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

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

Abstract

In this work, we used the ^{15}N labeling technique to identify the sources of N_2O emitted by a subtropical soil following application of mineral N fertilizer (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, namely: (1) Control (no crop residue or fertilizer N application); (2) ^{15}N-labeled cowpea (200 μg N g^{-1} soil); (3) ^{15}N-labeled urea (200 μg N g^{-1} soil); (4) ^{15}N-labeled cowpea (100 μg N g^{-1} soil) + unlabeled urea (100 μg N g^{-1} soil); and (5) unlabeled cowpea (100 μg N g^{-1} soil) + ^{15}N-labeled urea (100 μg N g^{-1} soil). Cores were analyzed for total N_2O formation, $\delta^{15}N$-N_2O and $\delta^{18}O$-N_2O by CF-IRMS, as well as for total NO_3^--N and NH_4^+-N. Legume crop residues and the mineral fertilizer increased N_2O emissions from soil to 10.5 and 9.7 μg N_2O-N cm^{-2}, respectively, which are roughly six times higher than the value for the control treatment (1.5 μg N_2O-N cm^{-2}). The amount of $^{15}N_2O$ emitted from labelled ^{15}N-urea (0.40–0.45% of ^{15}N applied) was greater than that 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 fertilization in climate-smart agriculture. Judging by our inconclusive results, however, using urea in combination with N-rich residues provides no clear-cut environmental advantage.

Keywords: ^{15}N; nitrous oxide; urea; cover crops.

Introduction

Nitrous oxide (N_2O) is a major greenhouse gas (GHG) with a global warming potential 298 times greater than 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 yr^{-1} and agricultural soils continue to be among the main emission sources for this gas owing to the widespread use of mineral N fertilizers (IPCC, 2014).

Nitrous oxide production 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). Nitrous oxide 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 oxidize ammonia (NH_3) to NO_2^- ion under aerobic conditions, and then nitrite being subsequently reduced to N_2O and N_2 (Wrage et al. 2005; Kool et al. 2011).

Organic and inorganic nitrogen added to soil alters N cycling and N flow by effect of microbial activity, thereby also potentially altering formation and emission of soil N_2O (Bowman 2008; Frimpong and Baggs 2010). Mineral N fertilizers rapidly increase soil available N, often boosting N_2O emissions as a result (Zanatta et al. 2010; Shchererbak et al. 2014). Legume cover crop residues have also been found to increase soil N_2O emissions (Jarecki et al. 2009; Gomes et al. 2009), but usually to a smaller extent than N fertilizers (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 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 N_2O in tropical and subtropical agriculture arising from application of N fertilizers and/or N-rich residues of legume cover crops is scant. This is largely the result of the differential dynamics of N from mineral fertilizers and crop residues, and of the also different impact of added N in accelerating mineralization of N in soil organic matter (Gentile et al. 2008; Chen et al. 2014). The starting hypothesis of this work was that mineral N fertilizer would boost soil N_2O emissions by rapidly increasing inorganic N levels and facilitating mineralization of N present in soil organic matter; conversely, legume residues would reduce N_2O emissions by effect of the slow mineralization of added N and of the N immobilization in microbial biomass having a less marked impact on N mineralization of soil organic matter.

The primary objectives of this work were to assess total soil N_2O-N emissions and identify their sources (soil, fertilizer or legume residues) following individual or joint addition of ^{15}N-labeled residues of cowpea —a summer legume cover crop— and ^{15}N-labeled 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 (30º 6′ S, 51º 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

93 soil properties and maize yield. The experimental plot used for soil sampling had been
94 managed under no-tillage with maize during summer and fallow in winter. No N
95 fertilization was used at any time during the experimental period.
96 Undisturbed soil cores from the 0–10 cm deep soil layer were collected by using
97 5 cm wide PVC tubes that were capped and transferred to Forschungszentrum (Jülich,
98 Germany) for incubation. The most salient properties of the soil were as follows: 220 g clay
99 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
100 available P and K, determined by the Mehlich-1 method, of 18 and 109 mg kg^{-1},
101 respectively.
102
103 *Cowpea biomass labeling*
104 The crop residues used were ^{15}N-labeled and unlabeled residues of cowpea [*Vigna*
105 *unguiculata* (L.) Walp.], a summer cover crop widely used in Southern Brazil. Seeds were
106 germinated in vermiculite substrate, transplanted to pots containing an aerated modified
107 Hoagland nutritive solution and grown until the three-leaf developmental stage was
108 reached. One half of the pots contained ^{15}N-labeled urea (60 atom% ^{15}N) and the other half
109 urea with natural abundance of ^{15}N. The concentration of nutrients in solution during plant
110 growth was monitored through electric conductivity. Cowpea aboveground biomass was
111 harvested at flowering stage, oven-dried at 60 ^{o}C, chopped into 2–8 mm pieces, and
112 analyzed for total C and total N by elemental analysis, and for ^{15}N by using an IsoPrime
113 EA-IRMS instrument from Elementar Analysensysteme (Hanau, Germany). The results
114 thus obtained are shown in Table 1.
115

116 *Treatments, experimental design and incubation procedure*

117 The soil cores were subjected to five different treatments, namely: (1) Control (no residue
118 or N fertilizer added); (2) ^{15}N-labeled cowpea (200 μg N g^{-1} soil); (3) ^{15}N-labeled urea (200
119 μg N g^{-1} soil) added as an aqueous solution; (4) ^{15}N-labeled cowpea (100 μg N g^{-1} soil) +
120 unlabeled urea (100 μg N g^{-1} soil); and (5) unlabeled cowpea (100 μg N g^{-1} soil) + ^{15}N-
121 labeled urea (100 μg N g^{-1} soil). The experiment was designed as a complete randomized
122 block, with three replications.

123 The incubation experiment was performed for 45 days in 1 L Duran glass bottles
124 fitted with lids having a three-way valve for gas sampling. PVC tubes containing
125 approximately 250 g of undisturbed soil each were placed inside the bottles. The
126 temperature was kept at 24 ºC and soil moisture at 60% water holding capacity (WHC) by
127 monitoring soil weight every other day. Air samples were withdrawn for analysis 0, 1, 2, 3,
128 4, 8, 10, 14, 18, 21, 25, 32 and 45 days after N application (DAA). The glass bottles were
129 closed 1 h prior to sampling, and ambient air samples collected in parallel to measure the
130 air N_2O concentration.

131

132 *Gas and soil analyses*

133 Air samples were analyzed for N_2O, $\delta^{15}N$-N_2O and $\delta^{18}O$-N_2O by using a trace gas
134 preparation unit coupled to an IsoPrime 100 CF-IRMS instrument (Elementar
135 Analysensysteme, Hanau, Germany). The soil was analyzed for total NO_3^--N and NH_4^+-N
136 by the central analytical laboratory of Forschungszentrum. Neither $^{15}NO_3^-$ nor $^{15}NH_4^+$ was
137 determined in soil owing to their extremely high content in ^{15}N label.

138

139 *Calculations*

140 Nitrous oxide fluxes were calculated as follows:

1. $$f = \frac{dC}{dt}\,\frac{PV}{RT}\,\frac{\overline{M}}{A}$$

141 where f is the gas production rate (g cm^{-2} h^{-1}), d*C*/d*t* the change in N_2O mixing ratio within
142 the glass bottle in 1 h (ppm h^{-1}), $\overline{M}$ the gas molar mass (g mol^{-1}), *P* the pressure inside the
143 incubation bottle (1 atm), *V* the headspace in the bottle (L), *T* temperature (K) and *R* the
144 ideal gas constant (0.08205 L atm K^{-1} mol^{-1}). Cumulative N_2O emissions were calculated
145 by trapezoidal integration of the daily N_2O fluxes over a period of 45 days with the aid of
146 SigmaPlot (Systat, San Jose, CA, USA).

147 The IRMS signal at the mass-over-charge ratio (*m*/*z*) 45 represents single-labeled
148 N_2O molecules ($^{14}N\,^{15}N^{16}O$ or $^{15}N^{14}N^{16}O$), whereas that at *m*/*z* 46, after subtraction of the
149 natural ^{18}O-background of N_2O ($^{14}N^{14}N^{18}O$), represents double-labeled N_2O molecules
150 ($^{15}N^{15}N^{16}O$). Excess ^{15}N and ^{18}O (atom%) in the sample, representing single- and double-
151 labeled N_2O molecules, was calculated with provision for the average contents of ^{15}N and
152 ^{18}O in the control samples:

153 2.

154 $$Excess\ ^{15}\mathrm{N}\ \text{sample}\ (\%) =$$

155 $$(^{15}\mathrm{N}\ \text{sample} - {}^{15}\mathrm{N}\ \text{background}) - (^{15}\mathrm{N}\ \text{control} - {}^{15}\mathrm{N}\ \text{background})$$

156 3.

157 $$Excess\ ^{18}\mathrm{O}\ \text{sample}\ (\%) =$$

158 $$(^{18}\mathrm{O}\ \text{sample} - {}^{18}\mathrm{O}\ \text{background}) - (^{18}\mathrm{O}\ \text{control} - {}^{18}\mathrm{O}\ \text{background})$$

where ^{15}N and ^{18}O sample are the amounts of ^{15}N and ^{18}O, respectively, in the ^{15}N-labeled sample (%); ^{15}N and ^{18}O background the natural abundance of ^{15}N (0.36764669 %) and ^{18}O (0.20011872%), respectively; and ^{15}N and ^{18}O control the average concentration of ^{15}N and ^{18}O (%), respectively, in the samples from the control treatment.

The excess of double-labeled N_2O molecules was multiplied by 2 because each molecule contained two ^{15}N atoms. The total excess of ^{15}N (%) in the samples was calculated as follows:

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

The recovery of ^{15}N applied in the residue and/or urea as N_2O gas at each air sampling event was calculated according to Gentile et al. (2008):

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

where *Q* input is the amount of N_2O-N derived from the labeled input; *Q* sample that of N_2O-N from the sample; ^{15}N sample the total ^{15}N concentration in the sample (%); ^{15}N background the natural abundance of ^{15}N (0.36764669%) and ^{15}N input the total ^{15}N concentration in the input (%). Total ^{15}N recovery was calculated by trapezoidal integration of the daily N_2O fluxes over a period of 45 days, using the software SigmaPlot (Systat, San Jose, CA, USA).

Statistical analyses

Because of the covariant nature of the relationships among N_2O flux, ^{15}N recovery in N_2O gas, and soil NO_3^--N and NH_4^+-N contents, these dependent variables were subjected to

180 analysis of variance with the Mixed Procedure in SAS® v. 9.4 (Statistical Analysis System
181 Institute, Cary, North Carolina, USA), using treatment, sampling date, and their respective
182 two-way interactions as fixed effects, and block as random effect. The analysis of variance
183 of total N_2O emissions, total soil-derived N_2O emissions, total soil plus unlabeled input-
184 derived N_2O emissions, total labeled input-derived N_2O emissions and total ^{15}N recovery
185 was done by using a generalized linear model (viz., the GLM Procedure in SAS).
186 Differences were considered to be statistically significant at the 5% confidence level
187 ($P < 0.05$) in Tukey's honestly significant different (HSD) test. The potential relationships
188 of N_2O fluxes with the soil NO_3^--N and N-NH_4^+-N contents during the incubation period
189 were assessed by regression analysis with SigmaPlot.
190
191 **Results**
192 *N_2O fluxes and cumulative emissions*
193 Soil N_2O fluxes were influenced by N source, sampling date, and the N source × sampling
194 date interaction (Table 2). Soil N_2O efflux rates were greater within the first 20 days of
195 incubation in the treatments with N addition; however, they decreased and levelled off at
196 values similar to those for the control treatment after 20 days (Figure 1a).
197 N_2O efflux rates peaked at 255, 4162, 1242, 1381, and 2029 ng N_2O-N cm^{-2} d^{-1} for
198 the control, ^{15}N-labeled cowpea, ^{15}N-labeled urea, ^{15}N-labeled cowpea + unlabeled urea,
199 and unlabeled cowpea + ^{15}N-labeled urea treatment, respectively. Although the ^{15}N-labeled
200 cowpea treatment exhibited a relatively high maximum efflux rate (4162 ng N_2O-N cm^{-2} d^{-}
201 ^{1}), the peak in N_2O-N emissions from the ^{15}N-labeled cowpea treatment was short-lived (5
202 days) relative to the treatments involving urea (10–20 days; Figure 1a).

Cumulative N_2O emissions were significantly influenced by N source (Table 2 and Figure 2). Thus, the greatest cumulative N_2O emissions were observed with the unlabeled cowpea + ^{15}N-labeled urea treatment (21.8 μg N_2O-N cm^{-2}) and were statistically identical with those for the ^{15}N-labeled urea treatment (18.1 μg N_2O-N cm^{-2}), followed by the ^{15}N-labeled cowpea (10.6 μg N_2O-N cm^{-2}) and ^{15}N-cowpea + unlabeled urea (7.2 μg N_2O-N cm^{-2}) treatments, and, finally, the control treatment (1.5 μg N_2O-N cm^{-2}; Figure 2).

Soil mineral N

A significant effect of N source, sampling date and their interaction on the dependent variables soil NO_3^--N and NH_4^+-N contents was observed (Table 2). One day after input application (DAA), soil NO_3^--N and NH_4^+-N contents were essentially similar in all treatments (Figure 3); however, a rapid increase in soil NH_4^+-N was observed from the first DAA to the third. The highest soil NH_4^+-N content measured on the third incubation day was that for the ^{15}N-labeled urea treatment (128.9 μg N g^{-1} soil), followed by those for the ^{15}N-labeled cowpea + unlabeled urea (65.2 μg N g^{-1} soil) and unlabeled cowpea + ^{15}N-labeled urea treatments (50.2 μg N g^{-1} soil), and, finally, the ^{15}N-labeled cowpea (1.7 μg N g^{-1} soil) and control (1.2 μg N g^{-1} soil) treatments. Soil NH_4^+-N contents started to decline 3 DAA (Figure 3) and, except for the ^{15}N-labeled urea treatment, were similar to those for the control treatment thereafter. The soil NH_4^+-N contents under cowpea and urea (unlabeled cowpea + ^{15}N-labeled urea and ^{15}N-labeled cowpea + unlabeled urea) were similar to those for control treatment over the period 18–25 DAA. Applying ^{15}N-labeled urea alone resulted in high soil NH_4^+-N values throughout the experiment; in fact, the soil NH_4^+-N content with that treatment was still greater than that for the control treatment 45 DAA (Figure 3).

As can be seen from Figure 3, NO_3^--N contents evolved differently. Thus, a gradual increase in soil NO_3^--N was observed with all urea based treatments. In contrast, the control and ^{15}N-labeled cowpea treatments exhibited a similar, very small increase in soil NO_3^--N over the incubation period (Figure 3). Soil NO_3^--N contents in the ^{15}N-labeled cowpea + unlabeled urea and unlabeled cowpea + ^{15}N-labeled urea treatments increased until 25 DAA and then levelled off at 94.3 and 89.5 μg N g^{-1} soil, respectively. However, the treatment involving ^{15}N-labeled urea exhibited a significant increase in soil NO_3^--N until 45 DAA, when it amounted to 198.4 μg N g^{-1} (Figure 3).

A significant relationship between soil NH_4^+-N content and total soil N_2O fluxes was observed [N_2O-N (ng cm^{-2} d^{-1}) =163.2 + 9.28 NH_4^+-N (μg N g^{-1} soil), $R^2 = 0.33$, $P = 0.001$], with about 33% of soil N_2O flux being explained by soil NH_4^+-N. On the other hand, soil NO_3^--N was not significantly related to soil N_2O fluxes ($P = 0.71$).

^{15}N recovery and N_2O emission sources

The effects of N source, sampling date and their mutual interaction on ^{15}N recovery in N_2O gas were significant (Table 2). The recovery of ^{15}N in N_2O gas fluxes was greatest with ^{15}N-labeled urea (i.e., with the treatments involving ^{15}N-labeled urea or unlabeled cowpea + ^{15}N-labeled urea). On the other hand, the lowest N_2O emissions were observed with cowpea residues, as confirmed by the low recoveries of ^{15}N in the treatments with ^{15}N-labeled cowpea alone or in combination with unlabeled urea. Adding urea did not increase N_2O emissions from cowpea residues; however, the residues increased the initial peak of ^{15}N recovery resulting from urea (Figure 1b), which was smaller than that for N_2O emissions with ^{15}N-labeled urea alone in terms of percent applied ^{15}N.

Cumulative ^{15}N recovery in N_2O was greatest with ^{15}N-labeled urea alone and in combination with unlabeled cowpea biomass at 0.4 and 0.45% of applied ^{15}N-urea (Fig. 2), respectively. Cumulative ^{15}N recovery from cowpea residues was significantly smaller than with ^{15}N-labeled urea, at 0.015% of applied ^{15}N with the ^{15}N-labeled cowpea treatment, and at 0.013% with the ^{15}N-labeled cowpea + unlabeled urea treatment (Figure 2).

Cumulative N_2O emission from soil under the control treatment was 1.5 μg N_2O-N cm^{-2} (Figure 2, Table 3). A significant increase in cumulative N_2O emission from unlabeled nitrogen in the soil to 10.5 and 9.7 μg N_2O-N cm^{-2} was observed with cowpea and urea, respectively. The treatments using a combination of organic and mineral inputs resulted in significantly increased N_2O emissions from the soil–unlabeled cowpea combination relative to the soil–unlabeled urea combination. However, N_2O emissions from ^{15}N-labeled urea were significantly higher than those from ^{15}N-labeled cowpea residues (Figure 2).

Discussion

Consistent with the results of previous studies (Frimpong and Baggs 2010; Bayer et al. 2015; Pimentel et al. 2015), the N_2O efflux peaks observed immediately after application of the N sources suggest increased N_2O production in soil by effect of microbial activity. Bayer et al. (2015) found 50–70% of annual soil N_2O emissions to occur within the first 40 days after winter cover crop management, whereas Frimpong and Baggs (2010) found 51–87% of such emissions to arise within the first 7 days after application of residues of three tropical plant species. Because N_2O 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 soil surface, in microcosm or field studies.

The significant relationship between NH_4^+-N and N_2O-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, where 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 denitrification and favoring nitrification (Bateman and Baggs 2005). This soil water content corresponded to approximately 45% of the water filled-pore space (WFPS) and was thus lower than the ideal level for denitrification (above 60% WFPS according to Davidson et al., 2000).

Based on our results, N_2O emission was dependent on whether legume residues or inorganic fertilizer 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 forms. In contrast, the peaks in ^{15}N recovery from N fertilizer indicate that a substantial portion of N_2O fluxes was derived from added fertilizer-N.

Soil N_2O emission was increased by a factor about 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 mineralization of native N in soil organic matter, which was significantly more marked than the effect 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 fertilizer to two types of soil (Arenosol and Lixisol), but no significant effect was observed with two other types of soil (Acrisol and Nitisol). The substantial effect of N

297 amendment on accelerating mineralization of native N in soil organic matter found here
298 was probably strengthened by the long-term (28 yr) cultivation of maize in summer and
299 fallow in winter, both without N fertilization. This management practice may in fact have
300 led to the depletion of mineral N forms in soil and to a strong dependence on microbial
301 activity of external mineral N forms in the amendment.

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

309 We could not determine the N_2O/N_2 product ratio of denitrification. Possibly, the
310 ratio was lower for the cowpea residues than it was for urea, which may have masked N
311 losses through denitrification. From a climate change standpoint, however, our data
312 strengthen the assumption that N inputs may be an attractive choice for mitigating N_2O
313 emissions during crop production (Bayer et al., 2015). Nevertheless, our results require
314 validation under field conditions in order to provide for the potential influence of other
315 variables such as the presence of growing plants with high N requirements. In fact, growing
316 plants actively absorbing nutrients from the soil solution can decrease the amount of N
317 available for N_2O formation. Therefore, the increase in N_2O emissions following
318 application of the N amendment might be less marked under field conditions by effect of
319 the reduction in available N caused by plant N uptake. This effect is likely to apply to all
320 types of N sources, but probably more markedly to urea than to legume residues.

321 N_2O emissions from either urea or cowpea residues alone were unaffected by the
322 use of combined N sources (Figure 2b). Some authors such as Gentile et al. (2008)
323 observed an interaction effect between organic and mineral N inputs that resulted in
324 transient immobilization of mineral N during biomass decomposition. Immobilized N was
325 subsequently mineralized and led to a better balance between soil N availability and plant N
326 requirements. No such interaction was observed here, however, possibly as a result of the
327 high N content of cowpea biomass (1.58 – 1.94%; Table 2) leading to net N mineralization
328 rather than to N immobilization (Pimentel et al. 2015).
329

330 **Conclusions**

331 Total N_2O emissions with urea-based treatments exceeded those with N-rich cowpea
332 residues as a result of the latter leading to much lower N_2O emissions. Both types of N
333 input increased N_2O emissions from soil by a factor of 6 relative to a control treatment
334 without N addition. Although our results are inconclusive as to whether using a
335 combination of N-rich legume cover crop residues and mineral N fertilizers is
336 environmentally advantageous, introducing legume cover crops in climate-smart soil
337 management strategies may help to mitigate N_2O emissions more efficiently than with
338 mineral N fertilization, and also to preserve organic matter levels and soil quality.
339

340 **Conflicts of interest**

341 The authors declare no conflicts of interest.
342

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348

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Table captions

Table 1. C, N and ^{15}N contents of labeled and unlabeled cowpea biomass; and N and ^{15}N contents of labeled and unlabeled urea. Contents are the averages of 3 replicates each and followed by one standard deviation.

Table 2. Summary statistics showing the significance of treatment (N source), sampling date and their mutual interaction (treatment × sampling date) on N_2O flux, ^{15}N recovery in N_2O gas, and soil NO_3^--N and NH_4^+-N contents, as well as the effect of treatment on total N_2O emissions, total soil-derived N_2O emissions, total soil plus ^{14}N input-derived N_2O emissions, total ^{15}N labeled input-derived N_2O emissions and total ^{15}N recovery in N_2O gas.

Table 3. N_2O emissions from soil, soil plus unlabeled input and ^{15}N-labeled input by treatment. Different letters for the same N_2O source indicate that means were statistically different as per Tukey's test at the 5% confidence level ($P < 0.05$).

Table 1. C, N and ^{15}N contents of labeled and unlabeled cowpea biomass; and N and ^{15}N contents of labeled and unlabeled urea. Contents are the averages of 3 replicates each and followed by one standard deviation.

Input	C (%)	N (%)	^{15}N enrichment
^{15}N-cowpea	41.5 ± 0.1	1.94 ± 0.02	10.19 ± 0.08
^{14}N-cowpea	41.6 ± 0.1	1.58 ± 0.02	0.376 ± 0.002
^{15}N-urea	–	45	15
^{14}N-urea	–	45	0.367

Table 2. Summary statistics showing the significance of treatment (N source), sampling date and their mutual interaction (treatment × sampling date) on N_2O flux, ^{15}N recovery in N_2O gas, and soil NO_3^--N and NH_4^+-N contents, as well as the effect of treatment on total N_2O emissions, total soil-derived N_2O emissions, total soil plus ^{14}N input-derived N_2O emissions, total ^{15}N labeled input-derived N_2O emissions and total ^{15}N recovery in N_2O gas.

Dependent variable	Fixed effect	df	*F* value	*p* value
N_2O flux	Treatment (T)	4	6.56	<0.0001
	Sampling date (SD)	12	5.87	<0.0001
	T × SD	48	2.82	<0.0001
^{15}N recovery in N_2O gas	T	3	23.67	<0.0001
	SD	12	4.33	<0.0001
	T × SD	36	2.73	<0.0001
Soil NO_3^--N content	T	4	14.44	<0.0001
	SD	5	10.9	<0.0001
	T × SD	20	2.5	0.0037
Soil NH_4^+-N content	T	4	34.83	<0.0001
	SD	5	13.88	<0.0001
	T × SD	20	3.71	<0.0001
Total N_2O emission	T	4	9.22	0.0043
Total soil-derived N_2O emission	T	2	8.13	0.039
Total soil plus ^{14}N input-derived N_2O emission	T	1	12.93	0.0228
Labeled ^{15}N input-derived N_2O emission	T	3	11.44	0.0068
Total ^{15}N recovery in N_2O gas	T	3	10.34	0.0087

Table 3. N_2O emissions from soil, soil plus unlabeled input and ^{15}N-labeled input by treatment. Different letters for the same N_2O source indicate that means were statistically different as per Tukey's test at the 5% confidence level ($P < 0.05$).

Treatment	N_2O emissions (μg N_2O-N cm^{-2})		
	From soil	From soil and unlabeled input	From ^{15}N-labeled input
Control	1.5 b	–	–
^{15}N-cowpea	10.5 a	–	0.23 b
^{15}N-urea	9.7 a	–	8.40 a
^{15}N-cowpea + ^{14}N-urea	–	7.1 b	0.10 b
^{14}N-cowpea + ^{15}N-urea	–	16.5 a	5.28 a

Figure Captions

Fig. 1. Soil N_2O-N flux (a) and ^{15}N recovery (b) following application of different N sources at different sampling dates. Error bars indicate one standard error.

Fig. 2. Total N_2O-N emissions derived from soil and unlabeled input and from ^{15}N-labeled sources (a) and total ^{15}N recovery for N-labeled sources (b). Error bars indicate one standard error. Different letters indicate that means were statistically significant as per Tukey's test at the 5% confidence level ($P < 0.05$).

Fig. 3. Soil NH_4^+-N (a) and NO_3^--N content (b) following application of different N sources at different sampling dates. Error bars indicate one standard error.

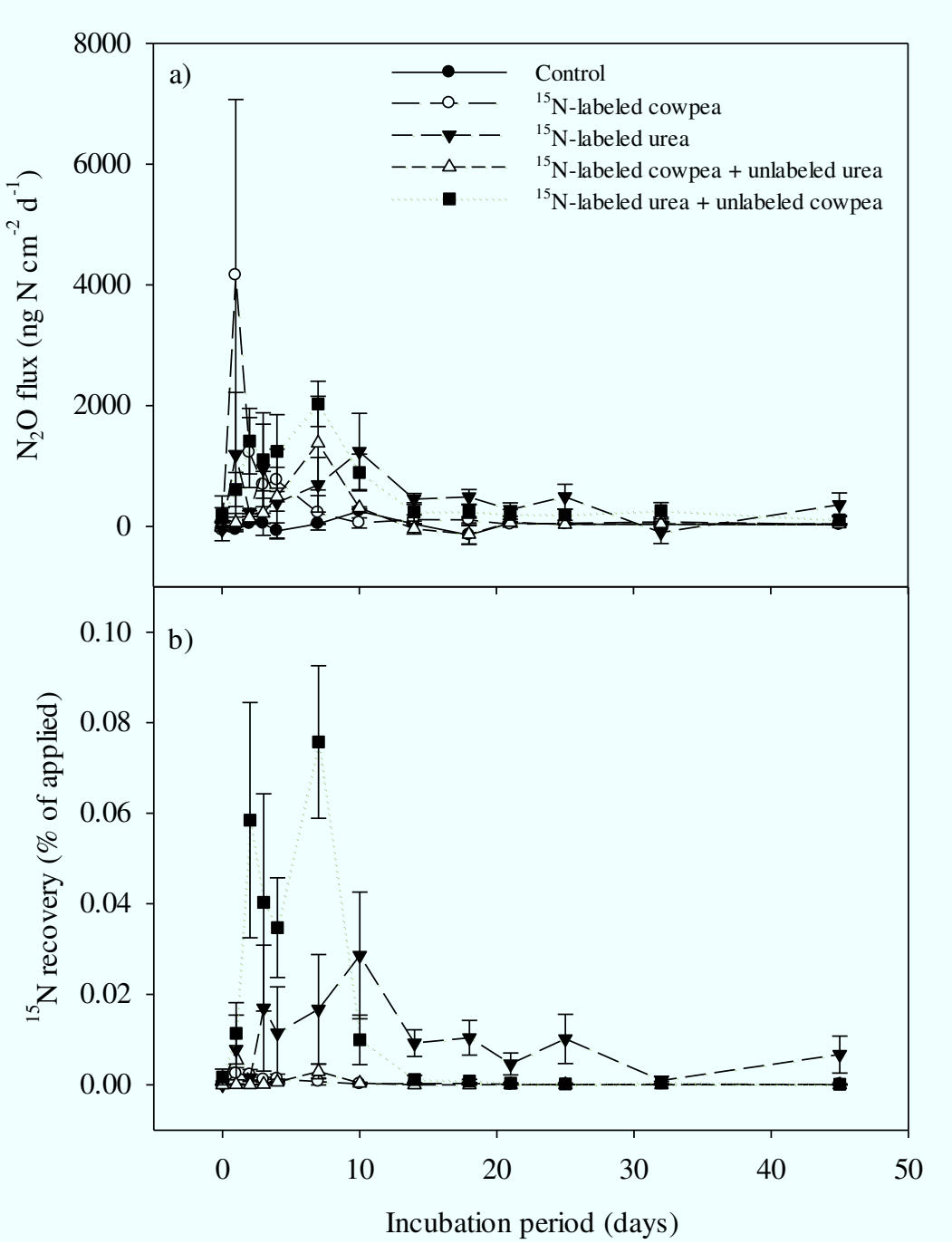
a)
b)
Control
^{15}N-labeled cowpea
^{15}N-labeled urea
^{15}N-labeled cowpea + unlabeled urea
^{15}N-labeled urea + unlabeled cowpea
N_2O flux (ng N cm^{-2} d^{-1})
^{15}N recovery (% of applied)
Incubation period (days)
8000
6000
4000
2000
0
0.10
0.08
0.06
0.04
0.02
0.00
0
10
20
30
40
50

Fig. 1.

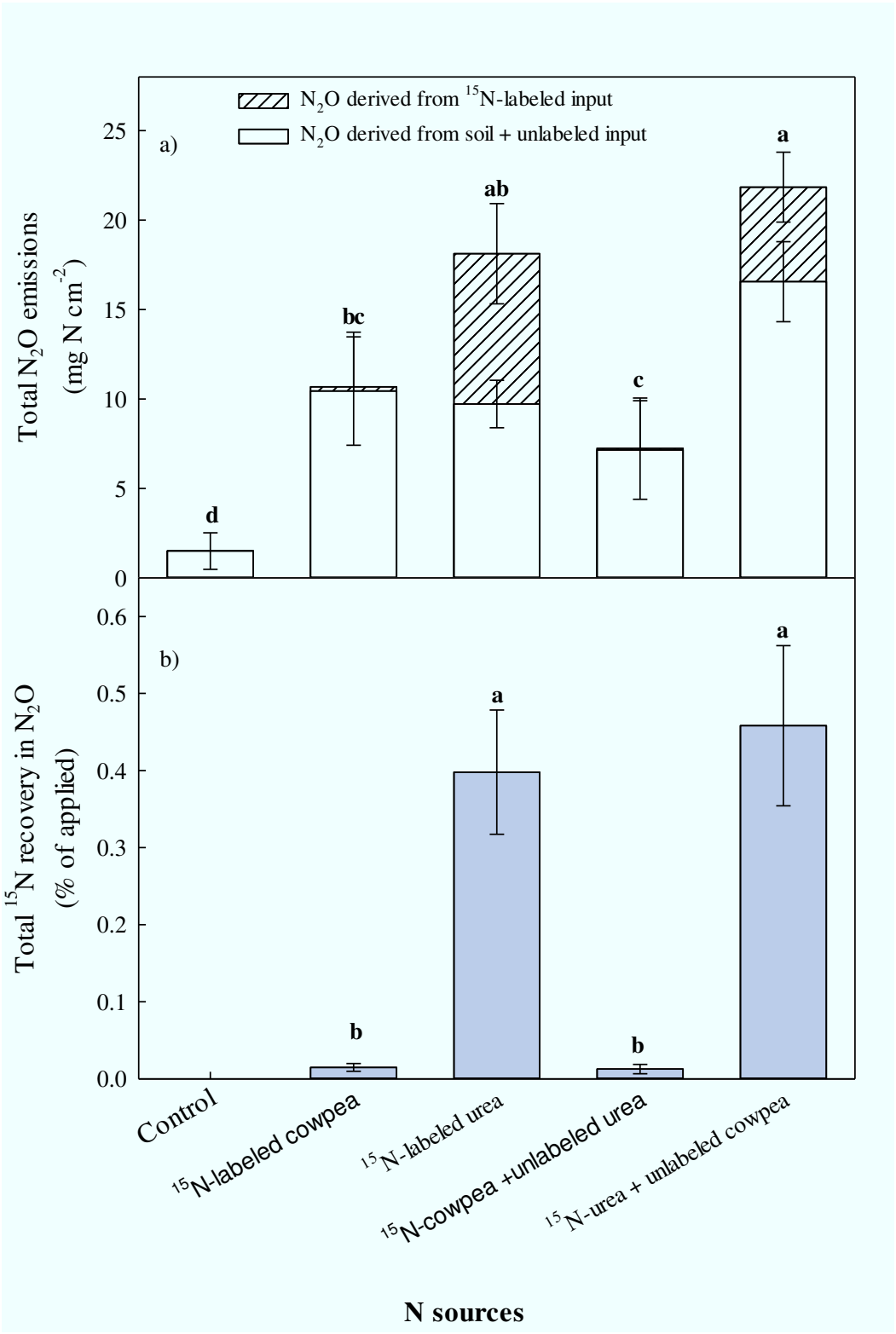
a)
N_2O derived from ^{15}N-labeled input
N_2O derived from soil + unlabeled input
Total N_2O emissions (mg N cm^{-2})
d
bc
ab
c
a
b)
Total ^{15}N recovery in N_2O (% of applied)
b
a
b
a
Control
^{15}N-labeled cowpea
^{15}N-labeled urea
^{15}N-cowpea +unlabeled urea
^{15}N-urea + unlabeled cowpea
N sources

Fig. 2.

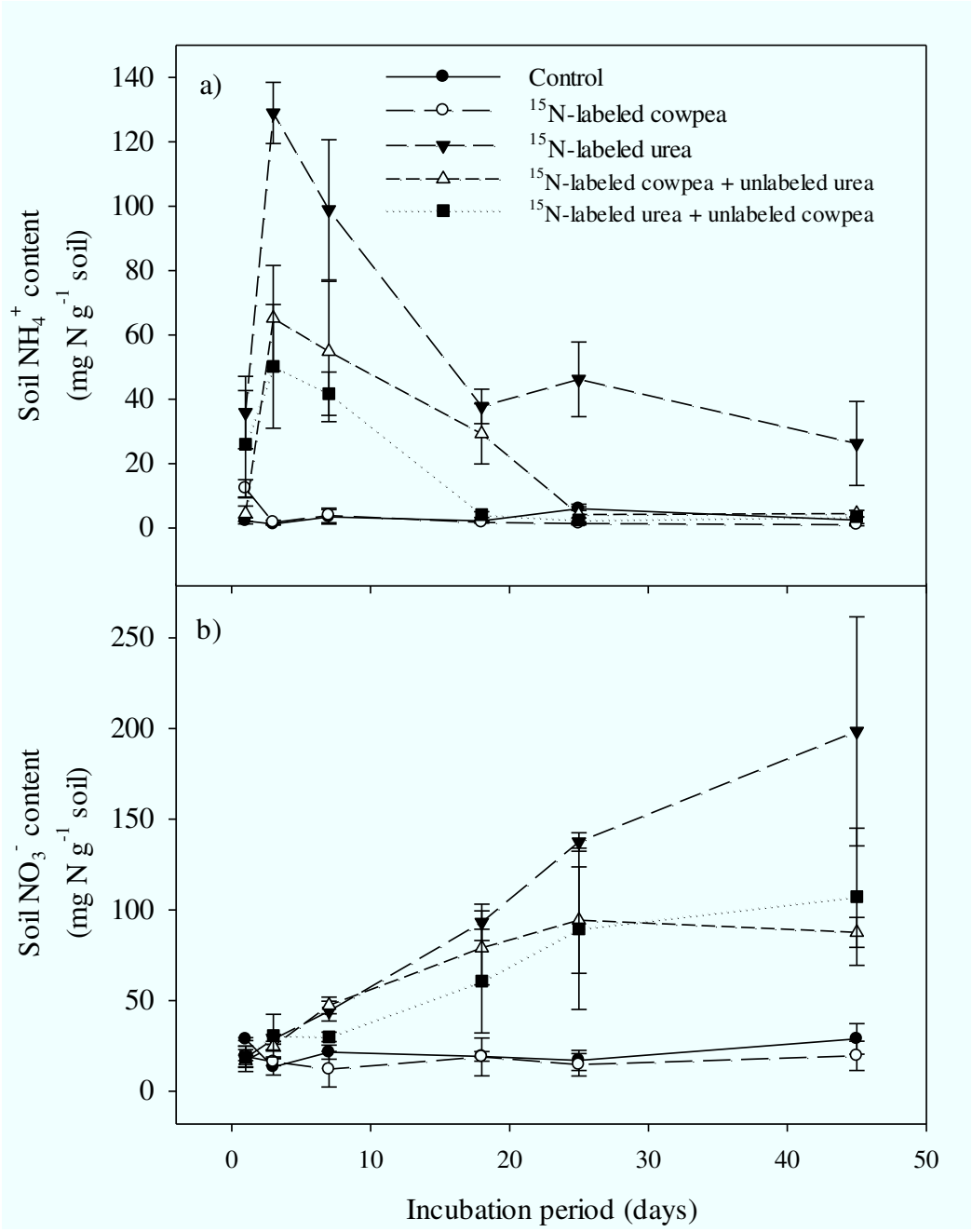
a)
b)
Control
15N-labeled cowpea
15N-labeled urea
15N-labeled cowpea + unlabeled urea
15N-labeled urea + unlabeled cowpea
Soil NH4+ content (mg N g-1 soil)
Soil NO3- content (mg N g-1 soil)
Incubation period (days)
0
20
40
60
80
100
120
140
0
50
100
150
200
250
0
10
20
30
40
50

Fig. 3.