ($\mu\text{g N}_2\text{O-N kg}^{-1}$ dry soil).^b TN, O-aryl C and carbonyl C as mg g⁻¹ plant material.^c No regression equation found between parameters.^d Net cumulative N₂O emission ($\mu\text{g N}_2\text{O-N kg}^{-1}$ dry soil).

Table 5Regression equations between cumulative N₂O emission (according to the total N application) and plant material properties during different periods of the incubation.

Treatment	Parameters	Days after start	Equation	R ²
Plant materials	C & N contents + Wet Chemical analysis	28	Y ^a = 184.0 + 0.6 (Cellulose) ^b	0.45*
		50	Y = 154.7 + 0.9 (Cellulose) + 5.3 (Lignin/N)	0.85**
		105	Y = 23.3 + 1.7 (Cellulose) + 0.8 (Lignin)	0.90**
		169	Y = -676.7 + 6.3 (Cellulose) + 3.9 (Lignin)	0.93**
	C & N contents + ¹³ C NMR	28	Y = -290.4 + 6.4 (TN) + 37.4 (O-aryl C)	0.83**
		50	Y = 150.5 + 713.5 (O-aryl/carbonyl C)	0.74**
		105	Y = 118.7 + 1055.8 (O-aryl/carbonyl C)	0.84**
		169	Y = -297.6 + 4104.0 (O-aryl/carbonyl C)	0.95**

Significance levels: *P < 0.05, **P < 0.01.

^a Cumulative N₂O emission (mg N₂O-N kg⁻¹ added N).^b TN, Cellulose, Lignin, O-aryl C and carbonyl C as mg g⁻¹ plant material.

throughout the incubation period for +N treatments, but only showed similar correlation ($P < 0.01$) in the first 105 days of incubation for -N treatments (Table 6). The results showed a negative correlation ($P < 0.05$) between cumulative N₂O emissions with MBC (first 28 days) and WSOC (first 105 days) for the -N treatments while, in the +N treatments only WSOC showed negative correlation ($P < 0.05$) with N₂O emission. The significant correlation between cumulative N₂O emission and soil NO₃⁻-N concentration, in the fluctuating soil moisture conditions of this experiment (55–85% WFPS), implied that denitrification was the dominant microbial process of N₂O production during the incubation. The negative correlation between MBC and WSOC with N₂O emissions in the early stages of decomposition also indicated that increasing soluble OC concentrations along with increasing size of the soil microbial community might enhance mineral N immobilisation and therefore reduce N₂O emissions, especially in plant materials with low TN contents.

4. Discussion

4.1. Effect of inorganic N fertiliser application on N₂O emission

The +N treatments had significantly higher cumulative N₂O emissions in the first 50 days compared with the -N treatments and this was reflected in the overall incubation period where +N treatments had higher cumulative N₂O emissions. However, the -N treatments tended to express higher N₂O emissions during the late stage of the incubation compared with the +N treatments. These results indicate that adding inorganic N fertiliser accelerated microbial activity including N₂O production at the early stages of decomposition, which might have reduced C availability after about 105 days of incubation. Wang et al. (2004a) in a 365 day incubation experiment found that the combined application of plant residues with mineral N initially increased residue decomposition for a short period of time (about 100 days), but substantially decreased the decomposition rates compared with the residue only application. In

the present study, the positive interactive effect between plant materials and inorganic N fertiliser during the early stages suggests that the combined application of plant materials and N fertilisers generally enhances N₂O emissions except when the applied residues have very low TN content and high N immobilisation potential. The findings of this study indicate that in order to minimise N₂O emissions, plant materials with high TN contents should be applied alone, but residues with low TN contents (e.g., carrot, sorghum and sweet corn) can be combined with inorganic N fertilisers in order to provide sufficient mineral N for plant growth without increasing N₂O emissions (Fig. 4f).

4.2. The influence of plant material amendment on MBC and WSOC

Amendment of plant materials into soil significantly increased WSOC concentration in the early stages of decomposition. Crop residues can be considered as one of the main sources of readily available C in the soil, and promote the formation of anaerobic microsites by increasing O₂ demand. Toma and Hatano (2007) reported a decrease in WSOC with time after the addition of plant materials to soil, but in contrast to this our findings did not show significant changes in WSOC concentration during the incubation period. The present findings suggest that WSOC may not be the major factor regulating microbial activity under our experimental setting. The positive correlation between WSOC and C:N ratios of the plant materials at the end of our incubation experiment (169 days) contrasts with the findings of Hadas et al. (2004) who indicated that residues with lower C:N ratios may produce higher WSOC, while Begum et al. (2014) observed no significant correlations between WSOC and C:N ratios of plant materials.

Plant material amendments in the current study significantly increased MBC in the early stages of decomposition compared with the control and urea only treatments. This increase in MBC and associated microbial activity would increase oxygen consumption in soil microsites and consequently promote N₂O production via denitrification. After this early peak, MBC contents in all treatments decreased significantly, which was probably due to a C limitation for microbial growth throughout the remaining incubation period. The similar pattern of reduction in MBC in both the -N and +N treatments demonstrated that N concentration was not a limiting factor for microbial growth in the late stages of the experiment. These findings indicate a shift in the key limiting factor for microbial growth from available N early in the incubation to available C as plant material decomposition progressed.

4.3. Effect of plant materials C:N ratio on N dynamics and N₂O emission

The C:N ratio of organic materials has been frequently used to

Table 6Relationships (r) between cumulative N₂O emission during different stages of incubation and the respective NO₃⁻, mineral N, MBC and WSOC contents in soil amended with different plant materials, with and without N fertiliser application.

Property	Plant materials only (days after start)				Plant materials + urea (days after start)			
	28	50	105	169	28	50	105	169
NO ₃ ⁻ -N	0.86**	0.90***	0.85**	0.06	0.94***	0.94***	0.95***	0.90***
mineral N	0.86**	0.90***	0.85**	0.06	0.94***	0.94***	0.95***	0.90***
MBC	-0.67*	—	-0.02	-0.21	0.18	—	-0.50	-0.06
WSOC	-0.63*	—	-0.64*	-0.24	-0.65*	—	-0.37	-0.28

Significance levels: *P < 0.05, **P < 0.01, ***P < 0.001.

predict their net C and N release after incorporation in soils. [Vigil and Kissel \(1991\)](#) summarised the results of several medium to long-term investigations and reported the C:N ratio of 41 as the break-even point between net N immobilization and mineralization, which is close to our findings (C:N ratio = 35) in the first 105 days of experiment. In general, the C content of plant materials does not vary widely across different plant species, but the ratios of their C components can vary considerably. Therefore, the net N immobilisation of plant materials with C:N ratio ≥ 35 in the first 105 days of the incubation can be attributed to their low initial N concentration and high contents of O-alkyl and O₂-alkyl C which mostly decompose in the first month after incorporation ([Bonanomi et al., 2013](#)) and consequently result in higher CO₂ release from plant materials and net N immobilisation.

[Chen et al. \(2013\)](#) suggested that the application of plant materials did not mitigate soil N₂O emissions even when C:N ratios were well above the threshold for net N immobilization, which is similar to the findings of this study. This behaviour can be attributed to the effect of plant material application on abiotic soil factors (such as oxygen depletion due to active microbial growth) other than its inorganic N content. The residues with higher C:N ratios (e.g. carrot, sorghum and sweet corn) produced lower N₂O emissions than others in the early stage of decomposition. The enhancement of N₂O emissions after incorporation of residues with higher N content, or lower C:N ratio, would be attributed to their higher decomposability and increase of N mineralization, which consequently results in more available C and N substrate for denitrification. The results of the current study also indicated an increase in the ratio of emitted N₂O to applied vegetable residue N with increasing C:N ratios.

4.4. Effect of total N content and biochemical characteristics of plant materials on N₂O emission

It is generally accepted that higher N₂O emissions are associated with plant materials with higher N content. [Novoa and Tejeda \(2006\)](#) and [Toma and Hatano \(2007\)](#) reported that the N dynamics and N₂O emissions of soil-incorporated plant materials are related to their initial N content, while the results of this study suggest that using the combination of TN and ¹³C NMR parameters of applied plant materials improved the correlation with cumulative N₂O emissions than using TN alone. This finding indicates that ¹³C NMR spectroscopy of plant materials, which identifies C atoms according to the chemical environment surrounding a C nucleus, seems to be a better predictor of N₂O emission compared to the conventional chemical analyses that divide organic components in terms of their molecular composition. Therefore, the Intergovernmental Panel on Climate Change (IPCC) approach which estimates N₂O emissions from plant materials based only on their total N content could possibly be improved by taking into account the C composition of applied residues, since plant materials with similar TN contents may show different patterns of N mineralisation and N₂O emissions during their decomposition process.

While the effect of biochemical quality of plant materials on N₂O emissions is still not completely understood, it has been suggested that lignin and cellulose contents of organic amendments affect their decomposition rates and N₂O emission ([Hadas et al., 2004](#); [Jalota et al., 2006](#)). The results of this study show a significant effect of cellulose, lignin and (lignin/N) contents of applied residues on N₂O emissions only when considered on the basis of their total N application. Therefore, it can be concluded that biochemical quality of vegetable plant materials is a more effective factor in predicting the relative N₂O emission to total N input than the total N₂O emissions. In the present study we did not observe any effect of residue polyphenol content in inhibiting N mineralisation and N₂O

emissions. This may have been due to the fact that the polyphenol contents of the applied plant materials in this study were below the generally accepted threshold of >3–4% for lowering N release by binding to soil microbial enzymes ([Constantinides and Fownes, 1994](#)).

4.5. Effect of soil moisture fluctuation and incubation time on cumulative N₂O emission

Fluctuations in soil moisture levels greatly alter gas (both N₂O and oxygen) transport in the soil matrix by affecting gaseous flow paths between soil pores. This subsequently determines the dominant process (nitrification or denitrification) of N₂O production at soil microsites and whether N₂O is likely to be further reduced to N₂ before emission. Varying the soil moisture content in this study, to simulate field conditions, facilitated the activity of soil nitrifiers and denitrifiers at different stages during the incubation, as the soil wetting and drying cycles are considered to be one of the main regulating factors of N₂O emissions in agricultural fields. Many researchers reported significant N₂O emissions only in the first month after plant residue amendment, followed by a gradual decrease in N₂O emissions to background levels when an aerobic ($\leq 60\%$ WFPS) condition is maintained ([Gentile et al., 2008](#); [Muhammad et al., 2011](#); [Toma and Hatano, 2007](#); [Zhu et al., 2013](#)). In the present study, a significant increase in the cumulative N₂O emissions compared with the control was observed after plant material amendment for the first 28 days in -N treatments, while generally no significant differences were observed after that time until the moisture was further increased (day 105) to the level favouring the denitrification process. When soil moisture was raised to 85% WFPS, plant materials with high O-alkyl C and low carbonyl C and alkyl C (i.e., green bean, lablab, sorghum, eggplant and sweet corn) showed greater N₂O emissions ([Fig. 4](#)), which consequently resulted in no significant difference in cumulative N₂O emissions between applied plant materials with different TN contents by the end of the experiment.

It has also been frequently reported from previous short-term (21–56 days) experiments that the application of plant materials with low N contents (high C:N ratios) promote N immobilisation and consequently reduce N₂O emissions ([Huang et al., 2004](#); [Pimentel et al., 2015](#); [Toma and Hatano, 2007](#); [Wu et al., 2016](#); [Zhu et al., 2013](#)). The reason for this can be attributed to the nutrient limitation caused by low N content plant materials on decomposer organisms during the early stage of decomposition ([Eiland et al., 2001](#)). Findings of the current study show that plant materials of different N contents decompose at different rates and higher N₂O emissions could result from the low N content materials during late stages. Therefore, it is possible that short-term experiments investigating a single incorporation of plant material of low N content into soil may not fully capture N₂O emission peaks exhibited in the longer-term.

5. Conclusion

In the present study the application of vegetable plant materials consistently resulted in higher cumulative N₂O emissions compared with the unamended treatments. Plant materials with high TN contents initially produced higher cumulative N₂O emissions than other residues, but those with low TN contents resulted in higher N₂O emissions during the late stage of the incubation. Consequently, there was no consistent and significant difference in cumulative N₂O emissions between applied plant materials with different TN contents in the -N treatments during the 169-day incubation. Compared with the urea only treatment, the application of plant materials plus inorganic N fertiliser consistently resulted in

higher cumulative N₂O emissions at the end of the incubation, although N₂O emissions were initially reduced following addition of a few low-N plant materials. In the fertilised soil, the application of plant materials with high TN contents generally produced higher cumulative N₂O emissions than those with low TN contents. Furthermore, plant materials with higher O-alkyl C and lower carbonyl C and alkyl C levels produced higher N₂O emissions at increased soil moisture levels. The stepwise multiple regression model for the -N and +N treatments showed the dominance of TN in regulating cumulative N₂O emissions. In comparison, the TN in combination with ¹³C NMR functional groups (O-aryl and carbonyl C) of applied plant materials gave much stronger correlations with cumulative N₂O emissions than using TN alone. Further field experiments are needed to extend the findings of this study to other environmental conditions. Also, the application of polymerase chain reaction (PCR)-based molecular methods for detecting the diversity and abundance of N functional genes in nitrification and denitrification processes may provide more insights into these complicated interactions.

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