GELTON GERALDO FERNANDES GUIMARÃES

EVALUATION OF OXIDIZED CHARCOAL AND UREASE INHIBITORS FOR INCREASING THE EFFICIENCY OF UREA-BASED FERTILIZERS

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Solos e Nutrição de Plantas, para obtenção do título de *Doctor Scientiae*.

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APROVADA: 27 de julho de 2015.

Leonardus Vergütz
(Coorientador)

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Reinaldo Bertola Cantarutti
(Orientador)

Dedico esta tese ao meu pai Antônio, à minha mãe Conceição (*in memoriam)*, aos meus irmãos e à minha namorada Camila, pelo amor, incentivo e apoio durante a concretização dos meus estudos.

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MUITO OBRIGADO!

BIOGRAFIA

Gelton Geraldo Fernandes Guimarães, filho de Antônio Agostinho Guimarães e Maria da Conceição Fernandes Guimarães, natural de Presidente Bernardes – MG, nasceu no dia 30 de janeiro de 1985.

Entre 2001 e 2003, estudou na Escola Agrotécnica Federal de Barbacena - MG, onde cursou o ensino médio e o curso Técnico em Zootecnia.

Em 2005, iniciou o curso de graduação em Agronomia pela Universidade Federal de Viçosa, recebendo o título de Engenheiro Agrônomo em janeiro de 2010. Durante a graduação, desenvolveu projetos de pesquisa na área de fertilidade do solo e nutrição de plantas.

Em março de 2010, iniciou o curso de mestrado pelo Programa de Pós-graduação em Solos e Nutrição de Plantas na Universidade Federal de Viçosa - MG, desenvolvendo trabalhos com objetivo de aumentar a eficiência da ureia.

Em julho de 2011, defendeu sua dissertação de mestrado, dando início ao curso de doutorado pelo mesmo Programa e Universidade, continuando os trabalhos nesta mesma linha de pesquisa, a fim de avaliar alternativas para o controle da hidrólise de ureia com objetivo de prolongar a disponibilidade e o fornecimento de N para a absorção pelas plantas.

Em 2010, recebeu o Prêmio Jovem Pesquisador do *International Plant Nutrition Institute*. Em 2013, recebeu o Prêmio Petrobras de Tecnologia Engenheiro Antônio Seabra Moggi oferecido pela PETROBRAS/CNPq. Ambas as premiações foram decorrentes de trabalhos desenvolvidos para melhoria da eficiência da ureia.

Em agosto de 2014, iniciou o programa de doutorado sanduíche no *Department of Natural Resources and Environmental Sciences na University of Illinois at Urbana-Champaign,* sob orientação do professor Dr. Richad L. Mulvaney. Permaneceu nesta instituição até maio de 2015, período em que desenvolveu estudos comparando os efeitos do carvão oxidado e inibidor de urease incorporados à ureia na transformação do N-ureia no solo.

Defendeu sua tese de doutorado em 27 de julho de 2015.

SUMÁRIO

ABSTRACT

GUIMARÃES, Gelton Geraldo Fernandes, D. Sc., Universidade Federal de Viçosa, July, 2015. **Evaluation of oxidized charcoal and urease inhibitors for increasing the efficiency of urea-based fertilizers.** Adviser: Reinaldo Bertola Cantarutti. Co-advisers: Leonardus Vergütz and Edson Marcio Mattiello

Urea fertilizer can have a low agronomic efficiency when applied to the soil surface, due to N losses through NH_3 volatilization. Because of the predominance of urea in the commercial fertilizer market in recent decades, different approaches have been proposed for controlling the hydrolysis of urea and thus prolong the availability and supply of N for crop uptake. Urease inhibitors have received most of the attention, particularly N- (n-butyl) thiophosphoric triamide (NBPT) that is marketed as Agrotain®. Alternatively, copper and zinc can be used to inhibit urease activity, which has the advantage of supplying plant-essential micronutrients. Another possibility, currently under investigation, is the use of compounds with high CEC and buffering capacity, such as oxidized charcoal, to prolong the availability of NH_4^+ released in soil by urea hydrolysis. The efficiency of oxidized charcoal was assessed (OCh, 150 g kg^{-1} fertilizer) when applied with or without Cu and/or Zn (~ 0.5-2 g kg^{-1} fertilizer), relative to the use of unamended urea, for increasing N uptake by Capim-mombaça (*Panicum maximum*). The forage was grown in a greenhouse on a coarse-textured Oxisol, and plant material was harvested 5, 14, 28, 42 and 56 days after surface application of ^{15}N urea granules with or without OCh, used alone or with Cu and/or Zn, for measurement of dry matter production, total N uptake and ^{15}N recovery. Soil samples were also collected along with the first and fourth harvests, to determine exchangeable NH_4^+ and NO_3^- concentrations. Under the conditions studied, dry matter production was unaffected by the use of OCh with Cu and/or Zn; however, NH_4^+ availability was prolonged by the presence of OCh. The application of Zn with urea significantly increased plant uptake of urea N and N use efficiency, presumably reflecting the antagonistic interaction of Zn with superphosphate that was applied to prevent P deficiency. A comparison was made of the effects of OCh and NBPT on the transformation of urea N in the soil. For this purpose, four soils differing in texture and CEC were incubated for 0, 1, 3, 7 and 14 days after application of pelletized ^{15}N urea applied with or without OCh (150 g kg^{-1} fertilizer) or NBPT (0.5 g kg^{-1} fertilizer). After each incubation period, ^{15}N recovery was determined as volatilized NH_3, exchangeable NH_4^+, (NO_3^- + NO_2^-)-N and immobilized organic N. The OCh had little effect on any parameter measured; however,

the efficacy of NBPT in retarding urea hydrolysis led to a gradual accumulation of NH_4^+ that reduced NH_3 volatilization, the accumulation of NO_2^- and immobilization of urea N.

RESUMO

GUIMARÃES, Gelton Geraldo Fernandes, D. Sc., Universidade Federal de Viçosa, julho de 2015. **Avaliação do carvão oxidado e inibidores de urease para aumentar a eficiência de fertilizantes base-ureia.** Orientador: Reinaldo Bertola Cantarutti. Coorientadores: Leonardus Vergütz e Edson Marcio Mattiello

A ureia pode apresentar baixa eficiência agronômica quando aplicada na superfície do solo, fato atribuído à intensa volatilização de NH_3. Devido à predominância da ureia no mercado de fertilizantes nitrogenados, nas últimas décadas, tem-se buscado alternativas para o controle da hidrólise de ureia e, assim, prolongar o fornecimento de N para a absorção das culturas. Inibidores da urease tem recebido a maior parte da atenção, particularmente N-(n-butil) tiofosfórico triamida **(**NBPT) que é comercializado como Agrotain®. Alternativamente, cobre e zinco podem ser utilizados para inibir a atividade da urease, tendo a vantagem de fornecer micronutrientes as plantas. Outra alternativa que vem sendo estudada é o emprego de compostos com elevada CTC e com alto poder tampão de acidez, como o carvão oxidado, com o propósito de prolongar a disponibilidade do NH_4^+ no solo proveniente da hidrólise da ureia. A eficiência do carvão oxidado (OCh, 150 g kg^{-1} fertilizante) foi avaliado quando aplicado com ou sem Cu e/ou Zn (~0,5-2 g kg^{-1} fertilizante) em relação ao uso de ureia sem alterações, para aumentar a absorção de N pela forrageira Capim-mombaça (*Panicum maximum*). A forragem foi cultivada em casa de vegetação em Latossolo de textura média, sendo o material vegetal colhido aos 5, 14, 28, 42 e 56 dias após a aplicação superficial de ^{15}N na forma de grânulos de ureia com ou sem OCh, enriquecido ou não com Cu e/ou Zn, para determinação da produção da matéria seca, do teor e conteúdo de N e de ^{15}N nas plantas proveniente da ureia. Amostras de solo foram também coletadas em conjunto com a primeira e quarta colheita do material vegetal, para determinação da concentração das frações trocáveis de NH_4^+ e NO_3^-. Nas condições estudadas, a produção de matéria seca não foi afetada pelo uso de OCh com Cu e/ou Zn; no entanto, a disponibilidade de NH_4^+ foi prolongada pela presença de OCh. A aplicação da ureia com Zn aumentou significativamente a absorção pelas plantas de N-ureia e o uso eficiente do N, presumivelmente refletindo a interação antagonista de Zn com superfosfato que foi aplicado para prevenir a deficiência de P. Também foi feita uma comparação dos efeitos de OCh e NBPT na transformação de N-ureia no solo. Para este propósito, quatro solos com diferentes texturas e CTC foram incubados durante 0, 1, 3, 7 e 14 dias após a aplicação de ^{15}N-ureia misturada ou não com OCh (150 g kg^{-1} fertilizante) ou NBPT

(0,5 g kg^{-1} fertilizante). Após cada período de incubação, foi determinada a recuperação do ^{15}N na forma de NH_3 volatilizada, do N trocável (NH_4^+, NO_3^- e NO_2^-) e do N imobilizado. O OCh teve pouco efeito sobre qualquer parâmetro medido; no entanto, a eficácia do NBPT no retardamento da hidrólise da ureia conduziu a um acúmulo gradual de NH_4^+ que reduziu a volatilização de NH_3, o acumulo de NO_2^- e a imobilização de N-ureia.

GENERAL INTRODUCTION

The importance of urea as the leading N fertilizer for world agriculture can be attributed to its low cost and high N content. Unfortunately, rapid hydrolysis can promote ammonia (NH_3) volatilization when urea is applied to the soil surface (Abalos et al. 2014); cause phytotoxicity or decrease germination when urea is applied at seeding, with adverse economic consequences for the producer (Bremner 1995; Haden et al. 2011; Singh et al. 2013); and have adverse environmental consequences that include acidification and eutrophication of surface and groundwater (Van der Stelt et al. 2005; Beusen et al. 2008). An additional problem arises from rapid nitrification of urea N (Soares et al. 2012; Rojas et al. 2014), which not only contributes to NO_3^- pollution of surface and ground water (Chen et al. 2012), but also promotes NO_2^- accumulation that can inhibit germination cause toxicity for seedlings and plants (Bremner 1995; Samater et al. 1998; Haden et al. 2011), and increased N loss through denitrification (Mulvaney et al. 1997). These difficulties become controllable with the use of amendments to inhibit soil urease activity or increase retention of NH_4^+ formed by hydrolysis.

Numerous compounds have been proposed and/or evaluated for decreasing urease activity and thereby increasing the fertilizer efficiency of urea (Kiss and Simihaian 2002). Of these, N-(n-butyl) thiophosphoric triamide (NBPT) has been studied most extensively and shows the greatest potential as a fertilizer amendment to slow urea hydrolysis (Chien et al. 2009; Suter et al. 2013; Tasca et al. 2011). An interesting alternative arises from the use of urease inhibitors that also supply plant-essential nutrients. The latter strategy becomes feasible by coating urea with copper and zinc salts to reduce volatilization of NH_3 relative to uncoated urea (Junejo et al. 2011); moreover, these coatings provide a practical means of correcting micronutrient deficiencies. Care is called for, however, in using proper proportions of these elements, so as to avoid phytotoxicity and minimize the risk of contaminating soil and water (Fuentes et al. 2007; Michaud et al. 2008; Reddy et al. 2013).

Another option for stabilizing urea N applied to soil is the use of amendments with high surface area, potential acidity and cation exchange capacity (CEC) (Paiva et al. 2012; Taghizadeh-Toosi et al. 2012; Güereña et al. 2013). These characteristics enhance the capacity for adsorptive retention of NH_4^+ in soil and thereby decrease losses by NH_3 volatilization (Liang et al. 2006; Chan et al. 2008; Yusuff et al. 2009). To obtain a suitable material, charcoal produced from carbonaceous materials can be oxidized with nitric acid (HNO_3) (Liu et al. 2007). Oxidation brings about a substantial increase in specific surface area, pore volume and amphoteric functional groups with high capacity for adsorption of cations and anions (Troca-Torrado et al. 2011), intensifying properties that influence N transformations in soil (Clough and Condron 2010).

The work reported herein was undertaken with the objective of increasing the agronomic efficiency of fertilizer urea. In chapter 1, this objective is addressed for pasture production with pelletized urea, through evaluations using oxidized charcoal applied with or without Cu and/or Zn. Chapter 2 evaluates the potential of oxidized charcoal for controlling soil transformations of urea N, in comparison to urease inhibition by NBPT.

CHAPTER 1

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Value of copper, zinc, and oxidized charcoal for increasing forage efficiency of urea N uptake

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Abstract

Volatilization losses reduce the efficiency of surface-applied urea for crop N uptake, and can be controlled using urease inhibitors to retard hydrolysis or by the presence of other amendments that enhance retention of NH_4^+ formed by urea hydrolysis. A greenhouse study was conducted to evaluate the effectiveness of oxidized charcoal (OCh; 150 g kg^{-1} fertilizer) applied with or without Cu and/or Zn (~0.5-2 g kg^{-1} fertilizer) for increasing uptake of urea ^{15}N by a common tropical pasture grass, Capim-mombaça (*Panicum maximum*), grown on a coarse-textured Oxisol. Cuttings were collected 5, 14, 28, 42, and 56 days after surface placement of amended or unamended urea pellets to estimate dry matter production, total N uptake, and ^{15}N recovery. Soil sampling was carried out in conjunction with the first and fourth cuts to evaluate exchangeable NH_4^+ and NO_3^- concentrations. At the concentrations studied, OCh was more effective than Cu and/or Zn for prolonging NH_4^+-N availability in urea-treated soil; however, dry matter production was unaffected. Owing to the antagonistic interaction of Zn with fertilizer P required for plant growth, the addition of Zn in conjunction with urea led to a significant increase in total N uptake and the efficiency of urea N fertilization. The use of Zn as a urea amendment has practical potential to improve forage quality in tropical regions.

Keywords: N uptake, Ammonia volatilization, Urease inhibitors, ^{15}N, P-Zn interaction

Introduction

The dominance of urea in the world fertilizer market can be explained not only by low cost, but also by the high N content, ease of handling, and inherent versatility that facilitate usage. Unfortunately, rapid hydrolysis promotes NH_3 volatilization when urea is surface-applied without incorporation, which can limit N uptake efficiency and reduce yield response (Bremner, 1995; Yusuff et al., 2009; Zerpa and Fox, 2011; Soares et al., 2012; Suter et al., 2013; Ni et al., 2014). Besides promoting agricultural nutrient loss, the volatilized NH_3 contributes to atmospheric N deposition that can have an adverse environmental impact by effecting eutrophication and acidification (Sommer and Hutchings, 2001; Van der Stelt et al., 2005; Beusen et al., 2008).

Considerable attention has been given to the use of urease inhibitors for reducing NH_3 losses and increasing the efficiency of fertilizer urea, involving a wide variety of compounds that include heavy metals, dithiocarbamates, hydroxamic acids, polyhydric phenols and quinones, and phosphoric and thiophosphoric triamides (Kiss and Simihaian, 2002). The latter group has generated more interest than the others focusing especially on N-(n-butyl) thiophosphoric triamide commonly known as NBPT (e.g., Bremner and Chai, 1986; Watson et al., 2008; Dawar et al., 2011). Ideally, the control of urea hydrolysis would be accomplished using an amendment that also supplies a plant-essential nutrient, such as $CuSO_4$ that was found to be an effective coating material for reducing NH_3 losses from pelletized urea (Volk, 1961) and subsequently patented for this purpose (Sor et al., 1966; Sor, 1968, 1969). The value of $CuSO_4$ as a urease inhibitor has repeatedly been confirmed (e.g., Pugh and Waid, 1969; Bremner and Douglas, 1971; Tabatabai, 1977; El-Din et al., 1985; Yadav et al., 1986; Hemida et al., 1997), and $ZnSO_4$ has also shown inhibitory potential, although not to the same extent as for $CuSO_4$ (e.g., Tabatabai, 1977; El-Din et al., 1985; Yadav et al., 1986; Hemida et al., 1997). In recent work by Junejo et al. (2011), $CuSO_4$ used alone (50 g kg^{-1}) or in a 1:1 mixture with $ZnSO_4$ proved effective for reducing NH_3 volatilization relative to uncoated urea; however, the high amendment rate employed (Cu or Zn 20 g kg^{-1}) could lead to a cumulative buildup causing toxicity problems for plants and the soil microflora and create the potential for heavy metal pollution (Fuentes et al., 2007; Michaud et al., 2008; Reddy et al., 2013).

Besides the use of urease inhibitors, volatilization of urea N can be controlled with amendments that have high specific surface area and high cation exchange capacity (CEC), which not only enhances retention of NH_4^+ formed by urea hydrolysis but also contributes buffer capacity to moderate the resulting rise in pH. The effectiveness of this strategy has been documented by studies reporting the value of biochar for increasing soil retention of urea N (Güereña et al., 2013; Huang et al., 2014), although the practical potential is limited by the need for high application rates (typically > 10 Mg ha^{-1}) that can promote microbial N immobilization and thus limit crop uptake of fertilizer N (Zavalloni et al., 2011; Nelissen et al., 2012; González et al., 2015). These limitations can be avoided by the use of chemically oxidized charcoal, which is higher than biochar in specific surface area and CEC while having a lower pH and C/N ratio. To maximize efficacy, the oxidized charcoal must be in microsite contact with the fertilizer urea during hydrolysis, suggesting the strategy adopted by Paiva et al. (2012) to coat fertilizer granules with material derived from *Eucalyptus grandis* (250 g kg^{-1}). This coating showed the practical potential of oxidized charcoal as a fertilizer amendment, for the high CEC (440 $cmol_c$ kg^{-1}) at pH 2 proved effective for decreasing NH_3 volatilization while delaying the maximum rate of loss. The same benefits were documented in subsequent work by Guimarães et al. (2015), and a lower rate (150 g kg^{-1}) was found to be sufficient when charcoal was brought into better contact with urea by granulation from a molten mix.

The present project was motivated by the possibility that loss of urea N through NH_3 volatilization might be more effectively controlled by applying Cu and/or Zn in conjunction with oxidized charcoal, as opposed to using these amendments separately. Owing to the high CEC of the latter material, there would be extensive adsorption of these two micronutrients, followed by gradual desorption to prolong the inhibitory effect and deliver plant nutrition without the risk of phytotoxicity or environmental pollution. Our primary purpose was to evaluate the effectiveness of oxidized charcoal applied with or without Cu and/or Zn for increasing crop uptake of urea ^{15}N. Additional objectives were to evaluate the efficacy of low Cu and Zn concentrations for inhibiting urea hydrolysis, and to compare the three amendments for their effects on retention of exchangeable NH_4^+ in urea-treated soil.

Materials and methods

Production and characterization of oxidized charcoal

Following the process described by Guimarães et al. (2015), wood from *Eucalyptus urophilla* was carbonized to obtain charcoal that was then oxidized using HNO_3, and the residue retained upon filtration was dissolved in deionized water without prior treatment with NaOH. The oxidized charcoal (OCh) was characterized by preparing a suspension with deionized water (OCh:water ratio, 1:10) that showed a pH of 2.7, and elemental concentrations determined with a PerkinElmer 2400 Series II CHNS/O Analyzer (PerkinElmer, Waltham, MA, USA) were 574 g kg^{-1} C, 24 g kg^{-1} H, and 30 g kg^{-1} N (C/N ratio, 19:1) when expressed on the basis of ash-free dry matter, giving an O content of 373 g kg^{-1} by difference. A CEC of 436 $cmol_c$ kg^{-1} was estimated by potentiometric titration with 0.1 mol L^{-1} NaOH, on the assumption that acidity was derived from carboxyl functional groups between pH 3 and 8 and from phenolic functional groups between pH 8 and 10 (Inbar et al., 1992; Bowles et al., 1994).

Urease inhibition by Cu and Zn

The soil used was a surface (0-5 cm) sample of a red-yellow Ultisol (Typic Hapludult) collected from a site under grassland. Before use, the soil was air-dried and crushed to pass through a 2 mm screen, and then characterized by analyses that gave the following results: pH (H_2O) measured with a glass electrode, 5.8; organic C by the Walkley-Black method (Nelson and Sommers, 1996), 35.6 g kg^{-1}; and texture by the pipette method (Kilmer and Alexander, 1949), 580 g kg^{-1} (sand), 60 g kg^{-1} (silt), and 360 g kg^{-1} (clay). Urease activity was measured as 70 mg kg^{-1} h^{-1} urea N hydrolyzed by a modified version of the buffer method of Tabatabai and Bremner (1972), in which 7.5 mL of phosphate buffer replaced 9 ml of THAM at the same pH (8.8), the incubation interval was increased from 2 to 3 h following a 10-min pre-incubation at 37 ºC prior to the addition of 10 mg of urea, phenylmercuric acetate (PMA) was used instead of Ag_2SO_4 to prevent urea hydrolysis during extraction with 2 mol L^{-1} KCl, and the soil suspension was centrifuged at 1.834 *g* for 10 min before pipetting an aliquot for steam distillation with NaOH.

To evaluate the inhibitory effects of Cu and Zn on soil urease activity, the modified buffer method was performed on triplicate 5-g samples of soil treated after pre-incubation with 1 mL of urea solution (10 mg mL^{-1}) and 1 mL of deionized water containing 0, 0.01, 0.05, 0.1, or 1 mg of Cu (as $CuSO_4$), Zn (as $ZnSO_4$), or Cu + Zn in a 1:1 ratio. Percentage inhibition was calculated as 100 × (C – I)/C, where the variables represent urease activities determined for control (C) and inhibitor (I)-amended samples. Using Statistica® software (StatSoft, Tulsa, OK, USA), data were subjected to analysis of variance (ANOVA) by assuming a factorial combination of three amendments and four rates, and treatment means were compared using the Tukey's honestly significant difference procedure at $P < 0.05$.

Production of pelletized urea containing Cu, Zn, and OCh

Solutions containing 0.5 g of Cu or Zn L^{-1} were prepared by dissolving $CuSO_4$ or $ZnSO_4$ in 10 mmol L^{-1} K_2SO_4, followed by addition of 1 mol L^{-1} H_2SO_4 to obtain a pH of 2.5. Using a batch technique, 1 g of OCh was added to 50 mL of each solution in a 50-mL centrifuge tube, and to the same volume of a 1:1 mixture. The resulting suspensions were gently agitated with an orbital shaker for 24 h, and then centrifuged at 1.834 *g* for 20 min. The residue consisting of OCh enriched with Cu (OChCu), Zn (OChZn), or Cu and Zn (OChCuZn) was dried for a few days at 45 °C, followed by grinding with a mortar and pestle to pass through a 74 µm screen. To verify amendment concentrations, 100 mg of each OCh mixture was boiled in 20 mL of 2.4 mol L^{-1} HCl, the resulting suspension was filtered through JP 42 filter paper (J. Prolab., São Jose dos Pinhais-PR, Brazil) in a Pyrex® filtering funnel, and the filtrate was transferred quantitatively to a 50-mL volumetric flask and brought to volume with deionized water. The content of adsorbed Cu and/or Zn (Table 1) was measured using a Varian SpectrAA 220FS atomic absorption spectrometer (Varian Medical Systems, Palo Alto, CA, USA). Unamended OCh was prepared by the same process, without the addition of $CuSO_4$ or $ZnSO_4$ to 50 mL of 10 mmol L^{-1} K_2SO_4.

Table 1 Contents of Cu and Zn adsorbed on OCh[a]

Charcoal mixture	Cu (g kg^{-1})	Zn (g kg^{-1})
OChCu	13.7	—
OChZn	—	8.1
OChCuZn	7.7	3.2

[a] Data reported as a mean of three replicate determinations

Labeled (98 atom % ^{15}N) and unlabeled urea obtained from Sigma-Aldrich (St. Louis, MO, USA) were dissolved in deionized water in a 500-mL beaker to obtain 200 mL of a solution containing 0.5 kg urea L^{-1} and enriched to approximately 1 atom % ^{15}N. So as to increase the surface to volume ratio, the urea solution was evenly allocated among four 500-mL beakers and then evaporated to complete dryness by heating on an electric hotplate at 70 ºC. The dried residue was scraped with a spatula from each beaker and composited as a homogeneous powder, followed by storage in a desiccator over Si gel.

To pelletize OCh-amended urea, 8.5 g of the labeled N powder was mixed in a 50-mL digestion tube with 1.5 g of OCh, OChCu, OChZn, or OChCuZn, the tube was immersed in a glycerin bath at 137 °C, and the liquefied contents were then dripped onto a stainless steel plate at room temperature using a mechanical pipetter, forming pellets that averaged 4 mm in diameter and 2 mm in height. The same technique was employed in pelletizing 10 g of urea with or without $CuSO_4$ and/or $ZnSO_4$, in which case care was taken to maintain the same proportion of urea relative to Cu and/or Zn as was achieved using the corresponding amendments with OCh (Table 1). Table 2 characterizes the eight pelletized fertilizers for their content of Cu and Zn determined as previously described, and for total N and ^{15}N enrichment measured by isotope ratio mass spectrometry using an ANCA-GSL instrument (Sercon, Crewe, UK).

Table 2 Characterization of ^{15}N-labeled fertilizer sources prepared by pelletizing urea with or without amendment

Fertilizer source[a]	N (g kg^{-1})	Cu (g kg^{-1})	Zn (g kg^{-1})	Atom % ^{15}N
U	466	—	—	0.96
UCu	468	2.12	—	0.96
UZn	463	—	1.17	0.96
UCuZn	461	0.88	0.53	0.96
UOCh	403	—	—	0.95
UOChCu	398	1.97	—	0.96
UOChZn	402	—	1.14	0.95
UOChCuZn	403	1.05	0.45	0.95

[a] U, unamended urea; UCu, urea + $CuSO_4$; UZn, urea + $ZnSO_4$; UCuZn, urea + $CuSO_4$ + $ZnSO_4$; UOCh, urea + oxidized charcoal; UOChCu, urea + oxidized charcoal + adsorbed Cu; UOChZn, urea + oxidized charcoal + adsorbed Zn; UOChCuZn, urea + oxidized charcoal + adsorbed Cu + adsorbed Zn

^{15}N uptake efficiency

For use in a greenhouse experiment, a red-yellow Oxisol (Typic Hapludox) was collected to a depth of 20 cm from a pasture site in Espírito Santo, Brazil. Before use, the soil was air-dried and crushed to pass through a 2 mm screen. Physicochemical characterization gave the following results: pH (H_2O), 6.1; organic C by the Walkley-Black method (Nelson and Sommers, 1996), 9.2 g kg^{-1}; total N by a semimicro Kjeldahl method (Bremner, 1996), 1.14 g kg^{-1}; available nutrients by Mehlich-1 extraction (Mehlich, 1953), 5.8 mg kg^{-1} P, 57 mg kg^{-1} K, 0.49 mg kg^{-1} Cu, and 3.1 mg kg^{-1} Zn; 1 mol L^{-1} KCl-extractable cations (EMBRAPA, 1979), 2.26 $cmol_c$ kg^{-1} Ca and 0.67 $cmol_c$ kg^{-1} Mg; potential acidity extractable with $CaC_2H_3O_2$ at pH 7 (EMBRAPA, 1979), 1.0 $cmol_c$ kg^{-1} H + Al; texture by the pipette method (Kilmer and Alexander, 1949), 810 g kg^{-1} (sand), 20 g kg^{-1} (silt), and 170 g kg^{-1} (clay); water-holding capacity (Bremner and Shaw, 1958), 76 g kg^{-1}.

To germinate seedlings for evaluating fertilizer N uptake efficiency, seeds of the tropical grass, Capim-mombaça (*Panicum maximum*) were sown in a plastic flat containing compost substrate that was moistened with distilled water supplied to the greenhouse bench. After a few days for germination, approximately 3 kg of the soil described was transferred into each of 36 4-l plastic pots lined with a polyethylene bag to prevent leaching losses, which provided experimental units for a completely randomized design with eight treatments and four replications, and four additional pots to serve as unlabeled controls. The soil in each pot was fertilized by incorporating 750 mg of P as powdered triple superphosphate [$Ca(H_2PO_4)_2$], followed by application to the entire soil surface of 55 mL of a solution supplying 7.7 mg kg^{-1} N (as urea), 58 mg kg^{-1} K (as KCl), 15 mg kg^{-1} S [as $(NH_4)_2SO_4$], 0.31 mg kg^{-1} B (as H_3BO_3), 0.51 mg kg^{-1} Cu (as $CuSO_4$), 0.60 mg kg^{-1} Fe (as FeEDTA), 1.4 mg kg^{-1} Mn (as $MnSO_4$), 0.06 mg kg^{-1} Mo (as H_2MoO_4), and 1.5 mg kg^{-1} Zn (as $ZnSO_4$), which was prepared according to Novais et al. (1991). The soil was then moistened with distilled water to 70 % of water-holding capacity, seven seedlings were transplanted per pot, and thinning was done four days later to obtain a final stand of five plants per pot. Soil moisture content was maintained as necessary by gently applying distilled water with a watering can. At the onset of tillering 20 days after transplanting, the fertilizers were applied to the surface of moist soil among plants, which supplied 450 mg of N to each of 32 pots. The uptake of ^{15}N was determined from cuttings made to a height of 5 cm for subsequent regrowth at 5, 14, 28, 42, and 56 days after fertilization, and soil samples were collected to quantify NH_4^+ and NO_3^- concentrations for the 5- and 42-day cuttings Soil sampling was accomplished between plants, by extracting a single 1.4-cm diâmetro core to a depth of 6 cm using a 10-mL disposable syringe that had been modified by removal of the Luer fitting. Immediately after collection, the hole left by sampling was filled with soil from the periphery of the pot, and the soil sample was treated with 2 mol L^{-1} KCl containing 5 mg of PMA L^{-1} for extraction of inorganic N, which was quantified by colorimetric analyses for NH_4^+ (Kempers and Zweers, 1986) and NO_3^- (Yang et al., 1998). The data obtained were analyzed statistically by ANOVA and Tukey's honestly significant difference at $P < 0.05$.

Plant material collected in each cut was dried in a forced-air oven at 70 °C for 72 h, and the material was then weighed and ground to < 1 mm with a Wiley mill. Total N and ^{15}N content were determined by isotope ratio mass spectrometry as specified previously. The milligrams of plant N derived from fertilizer (N_{dff}) was calculated as DM × TN × $(A_F - A_C)/(A_U - A_C)$, where DM is plant aboveground dry matter (g pot^{-1}), TN is plant total N concentration (mg g^{-1}), and A is atom % ^{15}N of the plant material analyzed from fertilized (F) or control (C) pots and of the labeled urea applied (U). The percentage recovery of labeled urea N in grass shoots was obtained as 100 × N_{dff}/urea N applied.

Dry matter accumulation, N content, and ^{15}N recovery were evaluated between 5 and 56 days after fertilizer application by fitting least squares regression equations using CurveExpert® software (Hyams Development, Chattanooga, TN, USA). These parameters were analyzed statistically by ANOVA as described previously, and treatment means compared for cumulative dry matter production with the use of Tukey's honestly significant difference at $P < 0.05$.

Results and discussion

As shown by Fig. 1, urea hydrolysis was inhibited over the entire range of Cu and/or Zn rates evaluated, although at the two lowest rates, no significant difference was observed among the three amendments, nor was inhibition significantly improved by a fivefold increase in the rate of addition. The latter findings are to be expected considering the high standard errors of the means obtained with 1 or 5 g kg^{-1} of urea, and can be attributed to the high organic C content of the pasture soil used to study urease inhibition, which would have promoted immobilization of metal ions through adsorption and chelation (Elliott et al., 1986; McBride et al., 1998; Arias et al., 2006; Vytopilová et al., 2015).

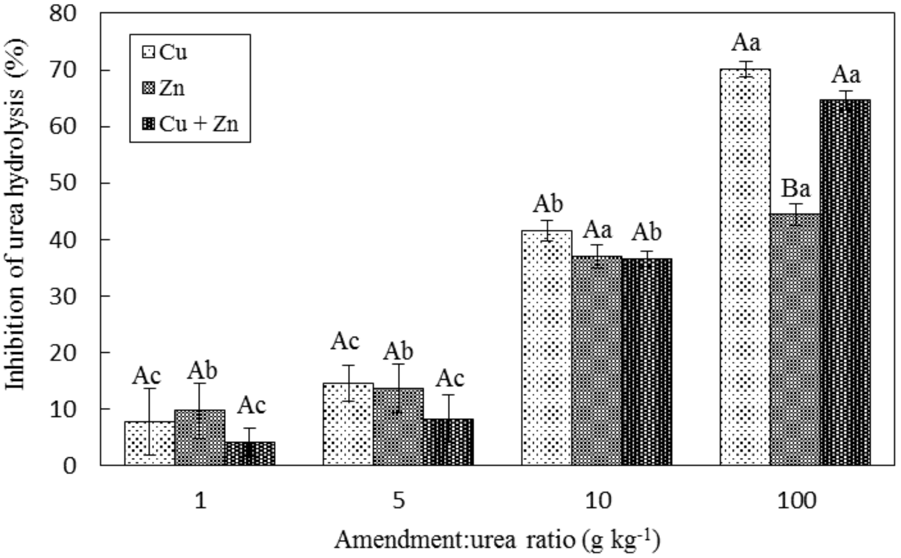

Fig. 1 Percentage inhibition of urea hydrolysis by $CuSO_4$ and/or $ZnSO_4$ applied to soil at a rate of 1, 5, 10, or 100 g kg^{-1} urea. For a single amendment:urea ratio, treatments having the same uppercase letter do not differ significantly ($P < 0.05$) by Tukey's test. For a single treatment, amendment:urea ratios having the same lowercase letter do not diffier significantly ($P < 0.05$) by Tukey's test. Data shown as a mean of triplicate incubations with bars to indicate the standard error of the mean

The immobilizing effect of soil organic C would have been expected to decrease with a higher concentration of Cu and/or Zn, and this is exactly what was observed as inhibition tripled when the application rate was doubled from 5 to 10 g kg^{-1} (Fig. 1). Inhibitory effects at the latter rate did not differ significantly, regardless of whether the two metals were applied individually or as an equimolar combination. When the application rate was increased tenfold to 100 g kg^{-1}, Cu, alone or in combination with Zn, became significantly more effective, whereas efficacy was unaffected for Zn alone. This finding is consistent with previous work documenting an advantage of Cu over Zn for inhibition of soil urease activity (Tabatabai, 1977; El-Din et al., 1985; Yadav et al., 1986; Hemida et al., 1997), which is believed to arise from a greater affinity for amino groups that occur in both urea and urease (Daif and van Beusichen, 1981).

Although high application rates enhance the effectiveness of Cu and/or Zn for inhibiting urea hydrolysis, low rates are more practical for utilization as a fertilizer amendment, so as to minimize the long-term risk of toxicity to plants and soil microorganisms and prevent heavy metal pollution (Giller et al., 1998; Fuentes et al., 2007; Michaud et al., 2008; Mallmann et al., 2014). Because of this constraint, inhibitor concentrations did not exceed 2 g kg^{-1} in the work reported (Table 2), and two strategies were adopted to enhance efficacy by: (1) incorporating Cu and/or Zn into pelletized urea, thereby improving the contact of inhibitor with substrate; and (2) utilizing the gradual desorption of metal ions from OCh-amended urea.

These strategies, and also the use of OCh without Cu and/or Zn, were evaluated by conducting a greenhouse pot study that utilized labeled urea to check ^{15}N recovery in multiple cuttings of a tropical forage grass (Capim-mombaça) grown on a low-fertility Oxisol. The amendments were also evaluated for their effects on soil concentrations of mineral N, by sampling the pots a few days after the urea had been applied and then again several weeks later. The soil data collected are summarized by Table 3, which shows that initial concentrations of exchangeable NH_4^+-N tended to be somewhat higher with than without OCh, although the only significant difference, apart from what was found for comparisons involving the control (C), occurred because of higher NH_4^+-N concentrations using urea pelletized with OCh and Cu (UOChCu) versus urea pelletized with Cu and Zn (UCuZn). At the 42-day cutting, only the UOCh treatment showed a significant accumulation of exchangeable NH_4^+-N, which can be attributed to the low pH and high CEC cited previously from characterization of the OCh used. Combining Cu and/or Zn with OCh would have reduced the CEC available to adsorb urea-derived NH_4^+, the result being a lower concentration of exchangeable NH_4^+-N that did not differ significantly from what was observed in the absence of OCh. In contrast to the initial sampling for the 5-day cutting, NH_4^+-N concentrations after 42 days were not significantly higher for urea treatments without OCh than for the control. Plant N uptake no doubt contributed to the latter finding, and also explains why NO_3^--N concentrations decreased over time without showing any treatment effects. Table 3 indicates that OCh has more potential to enhance the efficiency of fertilizer urea, as opposed to low concentrations of Cu and/or Zn.

Table 3 Soil concentrations of NH_4^+ and NO_3^- estimated for the 5- and 42-day cuttings grown with or without pelletized urea fertilization

Fertilizer source[a]	5-day cutting[b]		42-day cutting[b]	
	NH_4^+ (mg N kg^{-1})	NO_3^- (mg N kg^{-1})	NH_4^+ (mg N kg^{-1})	NO_3^- (mg N kg^{-1})
C	40 (3) c	9.2 (1.1) a	16.9 (1.4) b	5.0 (0.1) a
U	149 (23) ab	12.6 (1.4) a	16.6 (3.2) b	4.8 (0.1) a
UCu	147 (19) ab	11.1 (1.7) a	17.4 (1.5) b	5.0 (0.1) a
UZn	176 (18) ab	9.1 (0.1) a	16.7 (3.2) b	5.1 (0.3) a
UCuZn	132 (09) b	12.1 (0.9) a	21.1 (2.1) b	5.9 (0.8) a
UOCh	199 (23) ab	12.9 (1.9) a	37.3 (9.4) a	5.4 (0.1) a
UOChCu	237 (41) a	13.3 (1.4) a	22.2 (1.7) b	5.4 (0.3) a
UOChZn	189 (13) ab	12.6 (1.1) a	26.3 (3.8) ab	5.2 (0.1) a
UOChCuZn	153 (02) ab	12.9 (1.8) a	23.6 (1.3) ab	5.6 (0.3) a

[a] C, control; U, unamended urea; UCu, urea + $CuSO_4$; UZn, urea + $ZnSO_4$; UCuZn, urea + $CuSO_4$ + $ZnSO_4$; UOCh, urea + oxidized charcoal; UOChCu, urea + oxidized charcoal + adsorbed Cu; UOChZn, urea + oxidized charcoal + adsorbed Zn; UOChCuZn, urea + oxidized charcoal + adsorbed Cu + adsorbed Zn

[b] Mean values reported from four replicate soil cores, with standard deviations in parentheses. Values within a column followed by the same letter do not differ significantly by the Tukey test at $P < 0.05$

Considering the low N content of the soil used in the greenhouse study, crop response was expected to N fertilization, and to intensify over time as a growing number of cuttings increased crop N removal. Both aspects are apparent from Fig. 2a, which compares cumulative dry matter production for the control and urea treatments that followed supplemental fertilization intended to correct deficiencies of other macro- and micronutrients. In each case, plant growth decreased with time as represented by a quadratic model, reflecting a decline in plant N availability that can be attributed to the use of a sandy loam low in organic matter. This soil would have been inherently limited in buffer capacity as well as mineralization, creating conditions conducive to extensive volatilization loss from pelletized urea placed on the soil surface.

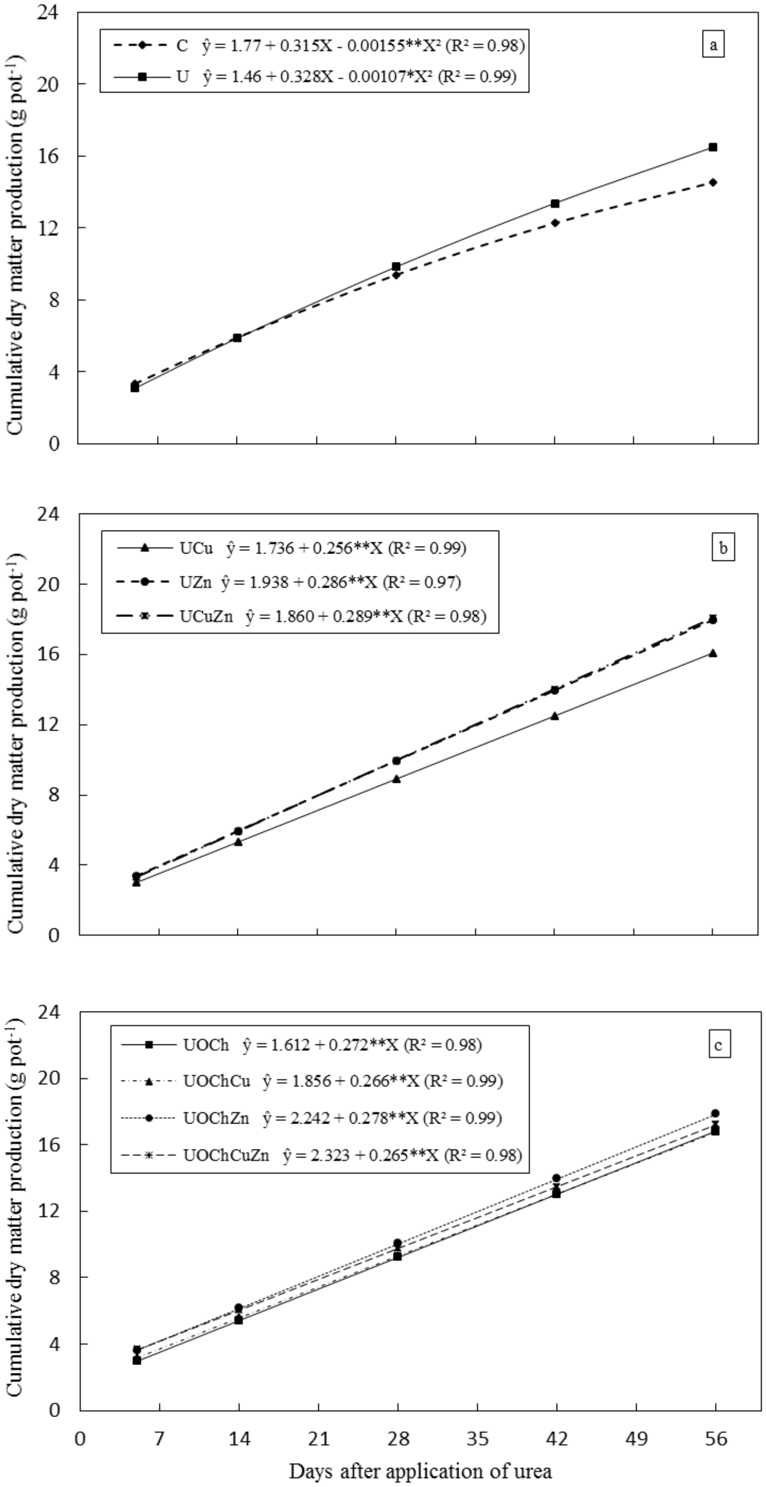

Fig. 2 Accumulation of aboveground dry matter in five cuttings of Capim-mombaça collected 5, 14, 28, 42, and 56 days after application of (a) pelletized urea (U), with simultaneous cuttings from an unfertilized control (C); (b) pelletized urea containing $CuSO_4$ (UCu), $ZnSO_4$ (UZn), or $CuSO_4$ and $ZnSO_4$ (UCuZn); or (c) pelletized urea containing oxidized charcoal (UOCh) or oxidized charcoal enriched with Cu (UOChCu), Zn (UOChZn), or Cu and Zn (UOChCuZn). Equations representing the observed values are shown for $P < 0.05$ (*) or 0.01 (**).

In contrast to what was observed with the control and urea treatments, Fig. 2b and 2c show a linear increase in dry matter production for all other treatments, indicating greater plant N availability. In the case of OCh-based amendments (Fig. 2c), this finding reflects buffer and exchange capacities that were enhanced in the vicinity of the urea pellet, prolonging the availability of fertilizer-derived NH_4^+-N (Table 3). For amendments without OCh (Fig. 2b), different factors were implicated in the linear trend documented for dry matter production. The beneficial effect of Cu probably originated from inhibition of urea hydrolysis previously documented by Fig. 1, which would have prolonged fertilizer N availability. A more likely benefit of applying Zn in conjunction with urea was to supply an essential nutrient increasingly removed in successive Capim-mombaça cuttings. Interestingly, Zn showed a consistent advantage over Cu for dry matter production that became more pronounced for later cuttings.

The same advantage is apparent from Fig. 3, which shows the daily rate of total N uptake between 5 and 56 days after urea fertilization. A marked decline invariably occurred in the first two weeks, followed by a more gradual reduction during the subsequent growing period. The decrease was greatest for the control treatment (Fig. 3a), as expected for a coarse-textured Oxisol having a low total N content. Among the N-fertilized treatments, the daily uptake rate followed a similar pattern except when urea was amended with Zn (UZn), in which case the decline in N uptake was considerably attenuated (Fig. 3b). The latter finding can be attributed to the synergistic interaction of these two nutrients, which originates from the occurrence of Zn as a constituent of carbonic anhydrase and other enzymes required for protein synthesis and carbohydrate metabolism (Marschner, 2012).

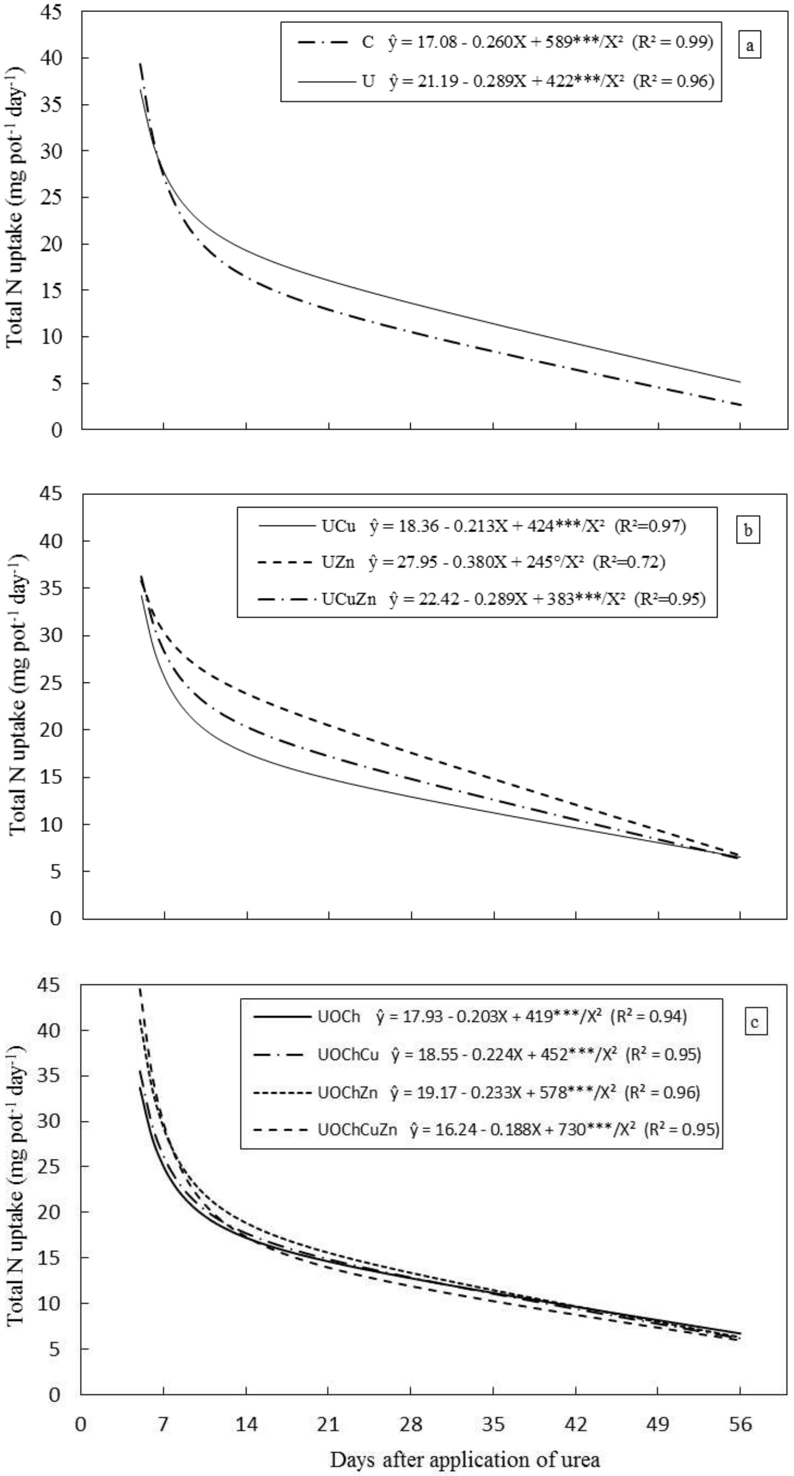

Fig. 3 Rate of N uptake in Capim-mombaça cuttings collected 5, 14, 28, 42, and 56 days after application of (a) pelletized urea (U), with simultaneous cuttings from an unfertilized control (C); (b) pelletized urea containing $CuSO_4$ (UCu), $ZnSO_4$ (UZn), or $CuSO_4$ and $ZnSO_4$ (UCuZn); or (c) pelletized urea containing oxidized charcoal (UOCh) or oxidized charcoal enriched with Cu (UOChCu), Zn (UOChZn), or Cu and Zn (UOChCuZn). Equations representing the observed values are shown for $P < 0.10$ (º) or 0.001 (***).

The marked influence of Zn in promoting total N uptake, as documented by Fig. 3b, is most likely explained by the incorporation of triple superphosphate supplying 250 mg P kg^{-1} prior to the application of a solution containing $ZnSO_4$ and other nutrients. Fertilization with P was necessitated by a nutrient limitation common for Oxisols; however, Zn deficiency was an unintended consequence of an antagonistic interaction exacerbated for such soils because phosphate enhances adsorption of Zn to hydrous oxides Fe and Al (Stanton and Burger, 1967; Loneragan et al., 1979).

As shown by Fig. 4, the daily rate of ^{15}N recovery followed a very different pattern than was observed for total N uptake, with maximal enrichment of cuttings collected 28 days after ^{15}N fertilization. This difference can be attributed in part to the fact that the pelletized urea was applied to the soil surface among plants with a well-established root system actively engaged in the uptake of mineralized soil N. A gradual increase in ^{15}N uptake occurred as urea N was incorporated into the soil by frequent irrigation; however, downward movement was limited by the absence of drainage. The gradual nature of this increase also reflects the dynamics of $^{15}NO_3^-$ production through nitrification, owing to the preference of heterotrophic microorganisms for immobilization of NH_4^+-N over NO_3^--N (Jansson et al., 1955; Rice and Tiedje, 1989; Recous et al., 1990). As expected, maximal ^{15}N uptake was followed by a decline due to ongoing ^{15}N removal in successive cuttings.

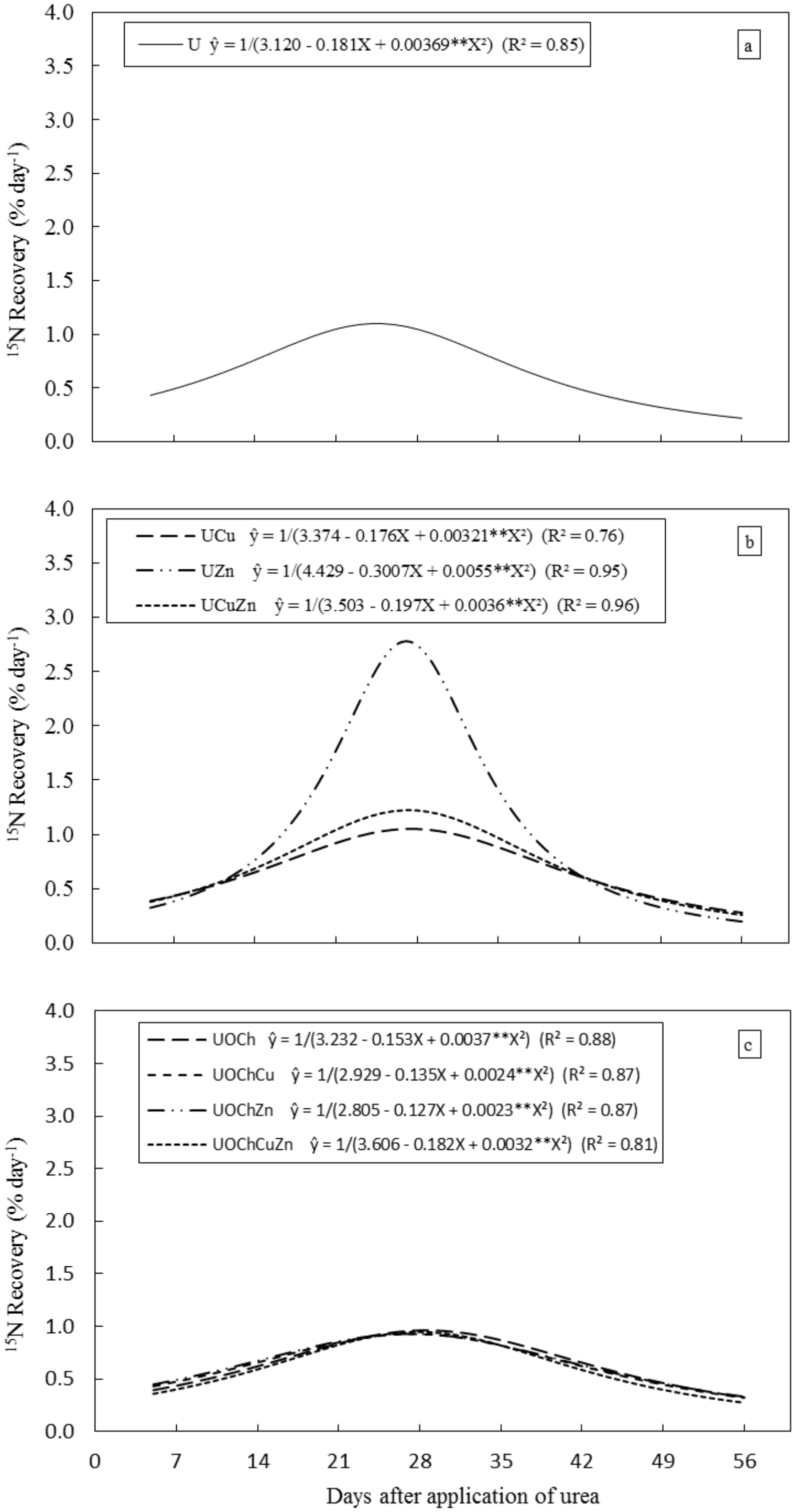

Fig. 4 Rate of ^{15}N recovery in Capim-mombaça cuttings collected 5, 14, 28, 42, and 56 days after application of (a) pelletized urea (U); (b) pelletized urea containing $CuSO_4$ (UCu), $ZnSO_4$ (UZn), or $CuSO_4$ and $ZnSO_4$ (UCuZn); or (c) pelletized urea containing oxidized charcoal (UOCh) or oxidized charcoal enriched with Cu (UOChCu), Zn (UOChZn), or Cu and Zn (UOChCuZn). Equations representing the observed values are shown for $P < 0.01$ (**).

Examination of Fig. 4 reveals that most of the treatments were very similar in the daily uptake of ^{15}N, except for unamended and Zn-amended urea. The increase in ^{15}N uptake occurred most rapidly for the urea (U) treatment (Fig 4a), in which case liberation of NH_4^+ for plant and microbial uptake was unimpeded by inhibition of urea hydrolysis or adsorption to OCh. Much more evident is the effect of Zn for increasing the rate of ^{15}N recovery (Fig. 4b), which is consistent with what was observed for total N uptake (Fig. 3b) and is no doubt attributable to the same interactions noted previously, involving synergism with N and antagonism with P.

Table 4 summarizes cumulative dry matter production, total N uptake, and ^{15}N recovery for all five of the cuttings collected. Urea fertilization led to a significant increase in dry matter production, as compared to the control treatment; however, Cu, Zn, and OCh were of no quantitative benefit in comparison to unamended urea. The Zn amendment provided the only significant benefit observed for total N uptake, indicating a potential advantage for improving the nutritional quality of pasture production. This was also the only amendment that significantly increased plant recovery of ^{15}N, by nearly 74 % as compared to unamended urea. Recovery of ^{15}N averaged 34 % for all other treatments, demonstrating the low efficiency inherent to surface placement of urea and the fundamental importance of indigenous soil N reserves to plant N uptake, which was apparent even for the coarse-textured Oxisol used in our work.

Table 4 Cumulative dry matter production of Capim-mombaça, total N uptake, and ^{15}N recovery for the 56-day experimental period

Fertilizer source[a]	Dry matter[b] (g pot^{-1})	Total N uptake[b] (mg pot^{-1})	^{15}N recovery[b] (%)
C	14.4 (0.43) b	619.6 (17.2) c	—
U	16.3 (0.33) a	752.1 (11.2) b	34.5 (1.66) b
UCu	15.8 (0.39) ab	743.7 (21.9) b	35.0 (2.64) b
UZn	17.4 (0.43) a	933.9 (17.9) a	58.9 (4.12) a
UCuZn	17.5 (0.67) a	817.9 (28.6) b	37.8 (1.45) b
UOCh	16.4 (0.36) a	735.2 (14.7) b	34.6 (0.82) b
UOChCu	16.3 (0.26) a	736.5 (17.9) b	34.1 (1.08) b
UOChZn	17.4 (0.23) a	789.3 (13.6) b	34.5 (0.91) b
UOChCuZn	16.7 (0.38) a	758.1 (24.8) b	32.7 (1.38) b

[a] C, control; U, unamended urea; UCu, urea + $CuSO_4$; UZn, urea + $ZnSO_4$; UCuZn, urea + $CuSO_4$ + $ZnSO_4$; UOCh, urea + oxidized charcoal; UOChCu, urea + oxidized charcoal + adsorbed Cu; UOChZn, urea + oxidized charcoal + adsorbed Zn; UOChCuZn, urea + oxidized charcoal + adsorbed Cu + adsorbed Zn

[b] Mean values reported from four replicate pots, with standard deviations in parentheses. Values within a column followed by the same letter do not differ significantly by the Tukey test at $P < 0.05$

Conclusions

In a greenhouse study using pelletized urea to enhance tropical pasture production, the supply of NH_4^+-N was prolonged more effectively by the use of oxidized charcoal (OCh) to increase CEC than by inhibiting urea hydrolysis with low concentrations of Cu and/or Zn; however, there was no significant effect on dry matter production. The additional Zn supplied by Zn-amended urea caused a significant increase in total N uptake and the efficiency of urea N fertilization. Incorporation of Zn into pelletized urea has practical potential to improve the concentration of N in forage grasses growing on tropical soils.

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CHAPTER 2

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Comparison of N-(n-butyl) thiophosphoric triamide and oxidized charcoal for conserving urea nitrogen applied to contrasting soils

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Abstract

Charcoal-based amendments have potential use in controlling NH_3 volatilization from urea fertilization, owing to a high cation-exchange capacity (CEC) that enhances the retention of NH_4^+. An incubation study was conducted to evaluate the potential of oxidized charcoal (OCh) for controlling soil transformations of urea N, in comparison to urease inhibition by N-(n-butyl) thiophosphoric triamide **(**NBPT). Four soils, ranging widely in texture and CEC, were incubated aerobically for 0, 1, 3, 7, and 14 days after application of ^{15}N-labeled urea with or without OCh (150 g kg^{-1} fertilizer) or NBPT (0.5 g kg^{-1} fertilizer), and analyses were performed to determine residual urea and ^{15}N recovery as volatilized NH_3, mineral N (as exchangeable NH_4^+, NO_3^-, and NO_2^-), and immobilized organic N. The OCh amendment reduced NH_3 volatilization by up to 12 % but had no effect on urea hydrolysis, NH_4^+ and NO_3^- concentrations, NO_2^- accumulation, and immobilization. In contrast, the use of NBPT to inhibit urea hydrolysis was markedly effective for moderating the accumulation of NH_4^+, which reduced immobilization and also controlled NH_3 toxicity to nitrifying microorganisms that otherwise, caused the accumulation of NO_2^- instead of NO_3^-. Oxidized charcoal is not a viable alternative to NBPT for increasing the efficiency of urea fertilization.

Keywords Ammonia volatilization · ^{15}N · Nitrification · Immobilization · Urease inhibitor

Introduction

Fertilizer use of urea is complicated by the rapid hydrolysis that often occurs in soil to produce an accumulation of NH_4^+ and a high pH, promoting NH_3 volatilization that compromises fertilizer N uptake efficiency when urea is allowed to decompose on the soil surface (Soares et al. 2012; Abalos et al. 2014). Volatilization is particularly problematic for coarse-textured soils limited in buffer capacity and CEC (Watson et al. 1994; Abalos et al. 2014; Ni et al. 2014), and in such cases the NH_3 liberated by hydrolysis can inhibit germination and lead to phytotoxicity when urea is applied at planting (Schenk and Wehrmann 1979; Bremner and Chai 1986; McCarty et al. 1990; Bremner 1995; Haden et al. 2011; Singh et al. 2013). Phytotoxicity can also arise from the accumulation of NO_2^- (Bremner 1995; Samater et al. 1998; Haden et al. 2011) by NH_4^+-oxidizing bacteria that utilize urea C as well as N (Marsh et al. 2005) and are favored by the alkalinity generated upon hydrolysis (Hauck and Stephenson 1965; McInnes and Fillery 1989). Rapid nitrification of urea N has important economic and environmental implications owing to the loss of NO_2^- and NO_3^- by leaching and denitrification, particularly since urea is the leading N fertilizer in world agriculture (FAO 2015).

One approach to controlling the problems associated with urea fertilization is to reduce the rate of hydrolysis using a urease inhibitor. Numerous compounds have been evaluated for this purpose; however, most have little practical potential because they are ineffective at low concentrations or prohibitively expensive, lack the necessary persistence, or pose a toxicity hazard. These limitations do not apply to N-(n-butyl) thiophosphoric triamide (NBPT), which remains the most successful inhibitor thirty years after the first evaluation by Bremner and Chai (1986). This compound is a urea derivative that decomposes within a few hours after application to soil, forming the oxon analog, N-(n-butyl) phosphoric triamide, which becomes the active inhibitor (Watson et al. 2008). Efficacy is typically retained for a week or two, and is inversely related to soil temperature and clay and organic matter content (Bremner and Chai 1986; Bremner et al. 1991).

In numerous field studies to date, NBPT has been evaluated for use with corn (*Zea mays* L.), wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), cotton (*Gossypium* sp.), barley (*Hordeum vulgare* L.), and forage and pasture grasses. Many of the findings document a reduction in NH_3 volatilization relative to unamended urea (e.g., Turner et al. 2010; San Francisco et al. 2011; Sanz-Cobena et al. 2011; Soares et al. 2012; Abalos et al.

2014), and some show an increase in the efficiency of urea N uptake and/or higher yield (Dawar et al. 2011; Franzen et al. 2011; Kawakami et al. 2012; Suter et al. 2013). Commercial formulations are marketed under the trade name, Agrotain® (Koch Agronomic Services, 2015), as a stabilizer to control NH_3 loss from granular urea, urea-NH_4NO_3 solutions, or liquid manure.

As an alternative to urease inhibitors, fertilizer N uptake efficiency can be improved by applying urea in conjunction with a much higher rate of biochar (typically > 10 Mg ha^{-1}), so as to enhance NH_4^+ adsorption for higher N uptake efficiency with lower losses by NH_3 volatilization, NO_3^- leaching, and N_2O emission (Taghizadeh-Toosi et al. 2012; Güereña et al. 2013; Huang et al. 2014). Much lower application rates will be essential if this strategy is to be widely adopted, utilizing a different carbonaceous material that can be applied with urea as an amendment.

One possibility is to activate charcoal by oxidation with HNO_3 (Trompowsky et al. 2005), which markedly increases the specific surface area, pore volume, carboxyl content, exchange acidity, and adsorptive capacity for cations and anions (Paiva et al. 2012). Oxidized charcoal prepared in this way was found to be effective for delaying and reducing NH_3 volatilization in a laboratory evaluation by Paiva et al. (2012) involving surface application of coated and uncoated granules to a Brazilian Ultisol collected from a no-tillage site. In subsequent work by Guimarães et al. (2015), the beneficial effects were enhanced when oxidized charcoal was blended with urea (150 g kg^{-1}), in lieu of being used as a coating.

The purpose of the work reported here was to further evaluate the potential of oxidized charcoal for controlling soil transformations of urea N, in comparison to urease inhibition by NBPT. The use of 15N-labeled urea ensured analytical specificity in assessing the fate of fertilizer-derived N due to immobilization as well as hydrolysis, NH3 volatilization, and nitrification, and the findings are based on some temperate and tropical soils.

Materials and methods

Soil samples

The soils used (Table 1) were surface (0-15 cm) samples that included a Brazilian Oxisol collected from a sugarcane (*Saccharum* spp.) field near Assis in the state of São Paulo (SP), and an Alfisol (Bloomfield) and two Mollisols (Elliott and Drummer) under corn-soybean (*Glycine max* L. Merr.) production in Illinois, USA. Before use, each sample was air-dried and then crushed to pass through a 2 mm screen. In the analyses reported in Table 1, pH was determined with a glass electrode (soil:water ratio, 1:1), organic C by the method of Mebius (1960), total N by the regular Kjeldahl method using a block digester (Bremner 1996) followed by diffusion with NaOH (Stevens et al. 2000), cation-exchange capacity (CEC) by the rapid saturation-diffusion method of Mulvaney et al. (2004), texture using the hydrometer method (Gee and Bauder 1986); water-holding capacity (WHC) as described by Bremner and Shaw (1958); and urease activity using the buffer method of Tabatabai and Bremner (1972) modified for NH_4^+-N determination by the accelerated diffusion technique of Khan et al. (1997).

Production and characterization of oxidized charcoal

Charcoal produced from *Eucalyptus urophilla* wood as described by Guimarães et al. (2015) was boiled under reflux for 4 h in 4 M HNO_3 (charcoal 45 g L^{-1} HNO_3), and after cooling the mixture was filtered under vacuum through JP 42 filter paper (J. Prolab., São Jose dos Pinhais-PR, Brazil) in a 12.5-cm diâmetro porcelain Büchner funnel. The residue was dissolved in deionized water and transferred to 25 mm dialysis tubing (MWCO 12400; Sigma-Aldrich, St. Louis, MO, USA), and excess acidity was removed by carrying out dialysis in distilled water that was repeatedly changed until the electrical conductivity exceeded 2 $\mu S\ cm^{-2}$. After oven-drying, the oxidized charcoal (OCh) was powdered with a mortar and pestle, and then screened to $< 74\ \mu m$. A more complete description of charcoal oxidation is available in Guimarães et al. (2015).

Table 1 Chemical and physical properties of soils studied

Soil	Soil subgroup	pH	Organic C (g kg^{-1})	Total N (g kg^{-1})	CEC[a] ($cmol_c$ kg^{-1})	Sand (g kg^{-1})	Silt (g kg^{-1})	Clay (g kg^{-1})	WHC[b] (g kg^{-1})	Urease activity (mg N kg^{-1} h^{-1})
Assis	Typic Hapludox	5.3	3.9	0.34	3.5	850	34	116	345	11.4
Bloomfield	Lamellic Hapludalfs	6.9	3.3	0.37	1.2	967	9	24	312	17.1
Elliott	Aquic Argiudolls	6.2	19.2	1.65	19.2	372	437	191	454	19.8
Drummer	Typic Endoaquolls	7.2	29.2	1.93	33.2	252	505	243	517	72.7

Data reported as a mean of triplicate determinations, except for unreplicated textural determinations

[a] CEC, cation-exchange capacity

[b] WHC, water-holding capacity

Upon characterization, the OCh was found to have a pH of 2.7 (OCh:water ratio, 1:10), and elemental analyses using a PerkinElmer 2400 Series II CHNS/O Analyzer (PerkinElmer, Waltham, MA, USA) showed 574 g C kg^{-1} C, 24 g kg^{-1} H, and 30 g kg^{-1} N when expressed on the basis of ash-free dry matter, giving a C/N ratio of 19.1 (w/w). An O content of 373 g kg^{-1} was estimated from the difference between the ash-free weight and the total content of C, H, and N. The CEC was determined as 436 cmol$_c$ kg^{-1} by potentiometric titration with 0.1 mol L^{-1} NaOH, assuming that the titratable acidity was derived from carboxyl functional groups between pH 3 and 8 and from phenolic functional groups between pH 8 and 10 (Bowles et al. 1994; Inbar et al. 1992).

Incorporation of urea amendments

A solution of ^{15}N-enriched urea (1 atom % ^{15}N, 44.44 g L^{-1}) was prepared using labeled reagent obtained from Sigma-Aldrich (St. Louis, MO, USA), for use with or without OCh or NBPT. To amend urea with OCh (150 g kg^{-1}), 0.233 g of the powdered material was weighed into 35 mL of the urea solution in a 100-mL beaker, and the OCh was largely dissolved by swirling the beaker. The same technique was employed in amending urea with NBPT, except that the amendment rate was reduced to 0.5 g kg^{-1} by dissolving 0.78 mg of NBPT in 35 mL of urea solution. To dry each of the amended urea mixtures and the unamended urea solution for soil application, 0.5-mL aliquots containing 10 mg of N were pipetted into 36 1.5-mL polypropylene microcentrifuge tubes (Fisher Scientific, Pittsburgh, PA, USA), and the tubes were transferred to a freezer and allowed to remain overnight at –13 ºC, followed by freeze-drying using a VirTis BenchTop 4K XL freeze dryer (VirTis, Gardiner, NY, USA). Preliminary work showed no loss of urea N by freeze-drying, and 97-99 % efficiency in delivering urea N, with or without the amendments, from the microcentrifuge tube.

Incubation procedure

To compare transformations of urea N applied with or without OCh or NBPT, triplicate 10-g samples of each soil were placed in 125-mL polyethylene screw-cap bottles and treated with 10 mg of N as urea (U), U + OCh (UOCh), or U + NBPT (UNBPT). The treatments were applied by inverting a microcentrifuge tube above the mouth of the bottle, tapping the tube to dislodge the contents, and using a spatula to detach any particles that might remain in the tube. To ensure complete incorporation of the fertilizer into the soil, the bottle was held horizontally after attaching the screw-cap, and then carefully rotated to bring the soil into complete contact with the walls of the bottle. After returning the bottle to an upright position, the cap was removed and deionized water was dispensed at a moderate rate from a Metrohm 665 Dosimat (Metrohm, Herisau, Switzerland), so as to bring the soil moisture content to 60 % WHC. With the exception of samples for zero-time incubation, the bottles were fitted with an aeration device having an acid trap for absorption of gaseous NH_3 liberated during incubation, similar in design to the unit described by Bremner and Douglas (1971) but utilizing a polycarbonate tube cemented to a disposable polystyrene beaker that contained 5 mL of 0.25 mol L^{-1} H_2SO_4. Incubation was performed for 1, 3, 7, and 14 days, after placing the bottles in a constant-temperature room maintained at 25 ºC.

Following incubation, the H_2SO_4 used to absorb volatilized NH_3 was transferred to a 473-mL wide-mouth Mason jar, and the transfer was completed by rinsing the beaker with 5 mL of deionized water from a mechanical pipetter. The diluted absorbent was then alkalized by addition of 2 mL of 1 mol L^{-1} NaOH, and the jar was sealed by attaching a lid modified to support a petri dish containing 5 mL of H_3BO_3-indicator solution (40 g H_3BO_3 L^{-1}), swirled to mix the contents, and placed on an electric hot plate maintained at 45-50 ºC. Volatilized NH_3 was quantified by titration with 0.0086 mol L^{-1} H_2SO_4 following a 4-h diffusion period, and then processed as described by Khan et al. (1997) for N-isotope analysis using a mass spectrometer equipped with an automated Rittenberg system (Mulvaney et al. 1990, 1997; Mulvaney and Liu 1991).

Mineral N produced during incubation or present in zero-time samples was extracted by shaking the soil sample for 1 h with 100 mL of 2 mol L^{-1} KCl containing phenylmercuric acetate (5 mg L^{-1}) as a urease inhibitor (soil:solution ratio, 1:10). The resulting suspension was filtered through folded Whatman no. 42 filter paper (GE Healthcare, Buckinghamshire, UK) in a Pyrex® funnel (Corning, Corning, NY, USA) for collection in a 125-mL glass bottle that was subsequently transferred to a refrigerator for storage at 5 ºC. The soil remaining on the filter paper was leached with two 25-mL aliquots of 0.5 mol L^{-1} K_2SO_4 to ensure the complete absence of mineral N, and allowed to dry overnight before transfer to a 125-mL polyethylene bottle. Drying was completed at room temperature while the bottle was left open for at least 5 days, and the residual soil was then pulverized with a mortar and pestle.

Determination of urea N, extractable inorganic N, and immobilized organic N

Soil extracts were analyzed for urea N by a modified version of the diacetyl monoxime method of Mulvaney and Bremner (1979), in which all volumes were reduced by 50 %, color development was carried out in a Pyrex® test tube (25 mm dia., 150 mm long) instead of a 50-mL volumetric flask, and the contents of the tube were homogenized by vortex mixing. Analyses for extractable inorganic N were performed using the accelerated diffusion methods of Khan et al. (1997) to determine exchangeable NH_4^+-N, $(NH_4^+ + NO_3^-)$-N, and $(NH_4^+ + NO_3^- + NO_2^-)$-N. The residual soil was analyzed for organic N by the regular Kjeldahl method using a block digester (Bremner 1996), followed by diffusion with NaOH (Stevens et al. 2000). Following organic and inorganic N analyses, the titrated samples were processed as described by Khan et al. (1997) to determine their N isotopic composition.

Statistical analysis

The experiment utilized a factorial design with three replications of four soils, three treatments (U, UOCh, and UNBPT), and five incubation periods. Replicate data were characterized by computing means and standard deviations, and the significance of treatment factors assessed by analysis of variance (ANOVA). In cases where the *F* test was significant, mean comparisons were performed by Tukey's procedure ($P < 0.05$) to detect treatment differences within the N fractions under analysis, and the differences were quantified whenever possible by fitting exponential regression models to urea recoveries, and logistic power or modified exponential regression models to $^{15}NH_3$ recoveries.

Results and discussion

Figure 1 plots the disappearance of urea applied to the four soils studied, with or without OCh or NBPT that had been incorporated into the fertilizer granules. The course of hydrolysis was unaffected by the presence of OCh, as evidenced by the graphical coincidence and very similar half-life values between this treatment and unamended urea. This is precisely what would be expected for an amendment that provides CEC and buffer capacity but has no inhibitory effect on urea hydrolysis, as previously documented by Paiva et al. (2012). In contrast, NBPT was highly effective for prolonging the presence of urea as shown by a much longer half-life for hydrolysis, and efficacy was considerably higher for the Assis and Bloomfield than for the Elliott and Drummer soils. The latter finding can be attributed to a substantial difference in organic C, clay, and CEC (Table 1), which have been found to be negatively correlated with urease inhibition by NBPT (Bremner and Chai 1986).

In the absence of OCh or NBPT, the rate of hydrolysis was very similar for the Assis, Bloomfield, and Drummer soils, and somewhat slower for the Elliott soil. This difference is consistent with the much higher urease activity reported in Table 1 for the Drummer soil, whereas hydrolysis was much more rapid for the Assis and Bloomfield soils than would have been predicted from their low urease activities. No definite explanation can be offered for the latter anomaly, but one factor could have been a rapid rise in pH owing to limited buffer capacity, which would have a beneficial effect on urea hydrolysis (Tabatabai and Bremner 1972; May and Douglas 1976; Longo and Melo 2005). The rise in pH, combined with low CEC (Table 1), created conditions favoring volatilization of NH_3, the result being a shift in equilibrium to compensate for the removal of an enzymatic product in accordance with Le Châtelier's principle.

The rapid hydrolysis observed in the first three days for the Assis sandy loam and Bloomfield sand (Fig. 1), combined with their low CEC (Table 1) and buffering capacities, created conditions that were highly conducive to NH_3 volatilization. This is readily apparent from Fig. 2, which shows that ^{15}N losses from unamended urea ultimately exceeded 50 % for the Assis soil, as compared to more than 70 % for the Bloomfield soil that was lower in CEC and higher in pH. In contrast, volatilization losses did not exceed 8 % for the two Mollisols (Elliott and Drummer), reflecting their much greater buffer capacities. Owing to a longer half-life for urea hydrolysis, NH_3 losses occurred more gradually from the Elliott soil than from the other soils studied.

Considering the high CEC and low pH of the OCh used, and the benefits documented for a similar material by Paiva et al. (2012), this amendment was expected to bring a considerable reduction in the gaseous loss of urea N as NH_3. That expectation was unfortunately not substantiated, for Fig. 2 shows that the UOCh treatment was only somewhat effective in reducing volatilization relative to unamended urea when evaluated with the Assis, Bloomfield, and Elliott soils, and totally ineffective with respect to the Drummer soil. The latter finding is indicative of a soil inherently high in organic C and 2:1 clay, such that the OCh had an insignificant effect for increasing CEC and buffer capacity.

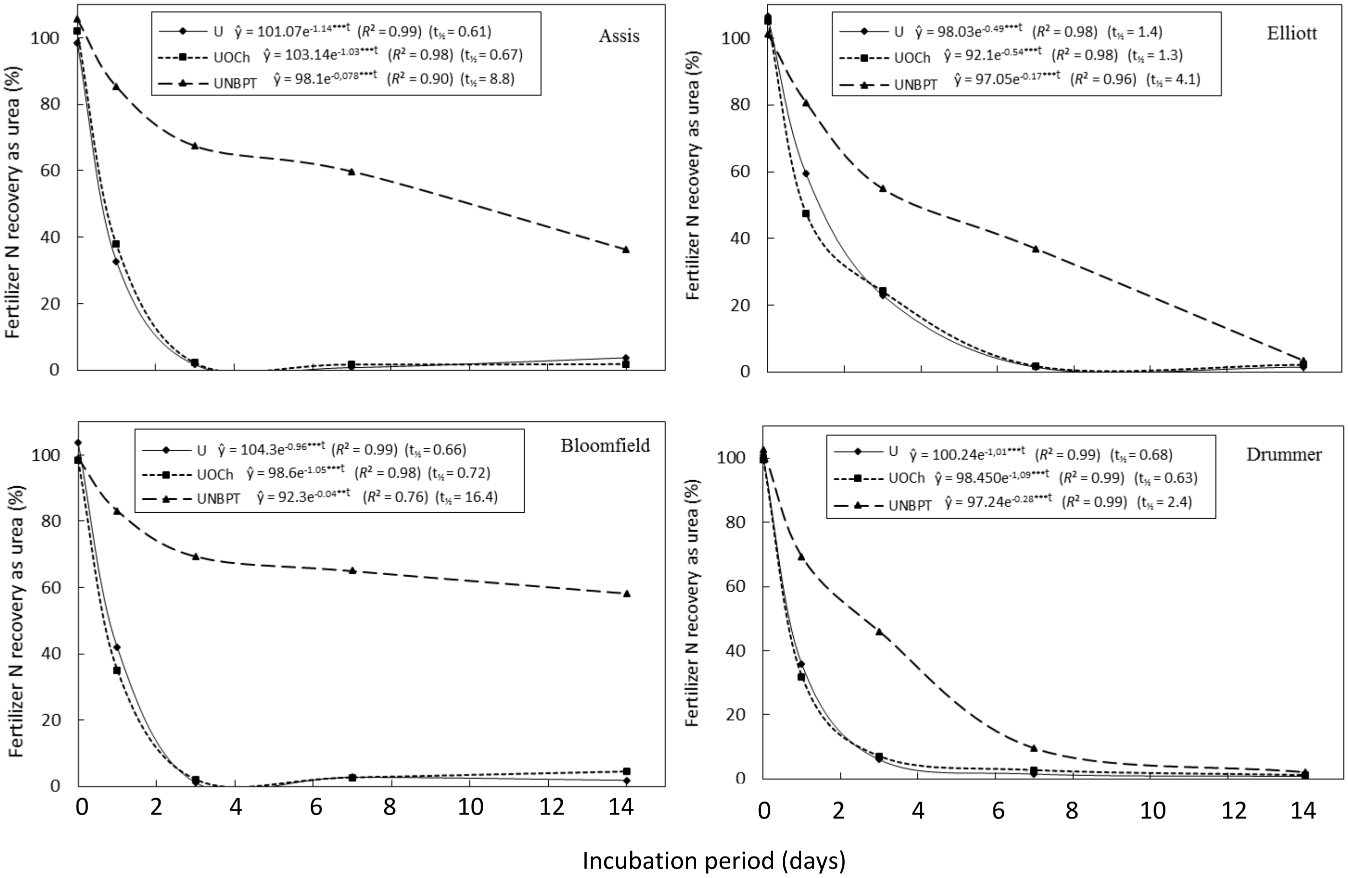

Fig. 1 Fertilizer N recovery as urea during aerobic incubation of Assis, Bloomfield, Elliott, and Drummer soils treated with urea (U), urea and oxidized charcoal (UOCh), or urea and NBPT (UNBPT). Exponential equations of the form, $\hat{y} = ae^{bt}$, are shown for $P < 0.001$ (***) or 0.01 (**), and were used to estimate a half-life ($t_{½}$ in days) for urea hydrolysis from the equation, $t = \ln(½)/b$.

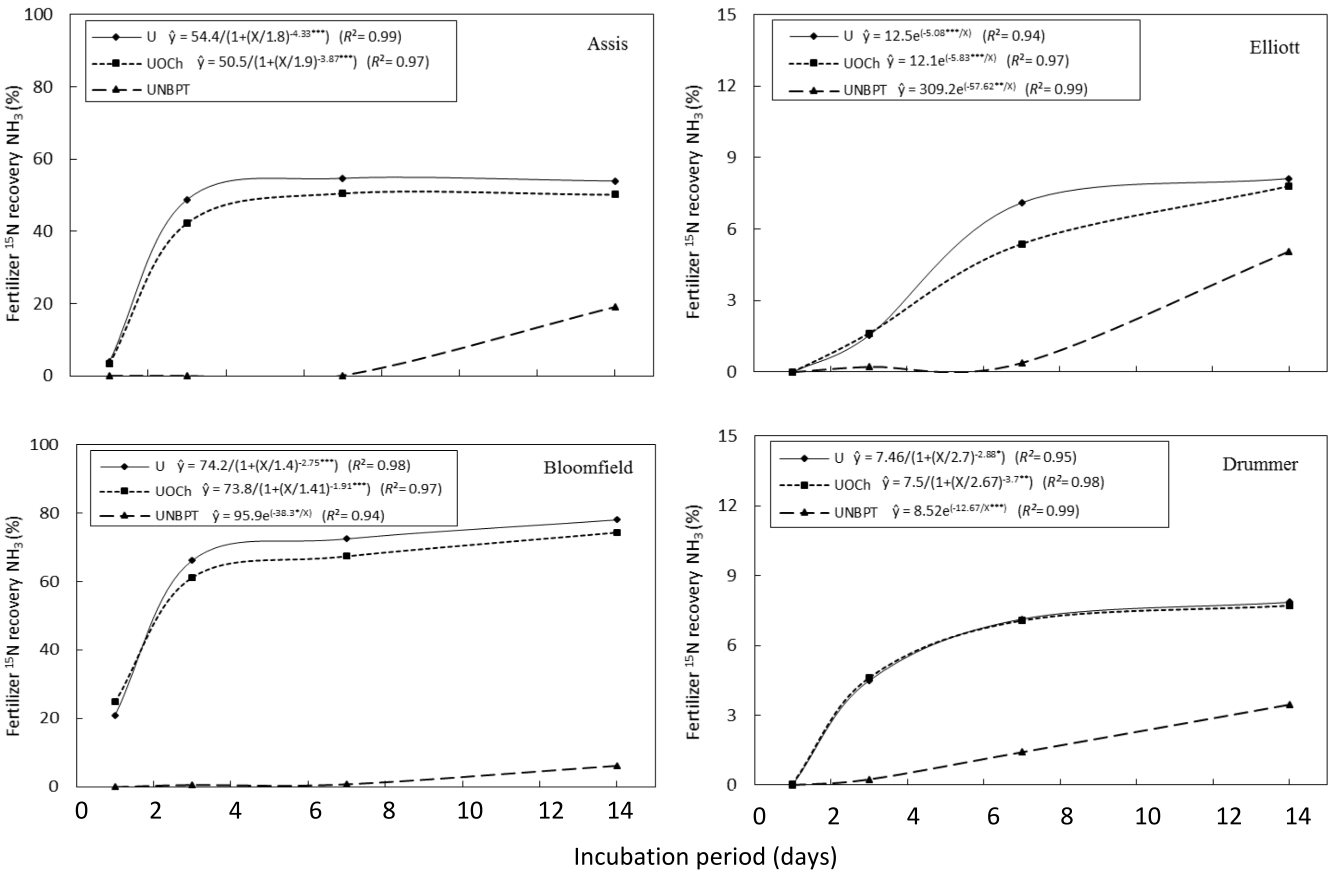

Fig. 2 Fertilizer ^{15}N recovery as NH_3 during aerobic incubation of Assis, Bloomfield, Elliott, and Drummer soils treated with urea (U), urea and oxidized charcoal (UOCh), or urea and NBPT (UNBPT). Equations representing the observed values are shown for *P* < 0.001 (***), 0.01 (**), or 0.05 (*), except when volatilization from the Assis soil was detected only once for the UNBPT treatment.

Numerous studies have demonstrated that NH_3 volatilization can be controlled by using NBPT to retard urea hydrolysis (Dawar et al. 2011; Franzen et al. 2011; Kawakami et al. 2012; Suter et al. 2013). Further evidence of this benefit is provided by Fig. 2, which shows that NH_3 losses from the Assis, Bloomfield, and Elliott soils were effectively prevented with the UNBPT treatment during the first week of incubation and thereafter remained considerably lower than what was observed using unamended urea or the UOCh treatment. The same advantage was evident for the Drummer soil; however, volatilization was detected somewhat sooner, presumably reflecting greater microbial activity that promoted decomposition of NBPT (McCarty et al. 1989, 1990; Bremner et al. 1991).

Table 2 compares the three treatments in terms of ^{15}N recovered as exchangeable NH_4^+, NO_3^-, and NO_2^- before and after the various incubation intervals. Slight recoveries sometimes occurred at the outset of the experiment, but these are undoubtedly an artifact of cross-contamination error. The actual recoveries would have been zero in all such instances, because the application of labeled urea was immediately followed by addition of 2 mol L^{-1} KCl containing a urease inhibitor (PMA) to prevent urea hydrolysis.

Recoveries reported in Table 2 for exchangeable $^{15}NH_4^+$ after 1- to 14-day incubations reflect production through urea hydrolysis, and consumption by NH_3 volatilization and/or nitrification. With unamended urea or the UOCh treatment, this fraction reached higher levels for the Elliott and Drummer soils than for the Assis and Bloomfield soils, and a longer period was required for maximal accumulation. Both differences can be attributed to a reduction in NH_3 loss, owing to the much higher CEC of the two Mollisols (Table 1). Unfortunately, OCh was ineffective for increasing retention of exchangeable NH_4^+ by any soil studied, which is consistent with the evidence in Fig. 2 that this amendment was of limited value for controlling volatilization.

As compared to urea applied alone or with OCh, the UNBPT treatment was significantly lower in exchangeable NH_4^+ after 1, 3, or 7 days, but this difference disappeared after the second week of incubation. For the Assis, Bloomfield, and Elliott soils, declining inhibition of urea hydrolysis by NBPT (Fig. 1) led to a gradual increase in

Table 2 Fertilizer ^{15}N recovery as exchangeable NH_4^+, NO_3^-, and NO_2^- during aerobic incubation of Assis, Bloomfield, Elliott, and Drummer soils treated with urea (U), urea and oxidized charcoal (UOCh), or urea and NBPT (UNBPT)

Soil	Treatment[a]	% Recovery of ^{15}N after incubation period (days)[b]				
		0	1	3	7	14
			Exchangeable NH_4^+			
Assis	U	0.3 a	47.9 b	37.6 b	30.1 b	27.7 a
	UOCh	0.1 a	43.4 b	36.2 b	28.8 b	29.3 a
	UNBPT	0.1 a	2.1 a	8.3 a	11.8 a	30.6 a
Bloomfield	U	0.2 a	29.9 b	29.5 b	13.7 b	10.4 a
	UOCh	0.1 a	34.6 b	21.9 b	15.6 b	11.2 a
	UNBPT	0.1 a	1.3 a	2.8 a	3.9 a	9.8 a
Elliott	U	0.4 a	15.3 b	61.0 b	68.8 b	59.9 a
	UOCh	0.3 a	16.9 b	58.8 b	64.6 b	50.3 a
	UNBPT	0.2 a	3.0 a	16.6 a	30.3 a	56.4 a
Drummer	U	0.3 a	36.4 b	70.2 b	45.8 b	32.2 a
	UOCh	0.4 a	34.2 b	68.6 b	46.0 b	28.6 a
	UNBPT	0.2 a	10.9 a	19.9 a	38.7 a	34.2 a
			NO_3^-			
Assis	U	0	0.5 a	0.8 a	1.2 a	0.2 a
	UOCh	0	0.7 a	1.0 a	1.4 a	0.1 a
	UNBPT	0	0.1 a	0.6 a	1.2 a	1.2 b
Bloomfield	U	0.1 a	2.1 b	0.6 a	0.1 a	0.1 a
	UOCh	0.1 a	1.3 ab	0.4 a	1.8 a	0.7 a
	UNBPT	0 a	0.1 a	0.2 a	0.5 a	0.8 a
Elliott	U	0.1 a	3.6 b	1.8 a	3.7 a	4.3 a
	UOCh	0.1 a	2.3 ab	2.5 a	4.9 a	2.5 a
	UNBPT	0.1 a	1.3 a	1.3 a	4.3 a	14.9 b
Drummer	U	0.1 a	1.6 a	1.4 a	11.1 a	26.4 a
	UOCh	0 a	2.2 a	1.7 a	14.0 a	27.9 a
	UNBPT	0.1 a	1.0 a	2.4 a	11.1 a	44.7 b
			NO_2^-			
Assis	U	0	0.04 b	0.05 a	0.1 b	2.8 b
	UOCh	0	0.04 b	0.06 a	0.1 b	3.1 b
	UNBPT	0	0 a	0.03 a	0.04 a	0.1 a
Bloomfield	U	0	0.05 b	0.07 a	1.2 b	10.5 b
	UOCh	0	0.06 b	0.09 a	0.1 a	9.8 b
	UNBPT	0	0.00 a	0.01 a	0.07 a	0.1 a
Elliott	U	0	0.05 b	0.44 b	5.6 b	18.8 b
	UOCh	0	0.05 b	0.50 b	4.3 b	17.5 b
	UNBPT	0	0.02 a	0.06 a	0.1 a	3.6 a
Drummer	U	0	0.2 b	2.2 c	16.1 b	10.2 b
	UOCh	0	0.2 b	1.8 b	13.7 b	8.7 b
	UNBPT	0	0.06 a	0.1 a	0.7 a	1.5 a

[a] U, urea; UOCh, urea + oxidized charcoal; UNBPT, urea + NBPT

[b] Mean values reported from triplicate incubations. Values within a column for a single soil followed by the same letter do not differ significantly by the Tukey test at $P < 0.05$

$^{15}NH_4^+$ recovery that continued throughout the study period. In the case of the Drummer soil, a downturn occurred during the second week of incubation, coinciding with an increase in the recovery of $^{15}NO_3^-$.

Incubation was expected to bring about a growing production of NO_3^-; however, this trend was much more consistent for the two Mollisols than for the Assis or Bloomfield soil (Table 2), in which case nitrification was limited by extensive loss of urea-derived NH_4^+-N through volatilization. Of the two amendments, only NBPT showed any promise for increasing recovery of $^{15}NO_3^-$, most notably with the two Mollisols in the second week of incubation. This increase corresponds to a significant decrease in $^{15}NO_2^-$ recovery that invariably occurred for the UNBPT treatment after 14 days (Table 2), and can be attributed to more gradual liberation of NH_4^+ than was achieved using unamended urea or the UOCh treatment, which would have limited the resulting rise in pH and thereby reduced NH_3 toxicity to NO_2^--oxidizing bacteria (Boullanger and Massol 1904; Smith 1964) that can lead to phytotoxic accumulations of NO_2^- (Court et al. 1962). Table 2 shows no inhibitory effect of NBPT on nitrification, and when considered in conjunction with Fig. 1 and 2, provides strong support for the practical value of this amendment to improve the efficiency of urea fertilization by controlling NH_3 volatilization and NO_2^- accumulation while enhancing NO_3^- availability for plant uptake.

Table 3 shows the recovery of ^{15}N immobilized by the soil microflora before and after the various incubation intervals. As noted previously for mineral N, cross-contamination error accounts for the slight recoveries obtained prior to incubation. A gradual increase thereafter occurred in the recovery of organic ^{15}N, as urea hydrolysis generated a growing supply of NH_4^+, the substrate preferred by the heterotrophic microflora (Jansson et al. 1955; Rice and Tiedje 1989; Recous et al. 1990). The extent of immobilization increased with soil organic C content (Table 1), reflecting the close coupling of microbial C and N demand. Additional organic C was supplied by the UOCh treatment, but with a C/N ratio of 19.1, this amendment showed no tendency to promote immobilization. Owing to inhibition of urea hydrolysis that reduced liberation of NH_4^+, organic ^{15}N recoveries during the first week of incubation were reduced somewhat by the use of NBPT, although the reduction was significant only when immobilization was enhanced by the high organic C content of the Drummer soil. The latter finding has obvious implications for improving the fertilizer value of urea applied to soils with heavy residue inputs from practices such as no-till cropping or pasture production.

Table 3 Fertilizer ^{15}N recovery as organic N during aerobic incubation of Assis, Bloomfield, Elliott, and Drummer soils treated with urea (U), urea and oxidized charcoal (UOCh), or urea and NBPT (UNBPT)

Soil	Treatment[a]	% Recovery of ^{15}N after incubation period (days)[b]				
		0	1	3	7	14
Assis	U	0 a	0.6 a	1.8 a	2.0 b	2.6 a
	UOCh	0.1 a	0.4 a	1.8 a	2.0 b	2.5 a
	UNBPT	0 a	0.2 a	1.1 a	1.1 a	2.2 a
Bloomfield	U	0.1 a	0.4 a	0.9 a	0.9 a	1.3 a
	UOCh	0 a	0.3 a	0.9 a	0.6 a	1.1 a
	UNBPT	0 a	0.2 a	0.6 a	0.8 a	1.3 a
Elliott	U	0 a	1.1 a	4.0 a	5.3 b	6.7 a
	UOCh	0.2 a	1.0 a	3.7 a	4.1 a	6.4 a
	UNBPT	0 a	0.7 a	3.1 a	4.0 a	6.2 a
Drummer	U	0.03 a	2.0 ab	5.4 b	6.6 b	8.4 a
	UOCh	0 a	2.3 b	5.1 b	7.1 b	8.3 a
	UNBPT	0 a	1.5 a	3.6 a	5.5 a	6.7 a

[a] U, urea; UOCh, urea + oxidized charcoal; UNBPT, urea + NBPT

[b] Mean values reported from triplicate incubations. Values within a column for a single soil followed by the same letter do not differ significantly by the Tukey test at $P < 0.05$

Conclusions

An incubation study was conducted to evaluate the use of oxidized charcoal (OCh) high in CEC and buffer capacity, relative to urease inhibition using NBPT, for controlling transformations of urea ^{15}N applied to contrasting soils. The latter strategy was considerably more effective than the former. The OCh amendment was of some benefit for reducing NH_3 volatilization from coarse-textured soils, but otherwise had no significant effects compared to unamended urea. Much lower NH_3 losses were achieved using NBPT to inhibit urea hydrolysis, and owing to the gradual accumulation of NH_4^+ that limited the rise in pH, nitrification produced more NO_3^- with very limited NO_2^- accumulation, and immobilization was reduced. Oxidized charcoal is not a viable alternative to NBPT for increasing the efficiency of urea fertilization.

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GENERAL CONCLUSION

The work reported showed the effects of different amendments on forage uptake and soil transformations of urea N. Oxidized charcoal (OCh) with or without low concentrations of Cu and/or Zn had no significant effect on production of tropical pasture in a greenhouse study using pelletized urea; however, soil concentrations of exchangeable NH_4^+-N were prolonged for urea pellets amended with OCh, which was attributed to an increase in CEC and buffer capacity in the soil microsite where urea hydrolysis occurred. At the concentrations studied, Cu and/or Zn had no significant effect on urea hydrolysis; however, the application of Zn increased crop uptake of urea N. The use of Zn-amended urea has practical potential to improve forage production on Brazilian Oxisols. The inhibitory effect of NBPT on urea hydrolysis proved effective for limiting the liberation of NH_4^+ and thereby reducing NH_3 volatilization, NO_2^- accumulation and N immobilization.

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