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J Gomes, N Brüggemann, D P Dick, G M Pedroso, Murilo Veloso, et al.. Urea and legume residues as ^{15}N - N_2O sources in a subtropical soil. *Soil Research*, 2019, 57, <10.1071/sr18300>. <hal-04260806>

HAL Id: hal-04260806

<https://normandie-univ.hal.science/hal-04260806v1>

Submitted on 26 Oct 2023

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Urea and legume residues as ^{15}N - N_2O sources in a subtropical soil

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Abstract. In this work, we used the ^{15}N labelling technique to identify the sources of N_2O emitted by a subtropical soil following application of mineral nitrogen (N) fertiliser (urea) and residues of a legume cover crop (cowpea). For this purpose, a 45-day incubation experiment was conducted by subjecting undisturbed soil cores from a subtropical Acrisol to five different treatments: (1) control (no crop residue or fertiliser-N application); (2) ^{15}N -labelled cowpea residue (200 $\mu\text{g N g}^{-1}$ soil); (3) ^{15}N -labelled urea (200 $\mu\text{g N g}^{-1}$ soil); (4) ^{15}N -labelled cowpea residue (100 $\mu\text{g N g}^{-1}$ soil) + unlabelled urea (100 $\mu\text{g N g}^{-1}$ soil); and (5) unlabelled cowpea residue (100 $\mu\text{g N g}^{-1}$ soil) + ^{15}N -labelled urea (100 $\mu\text{g N g}^{-1}$ soil). Cores were analysed for total N_2O formation, $\delta^{15}\text{N}$ - N_2O and $\delta^{18}\text{O}$ - N_2O by continuous flow isotope ratio mass spectrometry, as well as for total NO_3^- -N and NH_4^+ -N. Legume crop residues and mineral fertiliser increased N_2O emissions from soil to 10.5 and 9.7 $\mu\text{g N}_2\text{O-N cm}^{-2}$ respectively, which was roughly six times the value for control (1.5 $\mu\text{g N}_2\text{O-N cm}^{-2}$). The amount of $^{15}\text{N}_2\text{O}$ emitted from labelled ^{15}N -urea (0.40–0.45% of ^{15}N applied) was greater than from ^{15}N -cowpea residues (0.013–0.015% of ^{15}N applied). Unlike N-poor crop residues, urea in combination with N-rich residues (cowpea) failed to reduce N_2O emissions relative to urea alone. Legume cover crops thus provide an effective mitigation strategy for N_2O emissions in relation to mineral N fertilisation in climate-smart agriculture. Judging by our inconclusive results, however, using urea in combination with N-rich residues provides no clear-cut environmental advantage.

Additional keywords: ^{15}N , cover crops, nitrous oxide, urea.

Received 6 October 2018, accepted 11 February 2019, published online 19 March 2019

Introduction

Nitrous oxide (N_2O) is a major greenhouse gas with a global warming potential 298 times that of carbon dioxide (IPCC 2013); also, N_2O is the main ozone layer-depleting substance emitted in the 21st century (Ravishankara *et al.* 2009). In fact, atmospheric N_2O levels have increased steadily at a rate of 0.7 ppb year⁻¹ and agricultural soils continue to be among the main emission sources for this gas owing to the widespread use of mineral nitrogen (N) fertilisers (IPCC 2014).

Production of N_2O in soils is usually ascribed to microbial nitrification and denitrification (Wrage *et al.* 2005; Kool *et al.* 2011), the latter being the more effective process (Bateman and Baggs 2005; Pimentel *et al.* 2015). Under low O_2 availability conditions, nitrate ion (NO_3^-) acts as an electron acceptor and is gradually reduced to N_2O or N_2 (Knowles 1982). The N_2O is also a byproduct of the aerobic oxidation of ammonium ion (NH_4^+) to nitrite ion (NO_2^-), which is the first step in the nitrification process (Bock and Wagner 2006). Recently, N_2O production in soils has also been ascribed to nitrifier

denitrification. Thus, nitrifier autotrophic bacteria can oxidise ammonia (NH_3) to NO_2^- under aerobic conditions, and NO_2^- is subsequently reduced to N_2O and N_2 (Wrage *et al.* 2005; Kool *et al.* 2011).

Organic and inorganic N added to soil alters N cycling and N flow by affecting microbial activity, thereby also potentially altering formation and emission of soil N_2O (Bowman *et al.* 2008; Frimpong and Baggs 2010). Mineral N fertilisers rapidly increase soil available N, often boosting N_2O emissions as a result (Zanatta *et al.* 2010; Shcherbak *et al.* 2014). Legume cover crop residues have also been found to increase soil N_2O emissions (Gomes *et al.* 2009; Jarecki *et al.* 2009), but usually to a smaller extent than N fertilisers (Baggs *et al.* 2001; Bayer *et al.* 2015). Cover cropping has thus been deemed a useful tool for sustainable agriculture in tropical and subtropical developing countries, and also to provide advantages such as improved carbon (C) retention in soil organic matter (Veloso *et al.* 2018) and cash-crop yields (Lovato *et al.* 2004; Mahama *et al.* 2016).

Available knowledge about the specific sources of N₂O in tropical and subtropical agriculture arising from application of N fertilisers or N-rich residues of legume cover crops is scant. This is largely the result of the differential dynamics of N from mineral fertilisers and crop residues, and of also the different impact of added N in accelerating mineralisation of N in soil organic matter (Gentile *et al.* 2008; Chen *et al.* 2013). The starting hypothesis of this work was that mineral N fertiliser would boost soil N₂O emissions by rapidly increasing inorganic N levels and facilitating mineralisation of N present in soil organic matter; conversely, legume residues would reduce N₂O emissions by affecting the slow mineralisation of added N and of the N immobilisation in microbial biomass having a less marked impact on N mineralisation of soil organic matter.

The primary objectives of this work were to assess total soil N₂O-N emissions and identify their sources (soil, fertiliser or legume residues) following individual or joint addition of ¹⁵N-labelled residues of cowpea – a summer legume cover crop – and ¹⁵N-labelled urea in a 45-day incubation microcosm experiment with undisturbed soil cores of a subtropical Acrisol.

Material and methods

Soil sampling

Undisturbed cores were collected from a subtropical soil under a 28-year-old experiment in Eldorado do Sul (30°6'S, 51°41'W; 45 m above sea level), Southern Brazil. The long-term field study was originally designed to assess the effects of no-till cropping systems on soil properties and maize yield. The experimental plot used for soil sampling had been managed under no-tillage with maize during summer and fallow in winter. No N fertilisation was used at any time during the experimental period.

Undisturbed soil cores from the 0–10 cm deep soil layer were collected using 5-cm wide PVC tubes that were capped and transferred to Forschungszentrum (Jülich, Germany) for incubation. The most salient properties of the soil were as follows: 220 g clay kg⁻¹, 540 g sand kg⁻¹, 8.3 g total organic C kg⁻¹, 0.71 g total N kg⁻¹, pH_{water} = 4.9, and available phosphorus and potassium, determined by the Mehlich-1 method, of 18 and 109 mg kg⁻¹ respectively.

Cowpea biomass labelling

The crop residues used were ¹⁵N-labelled and unlabelled residues of cowpea (*Vigna unguiculata* (L.) Walp.), a summer cover crop widely used in Southern Brazil. Seeds were germinated in vermiculite substrate, transplanted to pots containing an aerated modified Hoagland nutritive solution and grown until the three-leaf developmental stage was reached. One-half of the pots contained ¹⁵N-labelled urea (60 atom% ¹⁵N) and the other half contained urea with natural abundance of ¹⁵N. The concentration of nutrients in solution during plant growth was monitored through electrical conductivity. Cowpea aboveground biomass was harvested at flowering stage, oven-dried at 60°C, chopped into 2–8 mm pieces and analysed for total C and total N by elemental analysis, and for ¹⁵N by using an IsoPrime EA-isotope ratio mass spectrometry (IRMS) instrument from Elementar Analysensysteme (Hanau, Germany). The results are shown in Table 1.

Table 1. The C, N and ¹⁵N contents of labelled and unlabelled cowpea biomass, and of labelled and unlabelled urea

Contents are averages of three replicates each and followed by one standard error

Input	C (%)	N (%)	¹⁵ N enrichment
¹⁵ N-labelled cowpea	41.5 ± 0.1	1.94 ± 0.02	10.19 ± 0.08
Unlabelled cowpea	41.6 ± 0.1	1.58 ± 0.02	0.376 ± 0.002
¹⁵ N-labelled urea	–	45	15
Unlabelled urea	–	45	0.367

Treatments, experimental design and incubation procedure

The soil cores were subjected to five different treatments: (1) control (no residue or N fertiliser added); (2) ¹⁵N-labelled cowpea residue (200 µg N g⁻¹ soil); (3) ¹⁵N-labelled urea (200 µg N g⁻¹ soil) added as an aqueous solution; (4) ¹⁵N-labelled cowpea residue (100 µg N g⁻¹ soil) + unlabelled urea (100 µg N g⁻¹ soil); and (5) unlabelled cowpea residue (100 µg N g⁻¹ soil) + ¹⁵N-labelled urea (100 µg N g⁻¹ soil). The experiment was designed as a complete randomised block, with three replications.

The incubation experiment was performed for 45 days in 1-L Duran glass bottles fitted with lids having a three-way valve for gas sampling. PVC tubes containing ~250 g of undisturbed soil each were placed inside the bottles. The temperature was kept at 24°C and soil moisture at 60% water holding capacity (WHC) by monitoring soil weight every other day. Air samples were withdrawn for analysis 0, 1, 2, 3, 4, 8, 10, 14, 18, 21, 25, 32 and 45 days after N application (DAA). The glass bottles were closed 1 h before sampling, and ambient air samples collected in parallel to measure the air N₂O concentration.

Gas and soil analyses

Air samples were analysed for N₂O, δ¹⁵N-N₂O and δ¹⁸O-N₂O using a trace gas preparation unit coupled to an IsoPrime 100 CF-IRMS instrument (Elementar Analysensysteme, Hanau, Germany). The soil was analysed for total NO₃⁻-N and NH₄⁺-N by the central analytical laboratory of Forschungszentrum. Neither ¹⁵NO₃⁻ nor ¹⁵NH₄⁺ was determined in soil owing to their extremely high content of ¹⁵N label.

Calculations

The N₂O fluxes were calculated as follows:

$$f = \frac{dC}{dt} \frac{PV}{RT} \frac{\bar{M}}{A} \quad (1)$$

where f is the gas production rate (g cm⁻² h⁻¹), dC/dt is change in N₂O mixing ratio within the glass bottle in 1 h (ppm h⁻¹), \bar{M} is gas molar mass (g mol⁻¹), P is pressure inside the incubation bottle (1 atm), V is headspace in the bottle (L), T is temperature (K) and R is the ideal gas constant (0.08205 L atm K⁻¹ mol⁻¹). Cumulative N₂O emissions were calculated by trapezoidal integration of the daily N₂O fluxes over a period of 45 days with the aid of SigmaPlot (Systat, San Jose, CA, USA).

The IRMS signal at the mass-over-charge ratio (m/z) 45 represents single-labelled N₂O molecules (¹⁴N¹⁵N¹⁶O or ¹⁵N¹⁴N¹⁶O), whereas that at m/z 46, after subtraction of the natural ¹⁸O-background of N₂O (¹⁴N¹⁴N¹⁸O), represents

Table 2 Summary statistics showing the significance of treatment (N source), sampling date and their mutual interaction (treatment × sampling date) on N₂O flux, ¹⁵N recovery in N₂O gas, and soil NO₃⁻-N and NH₄⁺-N contents, as well as the effect of treatment on total N₂O emissions, total soil-derived N₂O emissions, total soil plus unlabelled N input-derived N₂O emissions, total ¹⁵N labelled input-derived N₂O emissions and total ¹⁵N recovery in N₂O gas

Dependent variable	Fixed effect	df	F-value	P-value
N ₂ O flux	Treatment (T)	4	6.56	<0.0001
	Sampling date (s.d.)	12	5.87	<0.0001
	T × s.d.	48	2.82	<0.0001
¹⁵ N recovery in N ₂ O gas	T	3	23.67	<0.0001
	s.d.	12	4.33	<0.0001
	T × s.d.	36	2.73	<0.0001
Soil NO ₃ ⁻ -N content	T	4	14.44	<0.0001
	s.d.	5	10.9	<0.0001
	T × s.d.	20	2.5	0.0037
Soil NH ₄ ⁺ -N content	T	4	34.83	<0.0001
	s.d.	5	13.88	<0.0001
	T × s.d.	20	3.71	<0.0001
Total N ₂ O emission	T	4	9.22	0.0043
Total soil-derived N ₂ O emission	T	2	8.13	0.039
Total soil plus unlabelled N input-derived N ₂ O emission	T	1	12.93	0.0228
Labelled ¹⁵ N input-derived N ₂ O emission	T	3	11.44	0.0068
Total ¹⁵ N recovery in N ₂ O gas	T	3	10.34	0.0087

double-labelled N₂O molecules (¹⁵N¹⁵N¹⁶O). Excess ¹⁵N and ¹⁸O (atom%) in the sample, representing single- and double-labelled N₂O molecules, was calculated with provision for the average contents of ¹⁵N and ¹⁸O in control samples:

$$\text{Excess } ^{15}\text{N sample } (\%) = \frac{(^{15}\text{N sample} - ^{15}\text{N background})}{(^{15}\text{N control} - ^{15}\text{N background})} \quad (2)$$

$$\text{Excess } ^{18}\text{O sample } (\%) = \frac{(^{18}\text{O sample} - ^{18}\text{O background})}{(^{18}\text{O control} - ^{18}\text{O background})} \quad (3)$$

where ¹⁵N and ¹⁸O sample are the amounts of ¹⁵N and ¹⁸O in the ¹⁵N-labelled sample (%) respectively; ¹⁵N and ¹⁸O background the natural abundance of ¹⁵N (0.36764669%) and ¹⁸O (0.20011872%) respectively; and ¹⁵N and ¹⁸O control the average concentration of ¹⁵N and ¹⁸O (%) in the control samples respectively.

The excess of double-labelled N₂O molecules was multiplied by 2 because each molecule contained two ¹⁵N atoms. The total excess of ¹⁵N (%) in the samples was calculated as follows:

$$\text{Total excess } ^{15}\text{N sample } (\%) = \text{Excess } ^{15}\text{N sample} + (2 \times \text{Excess } ^{18}\text{O sample}) \quad (4)$$

The recovery of ¹⁵N applied in residue or urea as N₂O gas at each air sampling event was calculated according to Gentile *et al.* (2008):

$$Q \text{ input} = Q \text{ sample} \left[\frac{^{15}\text{N sample} - ^{15}\text{N background}}{^{15}\text{N input} - ^{15}\text{N background}} \right] \quad (5)$$

where *Q* input is the amount of N₂O-N derived from the labelled input, *Q* sample is that of N₂O-N from the sample, ¹⁵N sample is

total ¹⁵N concentration in the sample (%), ¹⁵N background is natural abundance of ¹⁵N (0.36764669%) and ¹⁵N input is total ¹⁵N concentration in the input (%). Total ¹⁵N recovery was calculated by trapezoidal integration of the daily N₂O fluxes over a period of 45 days, using SigmaPlot software.

Statistical analyses

Because of the covariant nature of the relationships among N₂O flux, ¹⁵N recovery in N₂O gas, and soil NO₃⁻-N and NH₄⁺-N contents, these dependent variables were subjected to analysis of variance (ANOVA) with the Mixed Procedure in SAS® v. 9.4 (Statistical Analysis System Institute, Cary, NC, USA), using treatment, sampling date and their respective two-way interactions as fixed effects, and block as random effect. The ANOVA of total N₂O emissions, total soil-derived N₂O emissions, total soil plus unlabelled input-derived N₂O emissions, total labelled input-derived N₂O emissions and total ¹⁵N recovery was done using a generalised linear model in the GLM Procedure in SAS. Differences were considered to be statistically significant at *P* < 0.05 in Tukey's honestly significant different (HSD) test. The potential relationships of N₂O fluxes with the soil NO₃⁻-N and N-NH₄⁺-N contents during the incubation period were assessed by regression analysis with SigmaPlot.

Results

N₂O fluxes and cumulative emissions

Soil N₂O fluxes were influenced by N source, sampling date and the N source × sampling date interaction (Table 2). Soil N₂O efflux rates were greater within the first 20 days of incubation in the treatments with N addition; however, they decreased and levelled off at values similar to those for the control after 20 days (Fig. 1a).

The N₂O efflux rates peaked at 255, 4162, 1242, 1381 and 2029 ng N₂O-N cm⁻² d⁻¹ for the control, ¹⁵N-labelled cowpea,

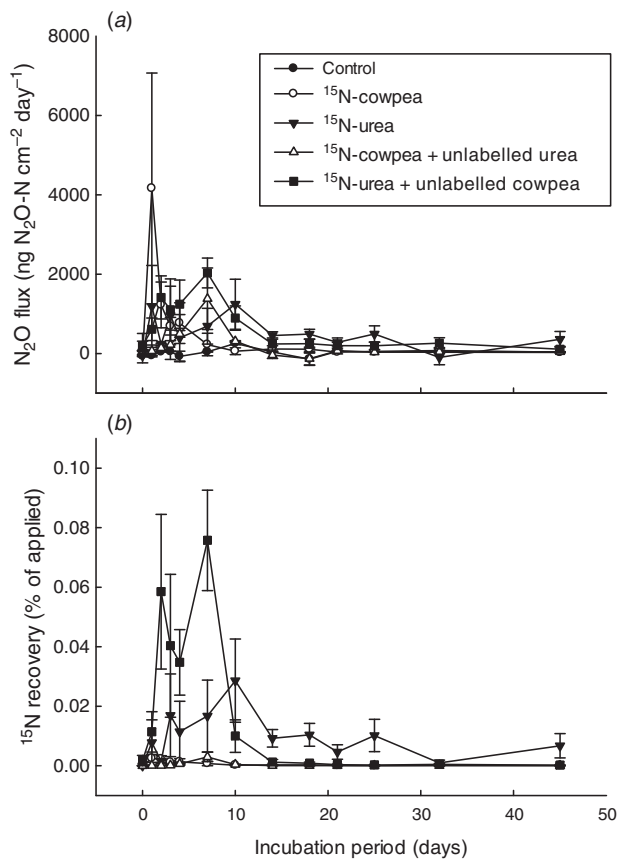


Fig. 1. Soil $\text{N}_2\text{O-N}$ flux (a) and ^{15}N recovery (b) following application of different N sources at different sampling dates. Error bars indicate one standard error.

^{15}N -labelled urea, ^{15}N -labelled cowpea + unlabelled urea and unlabelled cowpea + ^{15}N -labelled urea treatment respectively. Although the ^{15}N -labelled cowpea treatment exhibited a relatively high maximum efflux rate ($4162 \text{ ng N}_2\text{O-N cm}^{-2} \text{ d}^{-1}$), this peak in $\text{N}_2\text{O-N}$ emissions was short-lived (5 days) relative to treatments involving urea (10–20 days; Fig. 1a).

Cumulative N_2O emissions were significantly influenced by N source (Table 2 and Fig. 2). Thus, the greatest cumulative N_2O emissions were observed with the unlabelled cowpea + ^{15}N -labelled urea treatment ($21.8 \mu\text{g N}_2\text{O-N cm}^{-2}$) and were statistically identical with those for the ^{15}N -labelled urea treatment ($18.1 \mu\text{g N}_2\text{O-N cm}^{-2}$), followed by ^{15}N -labelled cowpea ($10.6 \mu\text{g N}_2\text{O-N cm}^{-2}$), ^{15}N -cowpea + unlabelled urea ($7.2 \mu\text{g N}_2\text{O-N cm}^{-2}$) and, finally, control ($1.5 \mu\text{g N}_2\text{O-N cm}^{-2}$; Fig. 2).

Soil mineral N

A significant effect of N source, sampling date and their interaction on the dependent variables soil $\text{NO}_3^- \text{-N}$ and $\text{NH}_4^+ \text{-N}$ contents was observed (Table 2). At 1 DAA, soil $\text{NO}_3^- \text{-N}$ and $\text{NH}_4^+ \text{-N}$ contents were similar in all treatments (Fig. 3); however, a rapid increase in soil $\text{NH}_4^+ \text{-N}$ occurred during 1–3 DAA. The highest soil $\text{NH}_4^+ \text{-N}$ content at 3 DAA was for the ^{15}N -labelled urea treatment ($128.9 \mu\text{g N g}^{-1}$ soil),

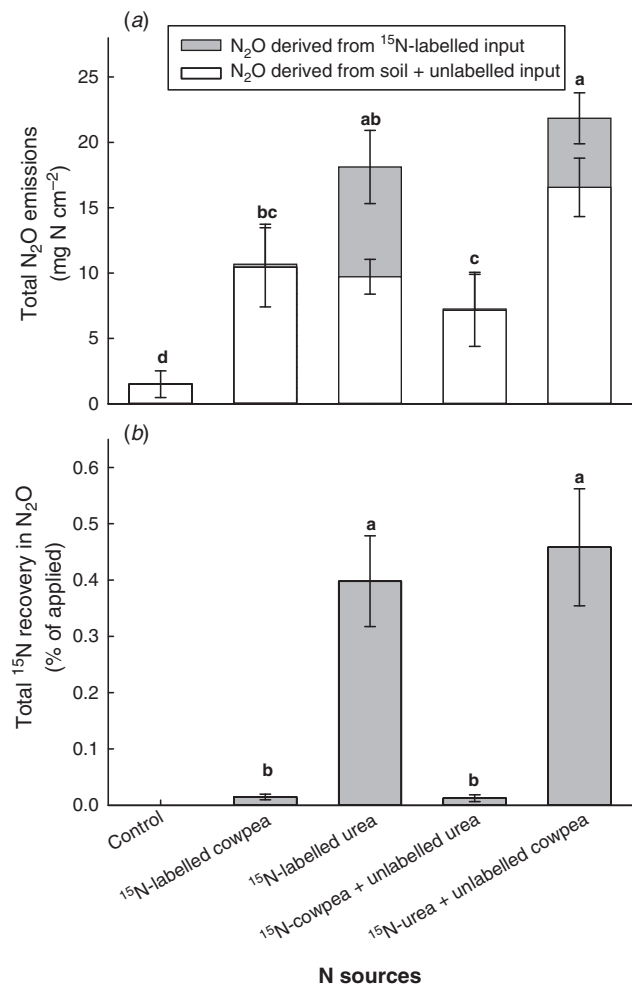


Fig. 2. Total $\text{N}_2\text{O-N}$ emissions derived from soil and unlabelled input and from ^{15}N -labelled sources (a) and total ^{15}N recovery for N-labelled sources (b). Error bars indicate one standard error. Different letters indicate that means were statistically different using Tukey's test at $P < 0.05$.

followed by ^{15}N -labelled cowpea + unlabelled urea ($65.2 \mu\text{g N g}^{-1}$ soil), unlabelled cowpea + ^{15}N -labelled urea ($50.2 \mu\text{g N g}^{-1}$ soil), ^{15}N -labelled cowpea ($1.7 \mu\text{g N g}^{-1}$ soil) and control ($1.2 \mu\text{g N g}^{-1}$ soil). Soil $\text{NH}_4^+ \text{-N}$ contents started to decline at 3 DAA (Fig. 3) and, except for the ^{15}N -labelled urea, were similar to those for the control thereafter. The soil $\text{NH}_4^+ \text{-N}$ contents under cowpea and urea (unlabelled cowpea + ^{15}N -labelled urea and ^{15}N -labelled cowpea + unlabelled urea) were similar to those for the control during the period 18–25 DAA. Applying ^{15}N -labelled urea alone resulted in high soil $\text{NH}_4^+ \text{-N}$ values throughout the experiment; in fact, the soil $\text{NH}_4^+ \text{-N}$ content in that treatment was still greater than for control at 45 DAA (Fig. 3).

The $\text{NO}_3^- \text{-N}$ contents evolved differently (Fig. 3). A gradual increase in soil $\text{NO}_3^- \text{-N}$ was observed with all urea-based treatments. In contrast, control and ^{15}N -labelled cowpea treatments exhibited a similar, very small increase in soil $\text{NO}_3^- \text{-N}$ over the incubation period (Fig. 3). Soil $\text{NO}_3^- \text{-N}$ contents in the ^{15}N -labelled cowpea + unlabelled urea and

unlabelled cowpea + ¹⁵N-labelled urea treatments increased until 25 DAA and then levelled off at 94.3 and 89.5 μg N g⁻¹ soil respectively. However, the ¹⁵N-labelled urea treatment significantly increased in soil NO₃⁻-N until 45 DAA, when it reached 198.4 μg N g⁻¹ (Fig. 3).

There was a significant relationship between soil NH₄⁺-N content and total soil N₂O fluxes - N₂O-N (ng cm⁻² d⁻¹) = 163.2 + 9.28 NH₄⁺-N (μg N g⁻¹ soil), R² = 0.33, P = 0.001 - with ~33% of soil N₂O flux explained by soil NH₄⁺-N. However, soil NO₃⁻-N was not significantly related to soil N₂O fluxes (P = 0.71).

¹⁵N recovery and N₂O emission sources

The effects of N source, sampling date and their mutual interaction on ¹⁵N recovery in N₂O gas were significant

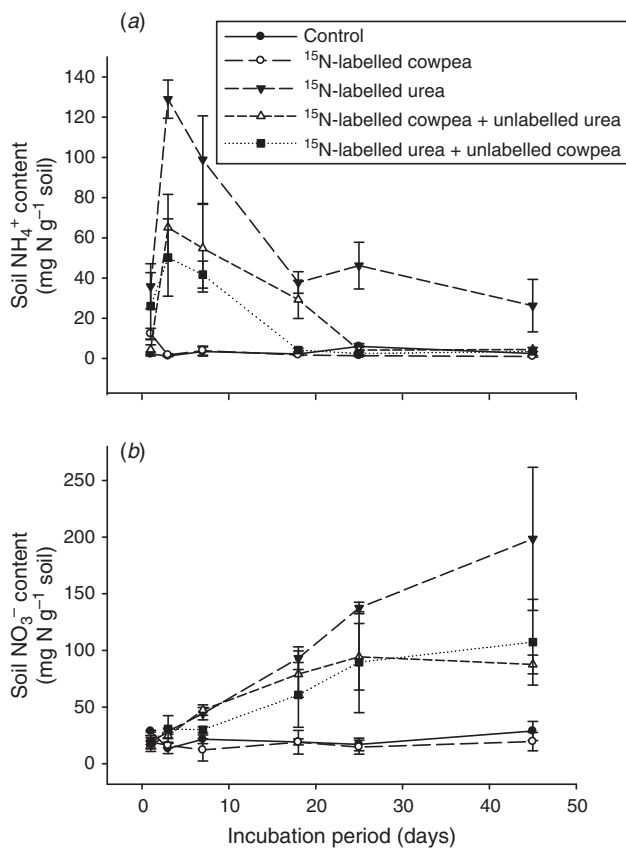


Fig. 3. Soil NH₄⁺-N (a) and NO₃⁻-N content (b) following application of different N sources at different sampling dates. Error bars indicate one standard error.

(Table 2). The recovery of ¹⁵N in N₂O gas fluxes was greatest with ¹⁵N-labelled urea (i.e. with the treatments involving ¹⁵N-labelled urea or unlabelled cowpea + ¹⁵N-labelled urea). In contrast, the lowest N₂O emissions were for cowpea residues, as confirmed by the low recoveries of ¹⁵N in treatments with ¹⁵N-labelled cowpea alone or combined with unlabelled urea. Adding urea did not increase N₂O emissions from cowpea residues; however, the residues increased the initial peak of ¹⁵N recovery resulting from urea (Fig. 1b), which was smaller than that for N₂O emissions with ¹⁵N-labelled urea alone in terms of percent applied ¹⁵N.

Cumulative ¹⁵N recovery in N₂O was greatest with ¹⁵N-labelled urea alone and combined with unlabelled cowpea biomass at 0.4 and 0.45% of applied ¹⁵N-urea (Fig. 2) respectively. Cumulative ¹⁵N recovery from cowpea residues was significantly smaller than with ¹⁵N-labelled urea, at 0.015% of applied ¹⁵N with the ¹⁵N-labelled cowpea treatment, and at 0.013% with the ¹⁵N-labelled cowpea + unlabelled urea treatment (Fig. 2).

Cumulative N₂O emission from soil under the control treatment was 1.5 μg N₂O-N cm⁻² (Fig. 2, Table 3). A significant increase in cumulative N₂O emission from unlabelled N in soil to 10.5 and 9.7 μg N₂O-N cm⁻² was observed for cowpea and urea respectively. The treatments using a combination of organic and mineral inputs resulted in significantly increased N₂O emissions from the soil-unlabelled cowpea combination relative to the soil-unlabelled urea combination. However, N₂O emissions were significantly higher from ¹⁵N-labelled urea than from ¹⁵N-labelled cowpea residues (Fig. 2).

Discussion

Consistent with results of previous studies (Frimpong and Baggs 2010; Bayer *et al.* 2015; Pimentel *et al.* 2015), the N₂O efflux peaks observed immediately after application of the N sources suggest increased N₂O production in soil by effect of microbial activity. Bayer *et al.* (2015) found 50–70% of annual soil N₂O emissions to occur within the first 40 days after winter cover crop management, whereas Frimpong and Baggs (2010) found 51–87% of such emissions arose within the first 7 days after application of residues of three tropical plant species. Because N₂O emissions usually peak immediately after an N amendment is applied, different rates may result from various soil and climate factors, and also from methodological aspects such as the extent of fractionation of the plant residues and whether they are mixed with the soil or deposited onto the soil surface, in microcosm or field studies.

Table 3. The N₂O emissions from soil, soil plus unlabelled input and ¹⁵N-labelled input by treatment
Different letters for the same N₂O source indicate that means statistically differ as per Tukey's test at P < 0.05

Treatment	N ₂ O emissions (μg N ₂ O-N cm ⁻²)		
	From soil	From soil and unlabelled input	From ¹⁵ N-labelled input
Control	1.5b	–	–
¹⁵ N-labelled cowpea	10.5a	–	0.23b
¹⁵ N-labelled urea	9.7a	–	8.40a
¹⁵ N-labelled cowpea + unlabelled urea	–	7.1b	0.10b
¹⁵ N-labelled urea + unlabelled cowpea	–	16.5a	5.28a

The significant relationship between $\text{NH}_4^+\text{-N}$ and $\text{N}_2\text{O-N}$ emissions during the incubation period suggests that N_2O was formed mainly by nitrification. This contradicts the results of previous field studies in Southern Brazil, in which denitrification was assumed to be the main process (Gomes *et al.* 2009; Zanatta *et al.* 2010; Bayer *et al.* 2015). This was probably a result of the constant, low soil moisture (60% WHC) maintained in this microcosm study restricting the denitrification and favouring nitrification (Bateman and Baggs 2005). This soil water content corresponded to ~45% of the water filled-pore space and was thus lower than the ideal level of >60% suitable for denitrification (Davidson *et al.* 2000).

Based on our results, N_2O emission was dependent on whether legume residues or inorganic fertiliser was applied on the soil. The very low recovery of ^{15}N with legume residues confirms that the N_2O peaks observed were not due to legume-N, but rather to N originally present in the soil – probably in mineral form. In contrast, the peaks in ^{15}N recovery from N fertiliser indicate that a substantial portion of N_2O fluxes was derived from added fertiliser-N.

Soil N_2O emission was increased by a factor of ~6 by the N-amendment treatments relative to the control (Table 3). This result is consistent with a significant, similar priming effect of both N sources on mineralisation of native N in soil organic matter, which was significantly more marked than that reported by Gentile *et al.* (2008). In their study, N_2O emissions from soil were increased 2–3 times by the addition of mineral N fertiliser to two types of soil (Arenosol and Lixisol), but with no significant effect for two other types of soil (Acrisol and Nitisol). The substantial effect of N amendment on accelerating mineralisation of native N in soil organic matter found here was probably strengthened by the long-term (28 years) cultivation of maize in summer and fallow in winter, both without N fertilisation. This management practice may in fact have led to the depletion of mineral N forms in soil and to a strong dependence on microbial activity of external mineral N forms in the amendment.

Cumulative N_2O emissions from urea were ~40–50 times those from cowpea biomass (Table 3). Approximately 0.42% of applied N in urea was lost as N_2O compared with only 0.014% from cowpea biomass. Our results suggest that addition of a mineral N fertiliser such as urea increases the availability of N in soil – and hence the potential for N_2O formation – as well as N losses as N_2O or N_2 . In contrast, adding an organic N input such as cowpea residues resulted in no N_2O formation from the input. These results suggest that cowpea residues are less prone to N losses than urea.

We could not determine the $\text{N}_2\text{O}/\text{N}_2$ product ratio of denitrification. Possibly, the ratio was lower for the cowpea residues than for urea, which may have masked N losses through denitrification. From a climate change standpoint, however, our data strengthen the assumption that N inputs may be an attractive choice for mitigating N_2O emissions during crop production (Bayer *et al.* 2015). Nevertheless, our results require validation under field conditions in order to provide for the potential influence of other variables such as the presence of growing plants with high N requirements. In fact, growing plants actively absorbing nutrients from the soil solution can decrease the amount of N available for N_2O formation. Therefore, the increase in N_2O emissions

following application of the N amendment might be less marked under field conditions by effect of the reduction in available N caused by plant N uptake. This effect is likely to apply to all types of N sources, but probably more markedly to urea than to legume residues.

The N_2O emissions from either urea or cowpea residues alone were unaffected by the use of combined N sources (Fig. 2b). Some authors such as Gentile *et al.* (2008) observed an interaction effect between organic and mineral N inputs that resulted in transient immobilisation of mineral N during biomass decomposition. Immobilised N was subsequently mineralised and led to a better balance between soil N availability and plant N requirements. No such interaction was observed here, however, possibly as a result of the high N content of cowpea biomass (1.58–1.94%; Table 2) leading to net N mineralisation rather than to N immobilisation (Pimentel *et al.* 2015).

Conclusions

Total N_2O emissions with urea-based treatments exceeded those with N-rich cowpea residues as a result of the latter leading to much lower N_2O emissions. Both types of N input increased N_2O emissions from soil by a factor of six relative to a control treatment without N addition. Although our results are inconclusive as to whether using a combination of N-rich legume cover crop residues and mineral N fertilisers is environmentally advantageous, introducing legume cover crops in climate-smart soil management strategies may help to mitigate N_2O emissions more efficiently than with mineral N fertilisation, and also to preserve organic matter levels and soil quality.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

The authors acknowledge funding from the German Academic Exchange Service (DAAD), Brazilian Coordination for the Improvement of Higher Education Personnel (CAPES), National Council for Scientific and Technological Development (CNPq) and Foundation for Research Support of Rio Grande do Sul State (Fapergs).

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Handling Editor: Ji-Zheng He