

Nitrapyrin effectiveness in reducing nitrous oxide emissions decreases at low doses of urea in an Andosol

Mayela MONGE-MUÑOZ¹, Segundo URQUIAGA², Christoph MÜLLER^{3,4}, Juan Carlos CAMBRONERO-HEINRICHS¹, Mohammad ZAMAN⁵, Cristina CHINCHILLA-SOTO¹, Azam BORZOUEI⁶, Khadim DAWAR⁷, Carlos E. RODRÍGUEZ-RODRÍGUEZ¹ and Ana Gabriela PÉREZ-CASTILLO^{1,*}

¹Environmental Pollution Research Center (CICA), University of Costa Rica, Montes de Oca 11501 (Costa Rica)

²Embrapa Agrobiologia, Rod. BR 465, km 7, Seropédica, RJ, CEP 23891-000 (Brazil)

³Institute of Plant Ecology, Justus Liebig University Giessen, Heinrich-Buff Ring 26, Giessen 35392 (Germany)

⁴School of Biology and Environmental Science, University College Dublin, Belfield, Dublin 4 (Ireland)

⁵Soil and Water Management & Crop Nutrition Section, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Department of Nuclear Sciences and Applications, International Atomic Energy Agency, Vienna International Centre, Vienna 1400 (Austria)

⁶Nuclear Agriculture Research School, Nuclear Science and Technology Research Institute (NSTRI), Karaj 3148644111 (Iran)

⁷Department of Soil and Environmental Sciences, University of Agriculture, Peshawar 25000 (Pakistan)

(Received October 22, 2019; revised December 18, 2019)

ABSTRACT

In the tropics, frequent nitrogen (N) fertilization of grazing areas can potentially increase nitrous oxide (N₂O) emissions. The application of nitrification inhibitors has been reported as an effective management practice for potentially reducing N loss from the soil-plant system and improving N use efficiency (NUE). The aim of this study was to determine the effect of the co-application of nitrapyrin (a nitrification inhibitor, NI) and urea in a tropical Andosol on the behavior of N and the emissions of N₂O from autotrophic and heterotrophic nitrification. A greenhouse experiment was performed using a soil (pH 5.9, organic matter content 78 g kg⁻¹, and N 5.6 g kg⁻¹) sown with *Cynodon nlemfuensis* at 60% water-filled pore space to quantify total N₂O emissions, N₂O derived from fertilizer, soil ammonium (NH₄⁺) and nitrate (NO₃⁻), and NUE. The study included treatments that received deionized water only (control, CK) and two doses of ¹⁵N-enriched urea (65 (UR) and 129 mg N kg⁻¹ (UD)) without or with 350 g nitrapyrin for each 100 kg N (UR + NI and UD + NI). No significant differences were observed in soil NH₄⁺ content between the UR and UR + NI treatments, probably because of soil mineralization and immobilization (influenced by high soil organic matter content). Nitrapyrin application failed to maintain a stable pool of labeled NO₃⁻ due to the additional NO₃⁻ produced by heterotrophic nitrification, which is not effectively inhibited by nitrapyrin. After 56 d, N₂O emissions in UR (0.51 ± 0.12 mg N₂O-N kg⁻¹) and UR + NI (0.45 ± 0.13 mg N₂O-N kg⁻¹) were not significantly different; by contrast, emissions were 36.3% lower in UD + NI than in UD. It was concluded that the soil organic N mineralization and heterotrophic nitrification are the main processes of NH₄⁺ and NO₃⁻ production. Additionally, it was found that N₂O emissions were partially a consequence of the direct oxidation of the soil's organic N *via* heterotrophic nitrification coupled to denitrification. Finally, the results suggest that nitrapyrin would likely exert significant mitigation on N₂O emissions only if a substantial N surplus exists in soils with high organic matter content.

Key Words: autotrophic nitrification, heterotrophic nitrification, mineral N, ¹⁵N tracer, nitrification inhibitor, tropical grass

Citation: Monge-Muñoz M, Urquiaga S, Müller C, Cambronero-Heinrichs J C, Zaman M, Chinchilla-Soto C, Borzouei A, Dawar K, Rodríguez-Rodríguez C E, Pérez-Castillo A G. 2021. Nitrapyrin effectiveness in reducing nitrous oxide emissions decreases at low doses of urea in an Andosol. *Pedosphere*. 31(2): 303–313.

INTRODUCTION

In the tropics, grassland systems used for grazing are frequently fertilized, which may potentially exert a high impact on nitrous oxide (N₂O) emissions. Nitrogen (N) losses to the environment as N₂O are of concern because of its warming potential, which is 265 times higher relative to that of carbon dioxide (CO₂), and its action as an ozone-depleting agent (Myhre *et al.*, 2013). Most of the data reported on this topic are from temperate systems, and information regarding tropical regions is limited (Breuer *et al.*, 2002), particularly

from grasslands with high soil organic matter content (above 50 g kg⁻¹) (Molina and Meléndez, 2002).

Production of N₂O in soils is governed by various complex microbial, physical, and chemical processes (Zaman *et al.*, 2012). Different environmental and biological conditions and physicochemical characteristics of soils influence the relative proportions of the end products resulting from these processes (Volpi *et al.*, 2017). Autotrophic nitrification (oxidation of ammonium (NH₄⁺) to nitrate (NO₃⁻)) and denitrification (dissimilatory reduction of NO₃⁻ and nitrite (NO₂⁻) to N₂O and dinitrogen (N₂)) are linked to N ferti-

*Corresponding author. E-mail: ana.perezcastillo@ucr.ac.cr.

lization (Wrage *et al.*, 2001; Zaman *et al.*, 2008; Saggar *et al.*, 2013; Zhu *et al.*, 2013), while a mechanism known as heterotrophic nitrification involves the oxidation of organic N to NO_3^- (Müller *et al.*, 2014; Zhang *et al.*, 2015).

In general, N application rates in grasslands are higher in temperate regions (*ca.* 57 kg N ha⁻¹ application⁻¹) (Koci and Nelson, 2016) compared to in the tropics (*ca.* 42–46 kg N ha⁻¹ application⁻¹) (Rivera, 2008; Zaman *et al.*, 2013). In tropical systems, N fertilization is subdivided into six or seven applications (Cabalceta, 1999), while in temperate farming systems, N is usually applied twice a year. Thus, the addition of nitrification inhibitors (NIs) to N fertilizers has been reported as an effective management practice for improving N use efficiency (NUE), given that N is retained in the root zone for a longer period, providing more time for plant uptake, and potentially reducing N loss from the soil-plant system under high application rates (Koci and Nelson, 2016).

The NIs, such as 2-chloro-6-(trichloromethyl) pyridine (nitrapyrin) and 3,4-dimethylpyrazole phosphate (DMPP), have been reported to mitigate N₂O emissions in agricultural systems with high N demand (Ruser and Schulz, 2015; Alonso-Ayuso *et al.*, 2016). The majority of NIs inhibit the enzyme ammonia monooxygenase (AMO) (Ruser and Schulz, 2015). The action on AMO delays the oxidation of NH_4^+ to NO_2^- , the first stage of autotrophic nitrification, thus reducing NO_3^- leaching losses and N₂O emission *via* nitrification (Trenkel, 2010; Dawar *et al.*, 2011).

The effect of NIs on N₂O emission may depend on experimental conditions. For instance, although Akiyama *et al.* (2010) and Ruser and Schulz (2015) reported in meta-analysis a significant reduction in N₂O, Dougherty *et al.* (2016) and Nauer *et al.* (2018) did not see any effect of applying urea with NIs in Australian dairy farms with ryegrass (*Lolium perenne* L.). Similarly, Pérez-Castillo *et al.* (2021) reported no effect of nitrapyrin in a Costa Rican Andosol dedicated to dairy farming, with *Cynodon nlemfuensis* as dominant grass. Contrary to these results, experiments on intact soil cores with addition of urea plus nitrapyrin revealed that addition of NIs increased NH_4^+ -N retention and supported a lower accumulation of soil NO_3^- -N content with a considerably reduced N₂O flux, and a 30%–40% increase in the recovery of the total mineral N, at moisture conditions close to field capacity (Abbasi *et al.*, 2003).

Thus, given the low mitigation action of nitrapyrin reported for Costa Rican Andosols (Pérez-Castillo *et al.*, 2021), this study was set up under controlled conditions (greenhouse) with the aims to: i) get more information on the behavior of N and the processes of N₂O production in soil, differentiating autotrophic and heterotrophic nitrification using ¹⁵N tracer technique, and ii) assess if the nitrapyrin mitigation effect on N₂O emissions is affected by urea application rates. In addition, potential differences in microbial community profiles were also investigated.

MATERIALS AND METHODS

Experiment setup

Soil samples (0–15 cm) were collected from a grassland site with *Cynodon nlemfuensis* as the dominant grass, in the Alfredo Volio Mata Experimental Station of the University of Costa Rica (UCR), located in Ochomogo, Cartago (9°54'39.08" N, 83°57'16.99" W, 1 584 m above sea level). The soil was classified as Typic Haplustands, with a sandy clay loam texture (28% clay, 12% silt, and 60% sand) (Mata *et al.*, 2016). The soil was air-dried, sieved through a 5-mm mesh, and analyzed for basic soil properties (Table I). Soil pH was measured at a soil:water ratio of 1:2.5. Soil organic carbon (C) and total N were determined by dry combustion (Dumas method) using a vario MACRO cube CN analyzer (Elementar Analysensysteme GmbH, Germany). Effective cation-exchange capacity (ECEC) was determined by extraction of cations using 1 mol L⁻¹ ammonium acetate buffered at pH 7. Copper was extracted by KCl-Olsen methodology and quantified with an atomic absorption spectrometer (novAA 400p, Analytik Jena, Germany). The soil was characterized by high fertility with a C/N ratio of 9.8 and a high organic matter (Cabalceta and Molina, 2006). The phosphorus content and minor elements were above the recommended levels for *C. nlemfuensis* (Cabalceta and Molina, 2006; Rivera, 2008). The high copper content is of particular interest because it acts as a cofactor of the AMO enzyme, the nitrapyrin's target (Duncan *et al.*, 2016).

TABLE I

Chemical properties of the sieved (< 5 mm) Andosol from the Alfredo Volio Mata Experimental Station of the University of Costa Rica

pH	Acidity	ECEC ^{a)}	Acidity	P	Cu	Total C	Total N
(H ₂ O)	cmol L ⁻¹	—	%	—	mg L ⁻¹	—	g kg ⁻¹
5.9	0.1	15.6	0.9	42.8	25.8	54.8	5.6

a) Effective cation-exchange capacity.

Based on the gravimetric soil water contents (five replications), fresh soil equivalent to 950 ± 5 g of dry soil was placed in each pot and packed to a bulk density of 0.65 g cm⁻³. Two months before the initiation of the treatments, *C. nlemfuensis*, from the original soil location, was transplanted to the pots.

During the period of grass establishment, the pots were kept in a protected environment. Water was applied to the grass in each pot three times a week, and the grass was cut to a height of approximately 5 cm after 28 and 56 d of planting. This ensured the proper development of the pasture and their root system before the treatments were applied and prevented pasture aging through successive cuts (Villalobos and Arce, 2014).

The trial was set up in two sets of pots, using a complete randomized block design. The first set, for measuring N_2O emissions, included five treatments and four replications (total 20 pots). The second set, for the destructive analysis of mineral N variation in six sampling days, included only three treatments and three replications (total 54 pots). The treatments, detailed in Table II, were applied once after the cut at day 56 of the transplanting of the grass. Nitrogen was applied as urea at two rates, 65 and 129 mg N kg⁻¹. The first dose, equivalent to 41.7 kg N ha⁻¹, represented one of six fractions that are usually applied in dairy farming to reach the recommended dose of 250 kg N ha⁻¹ year⁻¹ for *C. nlemfuensis*, while the second corresponded to twice that dose.

Nitrapyrin and ¹⁵N-labeled urea (2 atom%) were applied in solutions to achieve a homogenous distribution. Nitrapyrin working solution with a concentration of 32 mg L⁻¹ (below the water solubility of nitrapyrin) was prepared with deionized water at 25 °C. A volume of 25 mL of the working solution was added drop by drop on the soil surface of each pot according to the predefined treatment. Nitrapyrin was applied 2 d before the fertilization because previous studies had shown that in permanent pastures, the application of N process inhibitors a few days before the N application was more effective in reducing the combined losses of N as NH₃, N₂O, and NO₃⁻, with respect to simultaneous or subsequent application (Zaman and Nguyen, 2012). After urea was applied, deionized water was added by weight to achieve 60% water-filled pore space (WFPS) in all the pots.

Control of WFPS

The soil in each pot was maintained at 60% WFPS by weighing and adding deionized water. The gravimetric humidity (H_g) needed to reach that level of WFPS was determined using the following equation (Martins *et al.*, 2017):

$$\text{WFPS} = \frac{H_g \times \text{Bulk density}}{\text{Porosity}} \times 100 \quad (1)$$

The moisture adjustment by weight did not consider the change in the biomass of the grass and was carried out every day the first week after the application of the

treatments and three times a week from day 7 onwards. On the sampling dates for N₂O, the humidity adjustment was made 15–30 min before each flux was measured. During the test, NO₃⁻ leaching was prevented by keeping the soil moisture below field capacity, which had previously been determined by saturating the soil with water and measuring humidity and weight, after 48 h of drainage.

Soil mineral N analysis and isotopic monitoring

Soil mineral N, as NH₄⁺-N and NO₃⁻-N, was determined on days 0, 2, 5, 12, 28, and 56 after treatment application. The soil of each pot was homogenized, after which a 25-g sample was immediately extracted with 100 mL 2 mol L⁻¹ KCl (99.999% purity, Suprapur®, Merck, Germany) for 2 h using an orbital shaker at 200 r min⁻¹. The concentrations of NH₄⁺ and NO₃⁻ were determined by flow injection analysis on a Lachat FIA-8000 continuous flow analyzer (Hach, USA).

Contents of soil ¹⁵NH₄⁺ and ¹⁵NO₃⁻ + ¹⁵NO₂⁻ were analyzed by the ¹⁵N diffusion method modified by Mathieu *et al.* (2006a), in which NH₄⁺ and NO₃⁻ + NO₂⁻ were sequentially converted from KCl extracts by first alkalizing the solution with approximately 0.1 g MgO and then, with 0.5 g Devarda's alloy. Both NH₄⁺ and NO₃⁻ + NO₂⁻ were collected on a GF/D glass fiber disc suspended on a stainless-steel wire and impregnated with 10 μL 2.5 mol L⁻¹ KHSO₄. The ¹⁵N isotopic analyses were carried out by the Laboratory of Stable Isotope Facility of the Department of Plant Sciences of the University of California, USA.

The percentages of ¹⁵NH₄⁺ and ¹⁵NO₃⁻ derived from fertilizer (¹⁵NH₄⁺_{dff} and ¹⁵NO₃⁻_{dff}, respectively), *i.e.*, urea, were calculated using the following equations:

$$^{15}\text{NH}_4^+_{\text{dff}} = \frac{^{15}\text{N atom}\% \text{ excess in NH}_4^+ \text{ from sample}}{^{15}\text{N atom}\% \text{ excess in urea}} \times 100 \quad (2)$$

$$^{15}\text{NO}_3^-_{\text{dff}} = \frac{^{15}\text{N atom}\% \text{ excess in NO}_3^- \text{ from sample}}{^{15}\text{N atom}\% \text{ excess in urea}} \times 100 \quad (3)$$

Measurement of N₂O emissions and the isotopic signal of ¹⁵N₂O

Nitrous oxide fluxes were measured immediately after urea application, and then every day during the first week,

TABLE II

Details of the treatments in the pot experiment conducted with an Andosol from the Alfredo Volio Mata Experimental Station of the University of Costa Rica

Treatment	Description	N application rate g N kg ⁻¹ soil	Nitrapyrin application rate g kg ⁻¹ N	¹⁵ N enrichment in urea atom%
CK	Control, watering with deionized water	0	0	0
UR	Urea applied at the recommended dose	0.065	0	2
UR + NI	Urea applied at the recommended dose plus nitrapyrin	0.065	3.5	2
UD	Urea applied at approximately twice the recommended dose	0.129	0	1
UD + NI	Urea at approximately twice the recommended dose plus nitrapyrin	0.129	3.5	1

three times during the second week, twice a week during the third and the fourth weeks, and finally, every seven days until day 56 after fertilization. Emissions of N₂O and the isotopic signal of ¹⁵N₂O were monitored using a cavity ring-down spectrometer (CRDS, Picarro G5102-i, Santa Clara, USA) operated in close mode at default company settings, and the static chamber methodology. The chamber, a 12-L cylindrical plastic container, consisted of a lid with two ports, one for the air outlet to the analyzer and another for the re-entry of air into the chamber. A Nafion dryer (PermaPure LLC., Halma Holdings Company, USA) was placed to remove the excess water on the analyzer inlet.

Each pot was sampled for 15 min after the chamber was closed, and the system was purged with ambient air for 5 min between measurements. The air temperature inside the chamber was recorded during the measuring period, and the atmospheric pressure was taken from the average daily value reported by the Station at the Geophysical Research Center of the University of Costa Rica. Proper operation of the CRDS was checked by measuring the N₂O concentration of an ultrapure air cylinder and the concentration of a standard gas (0.45 μmol N₂O mol⁻¹ gas in air) with an uncertainty of 3%, at the beginning and end of each sampling day. The minimum detectable flux (MDF), 0.128 μg N₂O-N kg⁻¹ soil h⁻¹, was calculated following Christiansen *et al.* (2015) for the headspace volume of 0.0015 m³ (after subtracting the volume occupied by the pot), a dry soil weight of 0.95 kg, and a chamber mean air temperature of 23 °C. The quantification limit, 0.384 μg N₂O-N kg⁻¹ soil h⁻¹, was set to three times the MDF. The flux of N₂O (F , μg N₂O-N kg⁻¹ soil h⁻¹) was calculated using the following equation:

$$F = \frac{\delta C}{\delta t} \times \frac{\eta M}{M_s} \quad (4)$$

where $\delta C/\delta t$ is the change of N₂O concentration with time (μmol N₂O mol⁻¹ headspace air h⁻¹), η is the moles of gas in the headspace, calculated using the ideal gas law, M is the N mass (28 μg N μmol⁻¹ N₂O), and M_s is the dry soil weight (kg).

The change of N₂O in the headspace was evaluated using a second-order polynomial fit, with the obtained concentration *vs.* enclosure time. The flux at the time of coverage ($t = 0$) was estimated using the slope, *i.e.*, derivative of the polynomial function (Christiansen *et al.*, 2015). The parameter η was evaluated with temperature and pressure recorded during the sampling of the pot, and the calculated volume, taking into account the volume of the chamber, the volume occupied by the pots, the system of hoses, and the equipment cavity.

The quadratic regression coefficient of N₂O flux was used as an evaluation and rejection criterion for the N₂O flux data analysis. If the quadratic regression coefficient was less

than 0.96, and the flux data were lower than the detection limit, the values were replaced by the detection limit. If they were greater than 0.13 μg N₂O-N kg⁻¹ soil h⁻¹ but lower than the quantification limit, the reported values were kept. Finally, if the data were greater than the quantification limit, and the quadratic regression coefficient of concentration change rate of N₂O was lower than 0.96, the N₂O fluxes were rejected.

Cumulative N₂O emissions value (E_c , mg N₂O-N kg⁻¹ soil) were calculated following the trapezoidal method:

$$E_c = \sum_{i=1}^n \left[F_i + F_{i+1} + \frac{F_i + F_{i+1}}{2} \times (t_{i+1} - t_i - 1) \right] \times \frac{1}{10^3} \quad (5)$$

where F_i and F_{i+1} (μg N₂O-N kg⁻¹ soil h⁻¹) are the N₂O flux at time t_i and t_{i+1} (h), respectively. The conversion factor 10³ was required to obtain values in units of mg kg⁻¹ (Chen *et al.*, 2014).

The isotope value of the ¹⁵N₂O emitted ($\delta^{15}\text{N}_2\text{O}$, ‰) was obtained by Keeling plot, in which the X -axis corresponds to the reciprocal of the concentration ($1/\text{N}_2\text{O}$) and Y -axis to the value of the isotopic signal of the ¹⁵N₂O emitted ($\delta^{15}\text{N}$, ‰) accumulated at time t . In this linear graph of descending slope, the intercept is equal to $\delta^{15}\text{N}$ (Pataki *et al.*, 2003). Then, $\delta^{15}\text{N}_2\text{O}$ was converted to ¹⁵N abundance (atom‰) using the following equation:

$$^{15}\text{N abundance} = \delta^{15}\text{N}_2\text{O} \times \frac{0.3663\%}{1000} + 0.3663\% \quad (6)$$

where 0.3663% is the natural abundance of ¹⁵N in air.

Finally, the percentage of ¹⁵N₂O derived from fertilizer (¹⁵N₂O_{diff}) was calculated as the quotient of ¹⁵N enrichment (atom‰) from the sample and ¹⁵N enrichment (atom‰) in urea:

$$^{15}\text{N}_2\text{O}_{\text{diff}} = \frac{^{15}\text{N atom}\% \text{ excess in N}_2\text{O from sample}}{^{15}\text{N atom}\% \text{ excess in urea}} \times 100 \quad (7)$$

Analysis of NUE

By definition, NUE is the ratio of the N (derived from the ¹⁵N-enriched urea) taken up by plants to the total amount of N added (Tubert-Brohman *et al.*, 2013). The grass was cut 28 and 56 d after the application of the ¹⁵N-labeled urea. Each sample was dried in an oven at 60 °C for 72 h. After the weight of the dry foliage was determined, the whole sample was milled to powder with a ball mill. The total N content in the foliage samples was quantified by dry combustion in an N autoanalyzer using the Dumas method in the Agronomic Research Center, UCR. The ¹⁵N

content in the samples was analyzed using a continuous-flow isotope-ratio mass spectrometer (Finnigan DELTAplus, Thermo Fisher Scientific, Germany) coupled to the output of an ECS4010 Costech total C and N analyzer (Finnigan MAT, Germany) in the John Day Stable Isotope Laboratory at Embrapa Agrobiologia (RJ), Brazil. The amount of N derived from fertilizer in the plant (N_{dfp} , g) was calculated according to the following equation:

$$N_{dfp} = \frac{N_{dff} \times N_{AC}}{100} \quad (8)$$

where N_{AC} is the accumulated N (g) and calculated using Eq. 9 and N_{dff} is the percentage of N derived from the fertilizer (%) and calculated using Eq. 10:

$$N_{AC} = \frac{M_D \times TN}{100} \quad (9)$$

where M_D is the dry matter of the foliage (g) and TN is total N of the foliage (%);

$$N_{dff} = \frac{A_{foliage}}{A_{fertilizer}} \times 100 \quad (10)$$

where $A_{foliage}$ and $A_{fertilizer}$ are ^{15}N atom% excess (atom%) in foliage and fertilizer, respectively.

Finally, NUE (%) was calculated as:

$$\text{NUE} = \frac{N_{dfp}}{\text{N application rate}} \times 100 \quad (11)$$

Microbial community analysis

Total genomic DNA was obtained from soil samples (0.25 g) of the UR, UR + NI, UD + NI, and control treatments at days 0, 28, and 56 (12 samples). Extraction was carried out using PowerSoil DNA isolation kit (MO BIO Laboratories, USA) following the manufacturer's instructions. Extracted DNA was quantified, checked for purity with a 2000c NanoDrop spectrophotometer (Thermo Fisher Scientific, Germany), and maintained at -20°C prior to amplification of the 16S rRNA genes. Polymerase chain reaction (PCR) was performed with the primers 907r/341f and denaturing gradient gel electrophoresis (DGGE) analysis of the amplicons was performed with a DCode system (Bio-Rad, USA), both according to the protocol of Castro-Gutiérrez *et al.* (2016).

Statistical analysis

Data distribution normality was tested using the Shapiro-Wilk test. The outliers of the N_2O fluxes were evaluated using the Grubbs test. In total, 10% of all flows (34 data) were rejected. These values corresponded to pots with dead grass or with the pest *Phyllophaga* sp. The treatments were

compared using an analysis of variance (ANOVA). In the case of N_2O fluxes per day of measurement, the analysis was carried out using incomplete randomized blocks because of the rejected data. The comparison of N_2O cumulative emissions was done after 28 and 56 d of urea application. In all cases, significant differences between treatments were defined at $P < 0.05$, and the Tukey test was used to separate the means. Data are presented as mean \pm standard deviation. Statistical data analyses were performed using Infostat version 2017.

RESULTS AND DISCUSSION

Soil mineral N

The contents of soil mineral N, NH_4^+ , and NO_3^- increased rapidly during the first several days after urea application (Fig. 1a, b). The increase in soil NH_4^+ content up to $16.5 \pm 0.5 \text{ mg N kg}^{-1}$ for UR and $17.8 \pm 0.9 \text{ mg N kg}^{-1}$ for UR + NI, observed in the first 24 h of N fertilizer application, was likely due to the rapid urea hydrolysis at 60% WFPS (Martins *et al.*, 2017). Soil NO_3^- content reached its maximum ($20.9 \pm 5.9 \text{ mg N kg}^{-1}$ in UR and $22.7 \pm 1.8 \text{ mg N kg}^{-1}$ in UR + NI) after 5 d of fertilization. The increase in NO_3^- content and the simultaneous decrease in NH_4^+ content between days 0 and 5 in treatments UR and UR + NI, indicated rapid net nitrification following urea hydrolysis. This finding was corroborated by the enrichment of the $^{15}\text{NO}_3^-$ pool derived from the $^{15}\text{NH}_4^+$ pool (Fig. 1c, d) (Mathieu *et al.*, 2006b).

Ten days after fertilizer application, soil NH_4^+ and NO_3^- contents decreased quickly by more than 90%, and reached a baseline by day 28 after fertilization (Fig. 1a, b). This was partially attributed to rapid plant uptake. In addition, the reduction of NH_4^+ was due to nitrification, as mentioned earlier, and potentially to immobilization. The decrease of NO_3^- also reflected denitrification processes, given that leaching losses were avoided.

No significant differences were observed in soil NH_4^+ and NO_3^- contents between the UR and UR + NI treatments (Fig. 1a, b). A reason for the non-significant difference in NH_4^+ accumulation could be the immobilization of soil NH_4^+ , influenced by high soil organic matter content, which would mask any reduction in nitrification (McTaggart *et al.*, 1997). The dilution of $^{15}\text{NH}_4^+$ derived from ^{15}N -labeled urea confirmed the NH_4^+ production at low or natural ^{15}N abundance *via* mineralization in both treatments (Table III) (Mathieu *et al.*, 2006a). In addition, the significantly greater ($P < 0.05$) decrease in the $^{15}\text{NH}_4^+$ pool between days 2 and 5 in pots fertilized with urea only (Fig. 1c) indicated that mineralization was contributing to the accumulation of NH_4^+ caused by a reduction in the nitrification by nitrapyrin application.

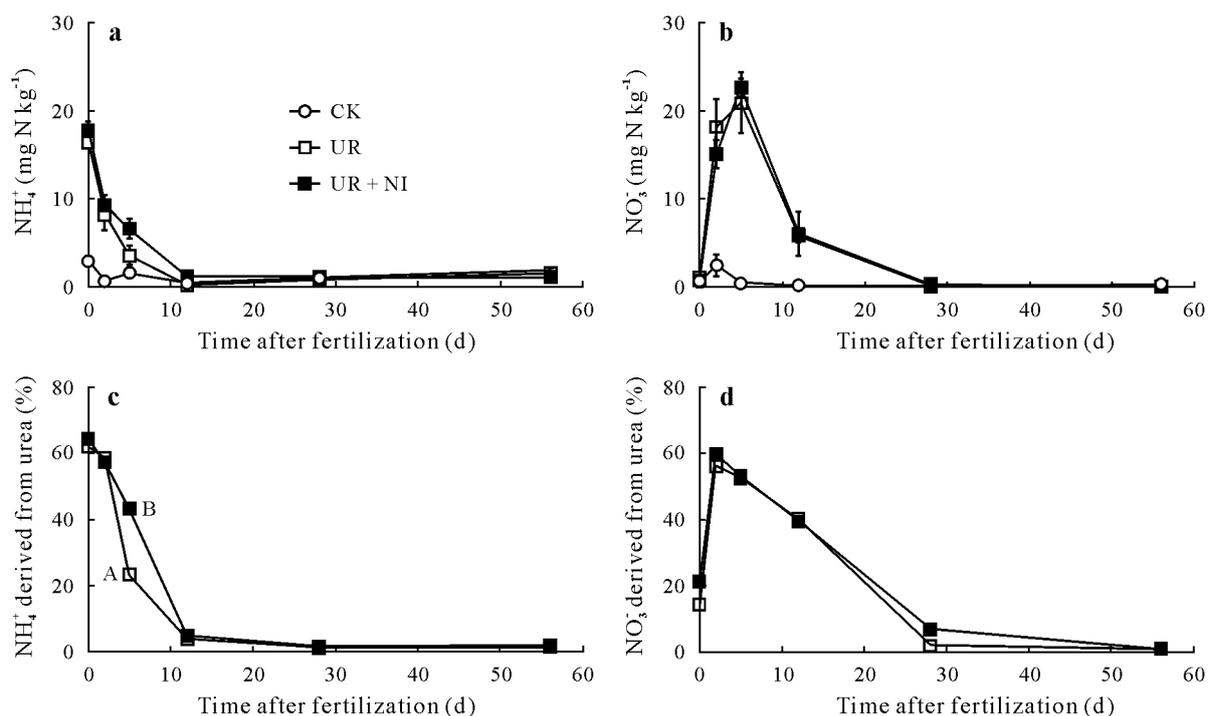


Fig. 1 Soil ammonium (NH_4^+) (a) and nitrate (NO_3^-) (b) and the percentages of NH_4^+ (c) and NO_3^- (d) that were derived from ^{15}N -labeled urea in the CK, UR, and UR + NI treatments of a pot experiment conducted with an Andosol from the Alfredo Volio Mata Experimental Station of the University of Costa Rica. See Table II for treatment details. Vertical bars indicate standard errors ($n = 3$). In subfigure c, the uppercase letters A and B beside the points at day 5 represent significant difference based on Tukey test ($P = 0.05$).

TABLE III

Variation of the ^{15}N enrichment in soil ammonium (NH_4^+) and nitrate (NO_3^-) in the treatments^{a)} of ^{15}N -labeled urea application (UR) and ^{15}N -labeled urea plus nitrapyrin application (UR + NI) of a pot experiment conducted with an Andosol from the Alfredo Volio Mata Experimental Station of the University of Costa Rica

Time after fertilization	NH_4^+		NO_3^-	
	UR	UR + NI	UR	UR + NI
	%			
115 min	1.24 ± 0.02 ^{b)}	1.29 ± 0.02	0.29 ± 0.04	0.43 ± 0.10
2 d	1.17 ± 0.02	1.15 ± 0.04	1.12 ± 0.10	1.20 ± 0.03
5 d	0.47 ± 0.14	0.87 ± 0.04	1.05 ± 0.03	1.06 ± 0.01
12 d	0.08 ± 0.01	0.10 ± 0.05	0.81 ± 0.05	0.79 ± 0.02
28 d	0.03 ± 0.00	0.03 ± 0.00	0.04 ± 0.01	0.14 ± 0.08
56 d	0.03 ± 0.00	0.04 ± 0.00	0.02 ± 0.00	0.02 ± 0.00

^{a)} See Table II for treatment details.

^{b)} Mean ± standard deviation ($n = 3$).

Close soil NO_3^- contents between UR and UR + NI were due to the additional NO_3^- produced by heterotrophic nitrification, a relevant mechanism in grassland soils with high organic matter content and pH below 6.5 (Müller *et al.*, 2014; Li *et al.*, 2015; Wang *et al.*, 2016). This conclusion is based on: i) the soil NO_3^- content change, which was larger than the variation in NH_4^+ content (Fig. 1a, b), ii) the decrease of the enrichment in $^{15}\text{NH}_4^+$ (Fig. 1c), likely caused by mineralization of organic matter (Mathieu *et al.*, 2006a), and iii) the percentage of soil NO_3^- derived from urea, which reached only a maximum value close to 60% (Fig. 1d).

Nitrapyrin application did not maintain the $^{15}\text{NO}_3^-$ pool stable from days 1 to 12 after fertilization (Table III), indica-

ting an inflow of unlabeled or low-abundance NO_3^- . These results do not agree with those reported by Fisk *et al.* (2015), who found that NI decreased gross nitrification rates in a Haplic Arenosol (organic matter 11–18 g kg^{-1} , pH 6.2). This difference in the decline of the ^{15}N enrichment of the NO_3^- pool is arguably related to the different nitrification processes (autotrophic vs. heterotrophic). For example, Fae-fen *et al.* (2016) compared the contributions of autotrophs and heterotrophs to nitrification in Oxisol (organic matter 22 g kg^{-1} , pH 5.0) and reported that autotrophic nitrification was the main nitrification pathway. In the Andosol from grassland, the main pathway of NO_3^- production was possibly heterotrophic nitrification, which is not effectively

inhibited by nitrapyrin.

At a low application dose of N as urea, the soil mineral N originated mostly from the fertilizer in the first several days after fertilization, with a maximum between 60% and 65%; this percentage decreased to less than 5% by around day 12. This finding suggests that the N fertilizer was quickly mineralized and extracted by the grass, which already had a well-developed root system, leading to low availability of NH_4^+ for the action of the inhibitor.

N₂O emissions

As expected, experimental pots fertilized with N exhibited N_2O fluxes higher than the emissions of the control. Emissions of N_2O reached their peak 2 d after fertilizer application (Fig. 2a), similar to results from tropical soils with C/N ratios between 10.6 and 9.8 (Martins *et al.*, 2017; Pérez-Castillo *et al.*, 2021). This finding was contrary to those reported by Abbasi *et al.* (2003) and Cui *et al.* (2013) from soils with higher C/N ratios (16.2–17.3) in temperate regions, where maximum rates of N_2O emissions were observed between days 14 and 28 after fertilization. The N_2O emission peaks at day 2 may be due to the lower content of humic acids in the soil organic matter (lower C/N ratio). According to Dong *et al.* (2009), high content of humic acids would suppress the growth of autotrophic ammonia-oxidizing bacteria and therefore, could delay soil nitrification.

The oxidation of accumulated organic N in high-fertility soil could contribute to N_2O emissions and explains the

slight increase in emissions from the control treatment, which reached the maximum flux 4 d after fertilization. Under the experimental conditions, heterotrophic denitrification was arguably favored by 60% WFPS (Aita *et al.*, 2015; Martins *et al.*, 2017). Moreover, heterotrophic nitrification has proven to be an important source of N_2O emissions in soils with a high content of organic matter and a slightly acidic pH in experiments carried out with fertilizers enriched with ^{15}N (Zhang *et al.*, 2015).

It should be noted that N_2O fluxes of UR and UR + NI only showed significant differences on day 0 ($0.86 \pm 0.14 \text{ mg N}_2\text{O-N kg}^{-1} \text{ soil d}^{-1}$ in UR vs. $0.19 \pm 0.04 \text{ mg N}_2\text{O-N kg}^{-1} \text{ soil d}^{-1}$ in UR + NI). Fluxes of N_2O from the UD pots were always higher than those from the UD + NI pots (Fig. 2a), and the differences were significant on day 1 ($0.33 \pm 0.03 \text{ mg N}_2\text{O-N kg}^{-1} \text{ soil d}^{-1}$ in UD vs. $0.13 \pm 0.01 \text{ mg N}_2\text{O-N kg}^{-1} \text{ soil d}^{-1}$ in UD + NI) and day 7 ($4.86 \pm 1.61 \text{ mg N}_2\text{O-N kg}^{-1} \text{ soil d}^{-1}$ in UD vs. $1.94 \pm 0.86 \text{ mg N}_2\text{O-N kg}^{-1} \text{ soil d}^{-1}$ in UD + NI). Nitrous oxide fluxes from all the treatments approached the baseline between days 16 and 20 (Fig. 2a). This condition mirrors mineral N depletion, as a result of N absorption by grass and N losses via gaseous N emissions (Fig. 1a, b).

Mean cumulative emissions of $\text{N}_2\text{O-N}$ (Fig. 2b) remained below the emission factor of 1% of N applied, established by the International Panel on Climate Change (IPCC) (2006), which is consistent with the low soil mineral N content

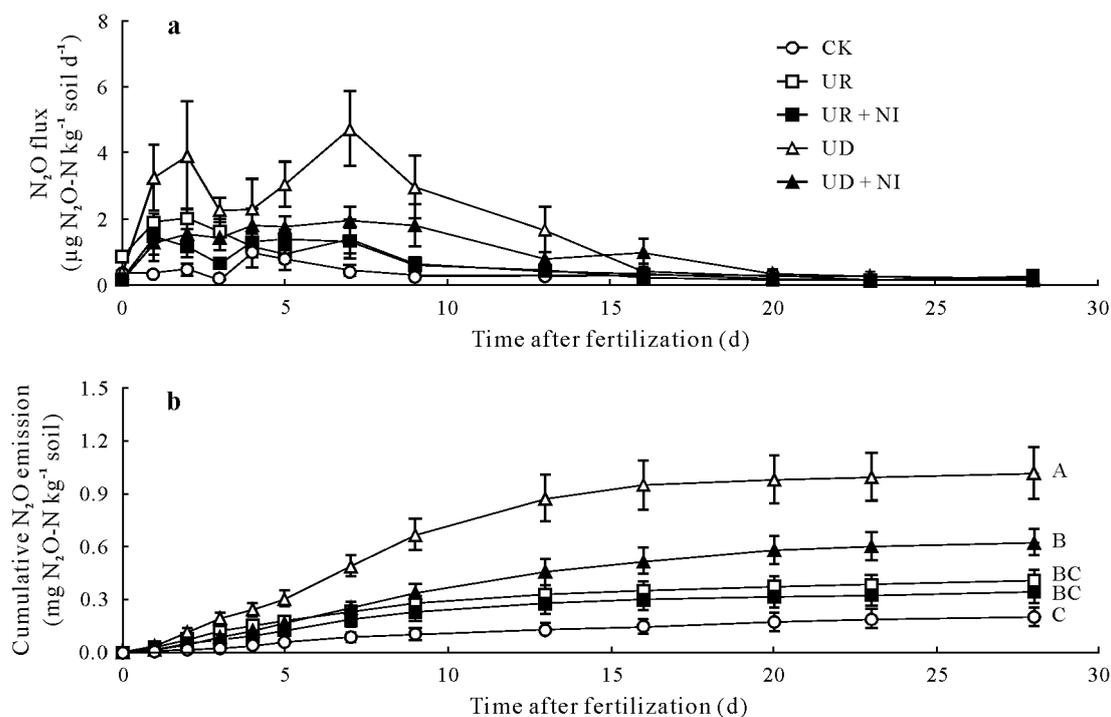


Fig. 2 Fluxes (a) and cumulative emissions (b) of nitrous oxide (N_2O) from the treatments in a pot experiment conducted with an Andosol from the Alfredo Volio Mata Experimental Station of the University of Costa Rica. See Table II for treatment details. Vertical bars indicate standard errors ($n = 4$). Different uppercase letters at the end of the lines in subfigure b represent significant differences based on Tukey test ($P = 0.05$).

discussed above. More than 90% of the N_2O emissions occurred in the first two weeks after the application of urea- ^{15}N , when Andosol presented the highest contents of mineral N (Fig. 1a, b). In particular, the cumulative emissions of the UD pots ($1.13 \pm 0.15 \text{ mg N}_2\text{O-N kg}^{-1} \text{ soil}$) were significantly higher than those of the UD + NI pots ($0.71 \pm 0.07 \text{ mg N}_2\text{O-N kg}^{-1} \text{ soil}$) since day 2, which represented a reduction of 37% N_2O emissions by the application of nitrapyrin. Therefore, the loss of N as N_2O decreased from 0.9% of the N applied in UD to only 0.55% in UD + NI. This trend is consistent with the substantial decrease in N_2O emissions reported by Abbasi *et al.* (2003) or McTaggart *et al.* (1997) when nitrapyrin was applied with N at doses higher than 100 mg N kg^{-1} (above 120 kg N ha^{-1}).

In contrast to the results presented for UD and UD + NI, the UR and UR + NI treatments did not exhibit significant differences in the cumulative N_2O emissions (Fig. 2b), suggesting that the inhibitory effect of nitrapyrin on N_2O emissions decreases in a tropical Andosol when the NH_4^+ content available for nitrification is low (Van Groenigen *et al.*, 2010). A non-significant difference between urea fertilization with and without nitrapyrin was reported in grasslands by others (Dougherty *et al.*, 2016; Nauer *et al.*, 2018; Pérez-Castillo *et al.*, 2021) when an equivalent dose of urea and NI was applied. In summary, an inhibitory effect of nitrapyrin was observed on N_2O emissions, but its impact was minimal when the soil mineral N content was low (Fig. 2b).

Nitrous oxide fluxes from UD + NI tended to be higher than those from UR or UR + NI from day 4 onwards; however, the cumulative N_2O emissions did not exhibit significant differences among the treatments, suggesting that the management of nitrogenous fertilization with the application of nitrapyrin could offset the environmental impacts of N surplus in the tropical Andosol studied.

Contribution of urea to N_2O flux

The isotopic ^{15}N signature of the emitted N_2O was lower than that of the fertilizer (Fig. 3). This suggests that part of the emitted N_2O originated from unlabeled N sources such as soil organic N. This finding contrasts with the results of Abbasi and Adams (2000) that all N_2O emitted in an incubation experiment (undisturbed soil with pH 5.1, total C 47.3 g kg^{-1} , and C/N ratio 13.9, receiving $70 \text{ mg urea-N kg}^{-1}$) was derived from the ^{15}N -labeled urea. Such difference is related to autotrophic and heterotrophic nitrification. In the present study, N_2O emissions may come partially from the oxidation of soil organic N to NO_3^- through heterotrophic nitrification and the reduction of this NO_3^- via denitrification in anaerobic microsites that coexist in the soil profile (Sey *et al.*, 2008; Müller *et al.*, 2014; Martins *et al.*, 2017). Moreover, the ^{15}N -enriched N_2O from the labeled urea demonstrates

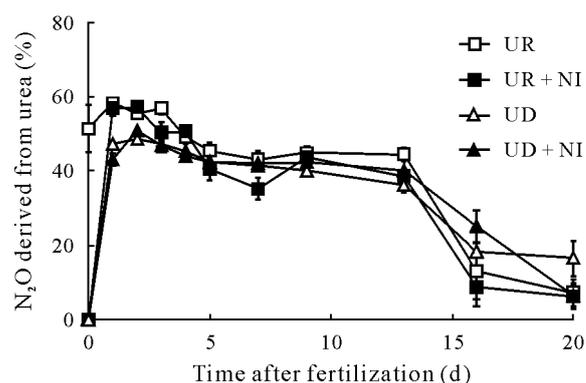


Fig. 3 Percentages of the emissions of nitrous oxide (N_2O) derived from ^{15}N -labeled urea applied in the treatments of a pot experiment conducted with an Andosol from the Alfredo Volio Mata Experimental Station of the University of Costa Rica. See Table II for treatment details. Vertical bars indicate standard errors ($n = 4$).

the production of NO_3^- via autotrophic nitrification (Stange and Döhling, 2005).

The N_2O originated from the ^{15}N -labeled urea (Fig. 3) showed the same trend as the content of soil NO_3^- derived from urea. Thus, during the first 5 d after fertilization, the percentages of N_2O flux derived from the fertilizer were similar to those of the NO_3^- produced, while 20 d later, the contribution of urea-N in both N_2O and NO_3^- was very low. One day after fertilization, urea-derived N_2O from the UR and UR + NI treatments was significantly higher ($P < 0.05$) than that from the UD + NI treatment but not significantly higher ($P > 0.05$) than that from the UD treatment. The UD treatment, with the higher dose of urea application, produced higher percentage of N_2O derived from the organic N of the soil, compared to the UR treatment. This finding could be explained by that urea-N increases soil organic N mineralization and particularly decreases the C/N ratio of the light fraction of the soil organic matter (Kuzyakov *et al.*, 2000).

NUE

The NUE obtained for *C. nlemfuensis* was low (Table IV), at around 24% at day 28 (first growing cycle) and around only 9.4% in the following 28-d period, with a global average of 33%, and a maximum cumulative efficiency of $41.0\% \pm 9.7\%$ for the UD treatment. These remarkably low values of NUE were at least partly due to the immobilization of N fertilizer by soil organic matter ($78 \text{ g kg}^{-1} \text{ C}$), and NH_3 losses, considering the rapid hydrolysis of urea and its application on the surface, which are favorable factors for the production of NH_3 .

No significant differences were observed in NUE between the doses of urea applied, or between the uses of urea with and without nitrapyrin, although treatments containing urea alone, contrary to expectations, showed the highest

TABLE IV

Nitrogen fertilizer use efficiency in the treatments of a pot experiment conducted with an Andosol from the Alfredo Volio Mata Experimental Station of the University of Costa Rica

Treatment ^{a)}	Days after fertilization		Cumulative
	28	56	
	%		
UR	24.1 ± 4.1 ^{b)}	8.5 ± 1.5	32.6 ± 5.6
UR + NI	21.9 ± 6.7	7.8 ± 4.2	29.8 ± 10.9
UD	29.3 ± 7.4	11.6 ± 2.3	41.0 ± 9.7
UD + NI	20.8 ± 1.9	9.5 ± 1.8	30.3 ± 3.7

^{a)} See Table II for treatment details.

^{b)} Mean ± standard deviation ($n = 4$).

NUE values. Application of nitrapyrin to the Andosol studied could increase N losses through NH₃ volatilization, and slightly reduce NUE in the UR + NI and UD + NI treatments (Abalos *et al.*, 2014).

Finally, the inhibitory effect of nitrapyrin on nitrification (especially when the dose of urea-N was doubled) did not affect the production of the forage biomass (data not shown). This was because, quantitatively, the losses of N in the form of N₂O were minimal (less than 1% of the applied N) compared to the doses of N applied.

Microbial population analysis

The DGGE analysis of all soil treatments and control showed diffuse band patterns. Such arrangement can be related to diverse microbial communities with populations in relatively equivalent proportions. It was also not possible to observe differences between experimental groups because their band patterns were mostly indistinguishable from each other (Fig. S1, see Supplementary Material for Fig. S1).

Applications of urea cause changes in soil pH and osmotic potential. These changes can cause short-term alterations in the biomass and composition of the soil microbial community (Geisseler and Scow, 2014). For example, *in vitro* and field studies where urea was applied to soil (90–120 kg N ha⁻¹), showed evident changes in microbial community composition with decreased biomass and functional diversity in less than 10 d. However, over time (*e.g.*, 90 d and even 3 years of continuous application), the communities would be reestablished and their composition profiles would become not distinguishable compared to those from pretreated soils (Stark *et al.*, 2007; Lupwayi *et al.*, 2011, 2012; Roberts *et al.*, 2011). Presumably, the monitoring time was too long to see this short-term effect.

CONCLUSIONS

In a tropical Andosol of high organic C and N contents and under pasture vegetation, the soil organic N mineralization and heterotrophic nitrification constitute the main processes for the formation of NH₄⁺ and NO₃⁻. In this study, the

¹⁵N technique allowed us to precisely determine the sources of N₂O emissions (direct oxidation of the soil organic N *via* heterotrophic nitrification or its production *via* heterotrophic denitrification), which are critical for mitigation of N₂O. Nitrapyrin would likely exert a significant mitigating effect on N₂O emission, only if a substantial N surplus exists in soils with high organic matter content. Further studies are needed to study tropical soils with different organic matter contents and other NIs, in order to find efficient mitigation practices to reduce N₂O emissions.

ACKNOWLEDGEMENTS

This research was supported by the International Atomic Energy Agency (IAEA), Vienna, Austria through a Coordinated Research Project (No. CRPD1.50.16) of Soil and Water Management and Crop Nutrition Section, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, and by the University of Costa Rica (No. VI-802-B7-505). We thank Miss María José Moya, Mr. José Manuel Monge, and Mr. José Rolando Montiel from the Environmental Pollution Research Center (CICA) of the University of Costa Rica for their help in fieldwork.

SUPPLEMENTARY MATERIAL

Supplementary material for this article can be found in the online version.

REFERENCES

- Abalos D, Jeffery S, Sanz-Cobena A, Guardia G, Vallejo A. 2014. Meta-analysis of the effect of urease and nitrification inhibitors on crop productivity and nitrogen use efficiency. *Agr Ecosyst Environ.* **189**: 136–144.
- Abbasi M K, Adams W A. 2000. Estimation of simultaneous nitrification and denitrification in grassland soil associated with urea-N using ¹⁵N and nitrification inhibitor. *Biol Fert Soils.* **31**: 38–44.
- Abbasi M K, Shah Z, Adams W A. 2003. Effect of the nitrification inhibitor nitrapyrin on the fate of nitrogen applied to a soil incubated under laboratory conditions. *J Plant Nutr Soil Sci.* **166**: 513–518.
- Aita C, Schirrmann J, Pujol S B, Giacomini S J, Rochette P, Angers D A, Chantigny M H, Gonzatto R, Giacomini D A, Doneda A. 2015. Reducing nitrous oxide emissions from a maize-wheat sequence by decreasing soil nitrate concentration: Effects of split application of pig slurry and dicyandiamide. *Eur J Soil Sci.* **66**: 359–368.
- Akiyama H, Yan X Y, Yagi K. 2010. Evaluation of effectiveness of enhanced-efficiency fertilizers as mitigation options for N₂O and NO emissions from agricultural soils: Meta-analysis. *Glob Change Biol.* **16**: 1837–1846.
- Alonso-Ayuso M, Gabriel J L, Quemada M. 2016. Nitrogen use efficiency and residual effect of fertilizers with nitrification inhibitors. *Eur J Agron.* **80**: 1–8.
- Breuer L, Kiese R, Butterbach-Bahl K. 2002. Temperature and moisture effects on nitrification rates in tropical rain-forest soils. *Soil Sci Soc Am J.* **66**: 834–844.
- Cabalaceta G. 1999. Fertilization and nutrition of highland forage. In XI National Agronomic Congress. III Soil Congress (in Spanish). Editorial of the State Distance University, San José. pp. 239–254.

- Cabalceta G, Molina E. 2006. Critical levels of nutrients in soils of Costa Rica using the Mehlich 3 extractor solution. *Agron Costarric* (in Spanish). **30**: 31–44.
- Castro-Gutiérrez V, Masís-Mora M, Caminal G, Vicent T, Carazo-Rojas E, Mora-López M, Rodríguez-Rodríguez C E. 2016. A microbial consortium from a biomixture swiftly degrades high concentrations of carbofuran in fluidized-bed reactors. *Process Biochem*. **51**: 1585–1593.
- Chen Z M, Ding W X, Luo Y Q, Yu H Y, Xu Y H, Müller C, Xu X, Zhu T B. 2014. Nitrous oxide emissions from cultivated black soil: A case study in Northeast China and global estimates using empirical model. *Global Biogeochem Cy*. **27**: 1311–1326.
- Christiansen J R, Outhwaite J, Smukler S M. 2015. Comparison of CO₂, CH₄ and N₂O soil-atmosphere exchange measured in static chambers with cavity ring-down spectroscopy and gas chromatography. *Agr Forest Meteorol*. **211–212**: 48–57.
- Cui P Y, Fan F L, Yin C, Li Z J, Song A L, Wan Y F, Liang Y C. 2013. Urea- and nitrapyrin-affected N₂O emission is coupled mainly with ammonia oxidizing bacteria growth in microcosms of three typical Chinese arable soils. *Soil Biol Biochem*. **66**: 214–221.
- Dawar K, Zaman M, Rowarth J S, Blennerhassett J, Turnbull M H. 2011. Urease inhibitor reduces N losses and improves plant-bioavailability of urea applied in fine particle and granular forms under field conditions. *Agr Ecosyst Environ*. **144**: 41–50.
- Dong L H, Córdova-Kreylos A L, Yang J S, Yuan H K, Scow K M. 2009. Humic acids buffer the effects of urea on soil ammonia oxidizers and potential nitrification. *Soil Biol Biochem*. **41**: 1612–1621.
- Dougherty W J, Collins D, Van Zwieten L, Rowlings D W. 2016. Nitrification (DMPP) and urease (NBPT) inhibitors had no effect on pasture yield, nitrous oxide emissions, or nitrate leaching under irrigation in a hot-dry climate. *Soil Res*. **54**: 675–683.
- Duncan E, O’Sullivan C, Roper M, Peoples M. 2016. The effect of nitrification inhibitors on wheat crop performance on coarse-grained soils in Mediterranean environments. In Proceedings of the 2016 International Nitrogen Initiative Conference “Solutions to Improve Nitrogen Use Efficiency for the World”. December 4–8, 2016, Melbourne. pp. 1–4.
- Faellen S J, Li S W, Xin X P, Wright A L, Jiang X J. 2016. Autotrophic and heterotrophic nitrification in a highly acidic subtropical pine forest soil. *Pedosphere*. **26**: 904–910.
- Fisk L M, Maccarone L D, Barton L, Murphy D V. 2015. Nitrapyrin decreased nitrification of nitrogen released from soil organic matter but not *amoA* gene abundance at high soil temperature. *Soil Biol Biochem*. **88**: 214–223.
- Geisseler D, Scow K M. 2014. Long-term effects of mineral fertilizers on soil microorganisms—a review. *Soil Biol Biochem*. **75**: 54–63.
- International Panel on Climate Change (IPCC). 2006. 2006 IPCC Guidelines for National Greenhouse Gas Inventories. Prepared by the National Greenhouse Gas Inventories Programme. IGES, Hayama.
- Koci J, Nelson P N. 2016. Tropical dairy pasture yield and nitrogen cycling: Effect of urea application rate and a nitrification inhibitor, DMPP. *Crop Pasture Sci*. **67**: 766–779.
- Kuzyakov Y, Friedel J K, Stahr K. 2000. Review of mechanisms and quantification of priming effects. *Soil Biol Biochem*. **32**: 1485–1498.
- Li B, Fan C H, Xiong Z Q, Li Q L, Zhang M. 2015. The combined effects of nitrification inhibitor and biochar incorporation on yield-scaled N₂O emissions from an intensively managed vegetable field in southeastern China. *Biogeosciences*. **12**: 2003–2017.
- Lupwayi N Z, Clayton G W, O’Donovan J T, Grant C A. 2011. Soil microbial response to nitrogen rate and placement and barley seeding rate under no till. *Agron J*. **103**: 1064–1071.
- Lupwayi N Z, Lafond G P, Ziadi N, Grant C A. 2012. Soil microbial response to nitrogen fertilizer and tillage in barley and corn. *Soil Till Res*. **118**: 139–146.
- Martins M R, Sant’Anna S A C, Zaman M, Santos R C, Monteiro R C, Alves B J R, Jantalia C P, Boddey R M, Urquiaga S. 2017. Strategies for the use of urease and nitrification inhibitors with urea: Impact on N₂O and NH₃ emissions, fertilizer-¹⁵N recovery and maize yield in a tropical soil. *Agr Ecosyst Environ*. **247**: 54–62.
- Mata R, Vázquez A, Rosales A, Salazar D. 2016. Costa Rica Digital Soil Map (in Spanish). Costa Rican Association of Soil Science, San José.
- Mathieu O, Hénault C, Lévêque J, Baujard E, Milloux M J, Andreux F. 2006a. Quantifying the contribution of nitrification and denitrification to the nitrous oxide flux using ¹⁵N tracers. *Environ Pollut*. **144**: 933–940.
- Mathieu O, Lévêque J, Hénault C, Milloux M J, Bizouard F, Andreux F. 2006b. Emissions and spatial variability of N₂O, N₂ and nitrous oxide mole fraction at the field scale, revealed with ¹⁵N isotopic techniques. *Soil Biol Biochem*. **38**: 941–951.
- McTaggart I P, Clayton H, Parker J, Swan L, Smith K A. 1997. Nitrous oxide emissions from grassland and spring barley, following N fertiliser application with and without nitrification inhibitors. *Biol Fert Soils*. **25**: 261–268.
- Molina E, Meléndez G. 2002. Interpretation Table of Soil Analysis (in Spanish). Agronomic Research Center, University of Costa Rica, San José.
- Müller C, Laughlin R J, Spott O, Rütting T. 2014. Quantification of N₂O emission pathways via a ¹⁵N tracing model. *Soil Biol Biochem*. **72**: 44–54.
- Myhre G, Shindell D, Bréon F M, Collins W, Fuglestedt J, Huang J, Koch D, Lamarque J F, Lee D, Mendoza B, Nakajima T, Robock A, Stephens G, Takemura T, Zhang H. 2013. Anthropogenic and natural radiative forcing supplementary material. In Stocker T F, Qin D, Plattner G K, Tignor M, Allen S K, Boschung J, Nauels A, Xia Y, Bex V, Midgley P M (eds.) Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge and New York. pp. 659–740.
- Nauer P A, Fest B J, Visser L, Arndt S K. 2018. On-farm trial on the effectiveness of the nitrification inhibitor DMPP indicates no benefits under commercial Australian farming practices. *Agr Ecosyst Environ*. **253**: 82–89.
- Pataki D E, Ehleringer J R, Flanagan L B, Yakir D, Bowling D R, Still C J, Buchmann N, Kaplan J O, Berry J A. 2003. The application and interpretation of Keeling plots in terrestrial carbon cycle research. *Global Biogeochem Cy*. **17**: 1022.
- Pérez-Castillo A G, Chinchilla-Soto C, Elizondo-Salazar J A, Barboza R, Kim D G, Müller C, Sanz-Cobena A, Borzouei A, Dawar K, Zaman M. 2021. Nitrification inhibitor nitrapyrin does not affect yield-scaled nitrous oxide emissions in a tropical grassland. *Pedosphere*. **31**: 265–278.
- Rivera A. 2008. Nutrient absorption curves during pasture establishment; total nutrient absorption and excreta effect, during rotational grazing with dairy cattle, in the Kikuyo (*Kikuyo chloa clandestinum*), African Star (*Cynodon nlemfluensis*) and Toledo (*Brachiaria brizantha* cv. TOLEDO) pastures (in Spanish). M.S. Thesis, University of Costa Rica.
- Roberts B A, Fritsch F B, Horwath W R, Scow K M, Rains W D, Travis R L. 2011. Comparisons of soil microbial communities influenced by soil texture, nitrogen fertility, and rotations. *Soil Sci*. **176**: 487–494.
- Ruser R, Schulz R. 2015. The effect of nitrification inhibitors on the nitrous oxide (N₂O) release from agricultural soils—a review. *J Plant Nutr Soil Sci*. **178**: 171–188.
- Saggar S, Jha N, Deslippe J, Bolan N S, Luo J, Giltrap D L, Kim D G, Zaman M, Tillman R W. 2013. Denitrification and N₂O:N₂ production in temperate grasslands: Processes, measurements, modelling and mitigating negative impacts. *Sci Total Environ*. **465**: 173–195.
- Sey B K, Manceur A M, Whalen J K, Gregorich E G, Rochette P. 2008. Small-scale heterogeneity in carbon dioxide, nitrous oxide and methane production from aggregates of a cultivated sandy-loam soil. *Soil Biol Biochem*. **40**: 2468–2473.
- Stange F, Döhling F. 2005. ¹⁵N tracing model SimKIM to analyse the NO and N₂O production during autotrophic, heterotrophic nitrification, and denitrification in soils. *Isotopes Environ Health Stud*. **41**: 261–274.
- Stark C, Condon L M, Stewart A, Di H J, O’Callaghan M. 2007. Influence of organic and mineral amendments on microbial soil properties and processes. *Appl Soil Ecol*. **35**: 79–93.

- Trenkel M E. 2010. Slow- and Controlled-Release and Stabilized Fertilizers: An Option for Enhancing Nutrient Use Efficiency in Agriculture. International Fertilizer Industry Association, Paris.
- Tubert-Brohman I, Sherman W, Repasky M, Beuming T. 2013. Improved docking of polypeptides with glide. *J Chem Inf Model.* **53**: 1689–1699.
- Van Groenigen J W, Velthof G L, Oenema O, van Groenigen K J, van Kessel C. 2010. Towards an agronomic assessment of N₂O emissions: A case study for arable crops. *Eur J Soil Sci.* **61**: 903–913.
- Villalobos L, Arce J. 2014. Agronomic and nutritional evaluation of African star grass-based pastures (*Cynodon nlemfuensis*) in the region of Monteverde, Puntarenas, Costa Rica. II. Nutritional value. *Agron Costarric* (in Spanish). **38**: 133–145.
- Volpi I, Laville P, Bonari E, Di Nasso N N O, Bosco S. 2017. Improving the management of mineral fertilizers for nitrous oxide mitigation: The effect of nitrogen fertilizer type, urease and nitrification inhibitors in two different textured soils. *Geoderma.* **307**: 181–188.
- Wang J, Wang L, Feng X J, Hu H F, Cai Z C, Müller C, Zhang J B. 2016. Soil N transformations and its controlling factors in temperate grasslands in China: A study from ¹⁵N tracing experiment to literature synthesis. *J Geophys Res Biogeosci.* **121**: 2949–2959.
- Wrage N, Velthof G L, van Beusichem M L, Oenema O. 2001. Role of nitrifier denitrification in the production of nitrous oxide. *Soil Biol Biochem.* **33**: 1723–1732.
- Zaman M, Nguyen M L. 2012. How application timings of urease and nitrification inhibitors affect N losses from urine patches in pastoral system. *Agr Ecosyst Environ.* **156**: 37–48.
- Zaman M, Nguyen M L, Gold A J, Groffman P M, Kellogg D Q, Wilcock R J. 2008. Nitrous oxide generation, denitrification, and nitrate removal in a seepage wetland intercepting surface and subsurface flows from a grazed dairy catchment. *Aust J Soil Res.* **46**: 565–577.
- Zaman M, Nguyen M L, Šimek M, Nawaz S, Khan M J, Babar M N, Zaman S, 2012. Emissions of nitrous oxide (N₂O) and di-nitrogen (N₂) from the agricultural landscapes, sources, sinks, and factors affecting N₂O and N₂ ratios. In Liu G X (ed.) *Greenhouse Gases—Emission, Measurement and Management*. IntechOpen, Shanghai. pp. 1–32.
- Zaman M, Zaman S, Adhinarayanan C, Nguyen M L, Nawaz S, Dawar K M. 2013. Effects of urease and nitrification inhibitors on the efficient use of urea for pastoral systems. *Soil Sci Plant Nutr.* **59**: 649–659.
- Zhang J B, Müller C, Cai Z C. 2015. Heterotrophic nitrification of organic N and its contribution to nitrous oxide emissions in soils. *Soil Biol Biochem.* **84**: 199–209.
- Zhu X, Burger M, Doane T A, Horwath W R. 2013. Ammonia oxidation pathways and nitrifier denitrification are significant sources of N₂O and NO under low oxygen availability. *Proc Natl Acad Sci USA.* **110**: 6328–6333.