UNIVERSIDADE FEDERAL DE LAVRAS

CÉSAR FERREIRA SANTOS

STRATEGIES FOR POTASSIUM FERTILIZATION TO IMPROVE COFFEE DRINK QUALITY AND FERTILIZER TECHNOLOGIES FOR EFFICIENT USE OF NITROGEN IN DIFFERENT CORN PLANTING SYSTEMS

LAVRAS – MG
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Dr. Douglas Ramos Guelfi Silva
Orientador

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STRATEGIES FOR POTASSIUM FERTILIZATION TO IMPROVE COFFEE DRINK QUALITY AND FERTILIZER TECHNOLOGIES FOR EFFICIENT USE OF NITROGEN IN DIFFERENT CORN PLANTING SYSTEMS

ESTRATÉGIAS PARA ADUBAÇÃO POTÁSSICA VISANDO MELHORIA NA QUALIDADE DE BEBIDA DO CAFÉ E TECNOLOGIAS DE FERTILIZANTES PARA USO EFICIENTE DO NITROGÊNIO EM DIFERENTES SISTEMAS DE PLANTIO DE MILHO

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ciência do Solo, área de concentração em Fertilidade do Solo e Nutrição de Plantas, para a obtenção do título de Doutor.

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Aos meus pais, Eva e Valdir, que apesar da simplicidade nunca deixaram de me incentivar na busca de uma educação de qualidade, mesmo que nunca a tiveram.

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“Faça algo que ame e nunca mais precisará trabalhar na vida.”

Willie Hill

RESUMO GERAL

A evolução no potencial produtivo das culturas, aliado ao clima favorável do Brasil, contribuíram para o sucesso da agricultura. O uso de tecnologias para fertilizantes, visando o aumento da eficiência de uso das principais fontes deve seguir por esse mesmo caminho. Neste sentido, as tecnologias para o uso eficiente de nutrientes podem contribuir para o fornecimento adequado de nutrientes as plantas e contribuir para a maior qualidade dos produtos colhidos. O Brasil se destaca como maior produtor de café do mundo, junto a isso está a responsabilidade de se alcançar produtividades cada vez melhores, aliado a melhoria na qualidade da bebida. Pensando nisso, e sabendo que dentre os nutrientes mais importantes para a qualidade, destaca-se o potássio (K), diversos estudos foram conduzidos com o objetivo de monitorar a influência do K na qualidade de bebida do café. Em alguns desses trabalhos, o cloreto (Cl), íon acompanhante do K na fonte cloreto de potássio (KCl), em elevadas concentrações, foi apontado como sendo capaz de reduzir a qualidade do café. Devido a isso, fontes alternativas, em relação ao KCl, devem ser estudadas, objetivando a melhoria da qualidade dos grãos colhidos. Além do café, o país ainda se destaca na produção de grãos, e cada vez mais sob o sistema plantio direto (PD). Os benefícios do PD são inúmeros, mas quando se pensa na eficiência de uso do nitrogênio (N), nutriente de maior demanda pelo milho, há uma redução da eficiência de uso do elemento com o aumento da palhada, principalmente com o uso da ureia como fonte de N. Isso se deve, a camada de palha presente, que pode aumentar a atividade da urease e reduzir o contato do fertilizante com o solo. O fato é que já se tem no mercado, produtos capazes de aumentar a eficiência do uso do N, como os inibidores de urease, por exemplo. O desafio então seria estudar o comportamento desses aditivos em relação aos sistemas de cultivo, pois a atividade da urease é maior no PD em relação ao PC. Desta forma, o objetivo do primeiro capítulo desse trabalho foi estudar o efeito da mistura física das fontes KCl e sulfato de potássio (K_2SO_4) em diferentes proporções e sua influência no estado nutricional, produtividade, composição química e qualidade de bebida do café. Já o segundo capítulo, teve como objetivo, avaliar inibidores de urease no tratamento da ureia aplicada em cobertura sob PC e PD e sua eficiência na mitigação das perdas de N-NH_3, e melhoria da nutrição e produtividade do milho cultivado em segunda safra. Para o primeiro capítulo, foi observado que, o estoque de Cl no solo reduz ao longo do tempo de avaliação; a adubação com KCl reduz a nota da prova de xícara e a atividade da PPO, provavelmente devido ao efeito negativo provocado pelo íon cloreto. O aumento da aplicação do Cl na adubação tem relação direta com o aumento da lixiviação de potássio, condutividade elétrica e acidez titulável. As análises de LK e CE, indiretamente, conseguem mostrar que algum dano ocorre no grão de café devido ao uso do Cl. Já com relação ao segundo capítulo, foi observado que em PD, as perdas de N-NH_3 foram 49% maiores do que no PC; sem diferenças para a produtividade do milho. Os fertilizantes nitrato de amônio e sulfato de amônio apresentaram as menores perdas de N-NH_3, independente do sistema de preparo do solo. A ureia + NBPT reduziu a perda média de N-NH_3 em 33% em comparação com ureia perolada. A ureia + NBPT (1.200 mg kg^{-1}) e ureia + NBPT (180 mg kg^{-1}) reduziram em 72% e 22% as perdas de N-NH_3 em relação a ureia perolada em sistema PD.

Palavras-chave: Tecnologias. Blends de fertilizantes. Qualidade. Inibidores de urease. Sistema de cultivo.

GENERAL ABSTRACT

The evolution in the productive potential of crops, combined with the favorable climate conditions, contributed to the success of agriculture in Brazil. The use of technologies for fertilizers, aiming to increase the use efficiency of the primary sources, should follow the same path. In this sense, technologies for the efficient use of nutrients can contribute to the proper supply of nutrients to plants and may improve the quality of harvested products. Brazil stands out as the largest coffee producer in the world, and the major challenge of Brazilian coffee production is to achieve increasing yields while improving the quality of the beverage. Potassium (K) is one of the most essential nutrients for the quality of the coffee beverage. Thus, several studies were conducted to monitor the influence of K on the quality of the coffee beverage. Some of these studies indicate that chloride (Cl), the accompanying ion of K in potassium chloride (KCl), can reduce the quality of the coffee beverage, when present in high concentrations. Therefore, other sources than KCl should be investigated, aiming to improve the quality of the harvested grains. Besides coffee, the country stands out in the production of grains under the no-tillage (NT) system. No-tillage promotes multiple benefits to soil health and crop yields. However, one potential drawback of NT systems is the reduced use efficiency of nitrogen (N), the most required nutrient by corn, when urea is used as an N source. Such reduced efficiency is due to the presence of the straw layer, which can enhance urease activity and reduce the contact of the fertilizer with soil. The fertilizer market already has products that can enhance N use efficiency, such as urease inhibitors. Thus, the challenge is to study the behavior of such additives in various cropping systems since the urease activity is higher in NT than in conventional tillage. Thus, the objective of the first chapter of this work was to study the effect of the physical mixture of KCl and potassium sulfate (K_2SO_4) sources in different proportions and their influence on the nutritional status, yield, chemical composition, and quality of the coffee beverage. As for the second chapter, the aim was to evaluate urease inhibitors in the treatment of urea applied as topdressing under NT and CT systems, and their efficiency in mitigating N-NH_3 losses and improving nutrition and yield of corn grown in the second crop season. For the first chapter, it was observed that the stock of Cl reduces the over time; fertilization with KCl reduces the cup quality grade and PPO activity, probably to the negative effect by the ion probably. The increased application of the cloro in top-dressing have of the direct relationship with the increase of potassium leaching and electrical conductivity. The analyses potassium leaching and electrical conductivity, it may show that coffee beans damage due to use of Cl. As for the second chapter, the fertilizers as ammonium nitrate, and ammonium sulfate had the lowest N-NH_3 losses, regardless of tillage system. The urea + NBPT reduced the mean N-NH_3 loss by 33% compared to prilled urea. The urea + NBPT (1,200 mg kg^{-1}) and urea + NBPT (180 mg kg^{-1}) reduced by 72% and 22% the N-NH_3 losses compared to prilled urea in the no-till system.

Keywords: Technologies. Blended fertilizers. Quality. Urease inhibitors. Cropping systems.

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO GERAL

O Brasil ocupa cenário de destaque na produção mundial de alimentos. Embora as questões que elevam o país a este patamar sejam, em parte, devidas às condições climáticas favoráveis que possibilitam o cultivo de duas ou três safras anuais, mas por outro lado, a aplicação de novas tecnologias no campo é igualmente fundamental para esse sucesso. Essas tecnologias vão desde a obtenção de materiais com maior potencial de produção, emprego de máquinas e equipamentos que possuem alto desempenho, até a melhoria das estratégias e tecnologias para fertilizantes.

Dentro deste escopo, a cafeicultura se destaca como uma atividade agrícola que consome quantidades elevadas de fertilizantes visando atender as demandas da cultura para produtividades crescentes. Dentre os nutrientes requeridos para uma adequada produtividade, o potássio (K) se destaca como o nutriente mais exportado pela cultura (MARTINEZ *et al.*, 2014). Além disso, o K possui importância relevante na ativação enzimática e processos metabólicos da planta, como fotossíntese, síntese de proteínas e carboidratos e manutenção da turgidez celular (CLEMENTE *et al.*, 2015; MALAVOLTA, 2006).

A importância do K nos processos metabólicos é de tamanha importância que o nutriente é reconhecido como elemento responsável pela qualidade em nutrição de plantas e por isso interfere diretamente na qualidade de bebida do café (GUIMARÃES *et al.*, 2011). Apesar de ser considerado primordial para a obtenção de uma boa qualidade de bebida do café, existem relatos de redução na qualidade de bebida quando se usa como fonte o fertilizante Cloreto de potássio (KCl) (DIAS *et al.*, 2018).

O cloro, íon acompanhante do K, na fonte KCl é um elemento essencial às plantas. Foi o penúltimo nutriente a ser considerado como nutrientes de plantas. Seu papel na nutrição de plantas está relacionado a fotólise da água no fotossistema II da fotossíntese, na ativação de enzimas (amilase, asparagina-sintetase e ATPase do tonoplasto) e ainda, na abertura e fechamento estomático (DECHEN *et al.*, 2018).

Apesar de essencial às plantas, a influência dos elevados níveis do íon Cl na planta e no solo, tem sido relatado como potencial responsável pela redução da qualidade de bebida do café. Esse efeito negativo, pode ser atribuído ao aumento da umidade na planta quando concentrações elevadas de Cl estão presentes, e isso favorece um ambiente adequado à proliferação de microrganismos que podem levar a fermentação indesejável dos frutos (GOUNY, 1973; LEITE, 1991).

Ainda sobre a importância da agricultura e das tecnologias presentes no campo para o sucesso do agronegócio no Brasil, destacam-se as culturas anuais como a soja e milho. Pensando na demanda nutricional destas culturas por nitrogênio, a soja brasileira já possui autossuficiência pela utilização do N atmosférico fixado por bactérias, no entanto, as demandas deste nutriente pelo milho são elevadas e crescentes, principalmente pela utilização de cultivares cada vez mais produtivos.

O fato é que a eficiência de uso do N nos sistemas agrícolas brasileiros é baixa, devido principalmente às perdas de N por volatilização na forma de amônia (N-NH_3) (SANTOS *et al.*, 2020, 2021; SOUZA *et al.*, 2017). A baixa eficiência de aproveitamento de N nesses sistemas se deve principalmente ao clima tropical, caracterizado por elevadas temperaturas, e também ao uso da ureia sem nenhum tipo de tecnologia ou estratégia que reduza a hidrólise e perda desta fonte aplicada ao solo (SANTOS *et al.*, 2020).

Além das condições tropicais que favorecem as perdas de N-NH_3, soma-se a isso, a crescente adoção do sistema de plantio direto, que favorece o aumento das perdas N-NH_3 por volatilização, que podem chegar a 25% de aumento em relação ao sistema de plantio convencional (PAN *et al.*, 2016). As perdas em sistema de plantio direto se devem, principalmente, ao reduzido contato da ureia diretamente com o solo, ao pH mais elevado da camada superficial e ainda a presença de resíduos orgânicos que favorecem a maior atividade da urease no solo (ROJAS *et al.*, 2012; VIERO *et al.*, 2017).

Nesse sentido, tem-se acima a exposição de duas situações de extrema importância para a agricultura brasileira. De um lado o inconveniente da influência do íon Cl aplicado via KCl na redução da qualidade de bebida do café, de outro lado, as questões relativas à eficiência do uso de N nos sistemas agrícolas brasileiros, que usam a ureia como fonte de N, principalmente em sistema de plantio direto.

Para enfrentar esses dois problemas é necessário saber quais são os diferentes tipos de estratégias e tecnologias existentes no mercado atualmente. Como principais tecnologias destacam-se os fertilizantes estabilizados, os de liberação controlada, liberação lenta, os de dupla função e seus blends.

Os estabilizados são mais comuns para fertilizantes nitrogenados e consistem no uso de algum aditivo que tem como função retardar ou bloquear algum tipo de reação do fertilizante no solo, como por exemplo, a inibição da urease (ADOTEY *et al.*, 2017; CANCELLIER *et al.*, 2016; HABALA; DEVÍNSKY; EGGER, 2018), retardando a quebra da molécula de ureia.

Os fertilizantes de liberação controlada consistem em produtos com revestimentos que possibilitam a redução da solubilidade do fertilizante, havendo uma liberação gradual do nutriente para o sistema (AZEEM *et al*., 2014; CHIEN; PROCHNOW; CANTARELLA, 2009; DIMKPA *et al.*, 2020; LAWRENCIA *et al.*, 2021; NAZ; SULAIMAN, 2016). Já os fertilizantes de liberação lenta são produzidos sob condições de temperatura e pressão controladas, onde há a reação do fertilizante com algum composto contendo carbono, fazendo com que se formem cadeias contendo carbono e o nutriente de interesse, sendo o exemplo mais comum à ureia formaldeído (AZEEM *et al.*, 2014; TIMILSENA *et al.*, 2015; YAMAMOTO *et al.*, 2016).

E existe ainda a tecnologia de dupla função, que consiste no uso de duas tecnologias no mesmo grânulo do fertilizante, como por exemplo, o tratamento da ureia com NBPT e posterior revestimento com polímero orgânico (SANTOS *et al.*, 2020). E por fim, temos os blends, que são a mistura física de diferentes tecnologias, mas em grânulos separados (CHAGAS *et al.*, 2016).

Diante dessa discussão, o primeiro capítulo deste trabalho teve como objetivo estudar a influência da aplicação de blends dos fertilizantes potássicos cloreto de potássio e sulfato de potássio na nutrição das plantas, produtividade e qualidade de bebida do café. Já o segundo capítulo, teve como objetivo o estudo de fertilizantes nitrogenados estabilizados com NBPT e Cu e B e seu potencial de aumentar a eficiência de uso da ureia como fonte de N em sistema de plantio direto.

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SEGUNDA PARTE – ARTIGOS

ARTIGO 1 - CHLORIDE ION APPLIED VIA POTASSIUM CHLORIDE FERTILIZER AFFECTS NUTRITION AND COFFEE BEVERAGE QUALITY

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Chloride ion applied via potassium chloride fertilizer affects nutrition and coffee beverage quality

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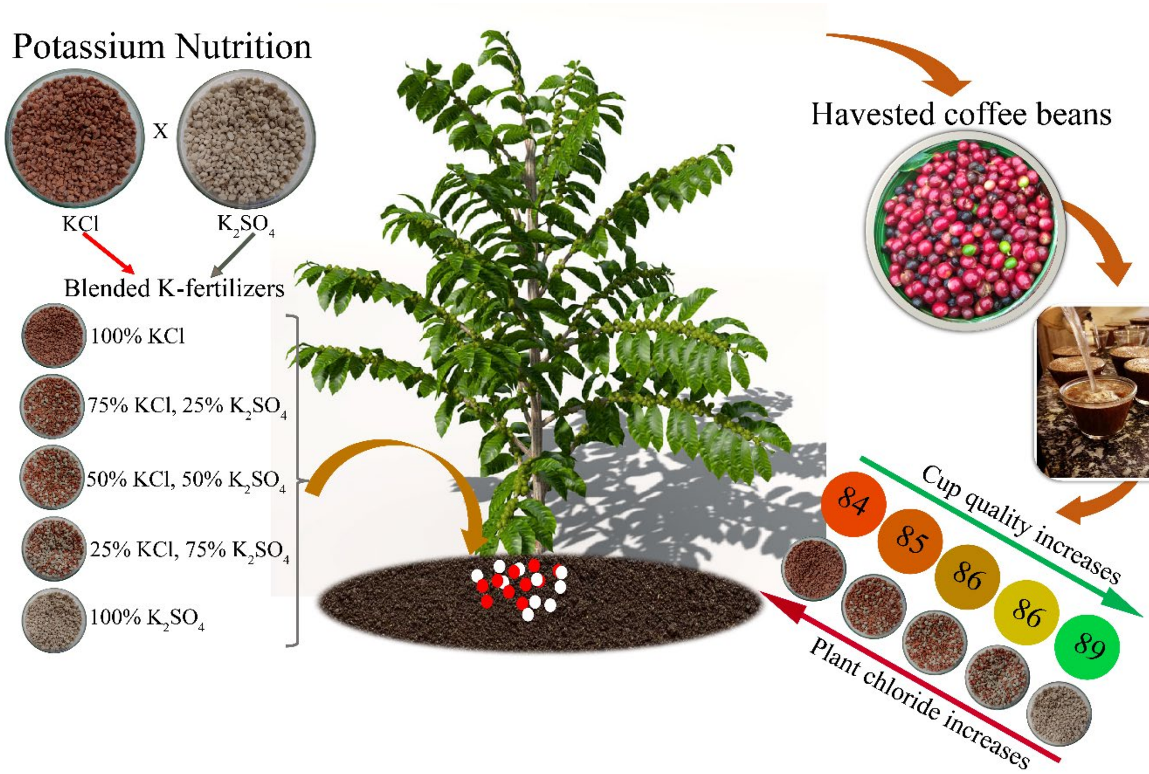
Abbreviations: K: potassium, Cl: chloride, PPO: polyphenol oxidase, KCl: potassium chloride, K_2O_4: potassium sulfate, BD: bulk density, ICP: inductively coupled plasma, KL: potassium leaching, EC: electric conductivity, TTA: total titratable acidity, TS: total sugars, Pol: total phenolic compounds, Caf: caffeine, $S.K^+$ = stocks of potassium, $S.Cl^-$ = stocks of chloride, K rem. by beans: potassium removal by the beans, Cl rem. by beans: chloride removal by the beans.

Graphical abstract



Abstract

There is a growing concern about the production and the economical return of better-quality coffees. Potassium is a nutrient responsible for the quality of crops. Its role in metabolic processes and enzymatic activation influences the chemical composition of the coffee beans and the quality of the beverage. In Brazil, the low-cost KCl fertilizer is the main source of K. However, excess Cl from KCl fertilizers can reduce the quality of the coffee beverage. Therefore, the present study had the objective to evaluate the effect of blends of KCl and K_2SO_4 fertilizers at different proportions and their influence on the yield and the nutritional state of coffee plants, as well as on the chemical composition and quality of the coffee beverage. An experiment was carried out for three consecutive harvests (2017/2018, 2018/2019, and 2019/2020) on a coffee plantation in Brazil. The experimental design was in randomized blocks with four repetitions and six treatments (T1: 100% KCl; T2: 75% KCl + 25% K_2SO_4; T3: 50% KCl + 50% K_2SO_4; T4: 25% KCl + 75% K_2SO_4; T5: 100% K_2SO_4; and a control without K_2O). The doses of K_2O applied as cover fertilization were 150, 200, and 300 kg ha^{-1} for the respective consecutive years. The following analyses were performed: K and Cl content in the leaves and the soil, stocks of Cl in soil, yield, removal of K and Cl by the beans, cup quality of the beverage, polyphenol oxidase activity, electric conductivity, potassium leaching, the content of phenolic compounds, the content of total sugars, and total titratable acidity. Results show a reduction of the stocks of Cl in the soil. The fertilization with KCl reduces the cup quality and the activity of the polyphenol oxidase, probably due to the ion Cl. The increase in the application of Cl directly relates to the increase in potassium leaching, electric conductivity, and titratable acidity. Indirectly, these variables indicate damages to the cells by the use of Cl in the fertilizer.

Keywords: Blend fertilizers. Chlorine. Cup test. Polyphenol oxidase.

1 INTRODUCTION

Coffee is one of the most popular beverages in the world and its cultivation is widespread in 80 countries. After petroleum, coffee is the second most commercialized product (FRIDELL, 2014; MURTHY and NAIDU, 2012). The marketing price is based on the quality of the beverage, which is related to the physical, chemical, and sensorial characteristics of the product (FRIDELL, 2014; SIMÕES et al., 2008; TOCI and FARAH, 2014).

Fertilization and crop nutrition can influence both yield and the chemical composition of the raw beans, which, consequently, interfere with the quality of the beverage (MARTINEZ et al., 2014). After nitrogen, potassium (K) is the most accumulated nutrient by coffee plant fruits, where it is demanded at high amounts. K is related to the enzymatic activation of several metabolic processes, such as photosynthesis, proteins, and carbohydrates synthesis, and in the maintenance of cell turgidity (ERNANI et al., 2007; MALAVOLTA, 2006).

The effects of the accompanying chloride ion (Cl) of the potassium chloride fertilizer (KCl) are currently under debate. Cl is demanded by the plants at low amounts, thus being one of the last micronutrients to enter the micronutrient list. Its role is related to the water photolysis on the photosystem II, enzyme activation (amylase, asparagine synthetase, and tonoplast ATPase), and stomatal control (DECHEN et al., 2018).

Despite Cl being essential to plant nutrition, when accompanying a highly demanded macronutrient such as K, it can reach excessive concentrations in the soil and plants and consequently reduce the quality of the beverage. In coffee plants, high concentrations of Cl are related to the increase in plant water, which favors an undesirable fermentation of the fruits by microorganisms (GOUNY, 1973; LEITE and CARVALHO, 1994).

The study of the Cl influence on the quality of the coffee beverage is not recent, but it is still inconclusive. For example, Dias et al. (2018) evaluated an alternative source of K (glauconite silicate mineral) to KCl in coffee fertilization. Despite finding similar yields and polyphenol oxidase activity (PPO) in the beans, the use of the glauconite did not improve the sensorial quality. Silva et al. (1999), along two seasons, verified that fertilization with potassium sulfate (K_2SO_4) increased PPO activity in comparison with KCl, which, according to the authors, is indicative of a better beverage quality. These studies suggest possible negative effects of the Cl on the quality of the coffee beverage and the necessity to use K sources without Cl as the accompanying ion, such as K_2SO_4 (48 % K_2O, 16 % S) and potassium nitrate (44 % K_2O, 13 % N). Nonetheless, this could increase the production costs, as these sources are more expensive than KCl. In turn, blends of KCl and K_2SO_4 (a physical mixture of the two less expensive sources in the market) could be an alternative to reduce Cl to thresholds that do not affect the quality of the coffee beverage without excessively increasing the costs of the fertilization.

Therefore, the present study had the objective to evaluate the effect of blends of KCl and K_2SO_4 fertilizers at different proportions and their influence on the yield and the nutritional state of coffee plants, as well as on the chemical composition and quality of the coffee beverage.

2 MATERIAL AND METHODS

2.1 Experimental area characterization

The experiment was performed through three consecutive years (harvests of 2017/2018, 2018/2019, and 2019/2020), in a commercial production system of coffee located in the municipality of Santo Antônio do Amparo-MG, Brazil (20°53’26.04” S and 44°52’04.14” W and mean altitude of 1,100 m). The plantation of *Coffea arabica* L., cultivar Catuaí Vermelho IAC 99, initiated in 2012 and spaced at 3.40 m × 0.65 m, is planted on a clayey Latossolo Vermelho distrófico (SANTOS et al., 2013).

Before the experiment, soil samples were collected for chemical attributes and texture analyses (TABLE 1). Samples from the 0 – 80 cm layer were collected to assess K and Cl stocks. Undisturbed soil samples were taken to assess bulk density (BD). For depths over 5 cm, multiple samples were taken followed by the weighted average of the BD values. After determining K and Cl concentrations (mg kg^{-1}), the values were multiplied by the BD to transform them into kg ha^{-1}.

Table 1. Soil analyses results on September 2017.

Depth	pH	K^+	P	Ca^{2+}	Mg^{2+}	Al^{3+}	H+Al	BS	ECEC	CEC	V	m
cm	$CaCl_2$	---mg dm^{-3}---		------------------------cmol$_c$ dm^{-3}----------------------							------%-----	
0-10	5.2	96	7.9	1.4	0.7	0.3	8.7	2.5	2.8	11.2	22.2	11.9
10-20	5.2	87	9.2	1.5	1.1	0.1	6.7	3.0	3.1	9.7	30.8	5.6
20-40	5.1	69	7.1	1.6	1.0	0.1	6.5	3.0	3.1	9.5	31.5	5.0

Depth	OM	P(rem)	Zn^{2+}	Fe^{2+}	Mn^{2+}	Cu^{2+}	B	S	Sand	Silt	Clay
cm	dag kg^{-1}	mg L^{-1}	--------------------mg dm^{-3}-----------------						---------------%---------------		
0-10	3.8	17.2	1.8	57.6	8.4	2.1	0.2	180	22	14	64
10-20	3.7	15.9	2.2	52.4	7.7	2.1	0.3	90	22	16	62
20-40	3.6	14.6	1.3	39.3	4.2	2.0	0.3	48	22	18	60

P, K^+, Fe^{2+}, Zn^{2+}, Mn^{2+}, Cu^{2+} – Mehlich extractor. Ca^{2+}, Mg^{2+}, Al^{3+} – 1 mol L^{-1} KCl extractor. H+Al – SMP extractor. B – hot water extractor. S – monocalcium phosphate in acetic acid extractor. BS = exchangeable bases sum. ECEC = effective cation exchange capacity. CEC = cation exchange capacity at pH 7.0. V = base saturation. m = aluminum saturation. P-rem = remaining phosphorus. OM = organic matter (oxidation with $Na_2Cr_2O_7$ 0.57 mol L^{-1} + H_2SO_4 5mol L^{-1}).

2.2 Experimental design

The experimental design was in randomized blocks, with four blocks disposed at 90 degrees with the slope of the area. The treatments were composed of blends of KCl and K_2SO_4 (both in terms of K_2O) as follows: T1 – 100 % as KCl; T2 – 75 % as KCl + 25 % as K_2SO_4; T3 – 50 % as KCl + 50 % as K_2SO_4; T4 –25 % as KCl + 75 % as K_2SO_4; T5: 100% as K_2SO_4; and a control without K_2O application. Each plot was composed of three planting lines with 16 plants and the 10 central plants were considered as useful area (Fig. 1).

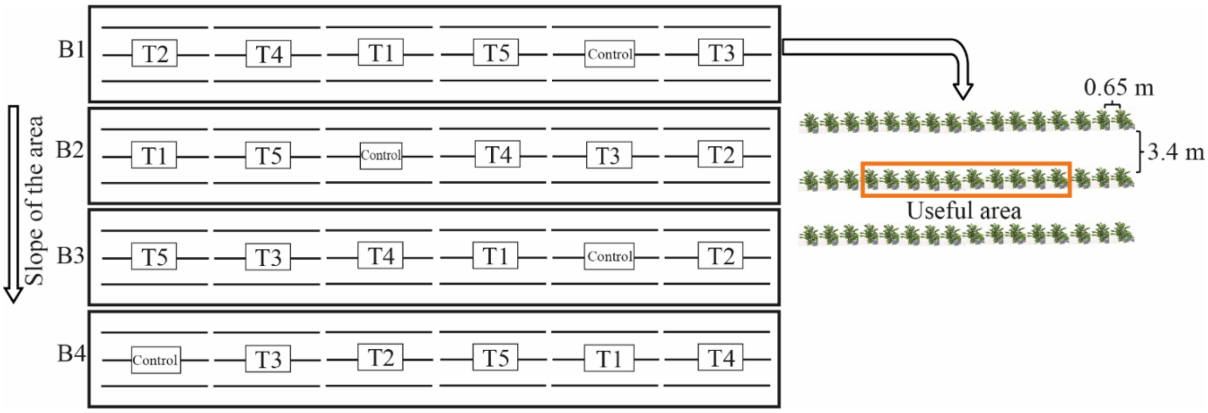


Figure 1: Schematic representation of the experimental design, number of plants in each plot, and the useful area used to collect the data.

2.3 Experiment conducting

2.3.1 Liming, fertilization, and gypsum application

After the coffee harvest of each studied year, soil samples from the 0 – 10 cm, 0 – 20 cm, and 20 – 40 cm layers were collected to evaluate the needs for liming, fertilization, and gypsum application, respectively (RIBEIRO et al., 1999). Liming was applied at 1.0 t ha^{-1}, 1.2 t ha^{-1}, and 1.5 t ha^{-1} on the first, second, and third harvest years, respectively. Gypsum was applied at 1.1 t ha^{-1} and 2.0 t ha^{-1} in the second and third years, respectively. Both dolomite lime and gypsum were applied unerneath the projection of the tree canopies. P was applied at 120, 90, and 90 kg ha^{-1} of P_2O_5 as triple superphosphate on each consecutive year. N was applied at 350, 350, and 400 kg ha^{-1} of N as ammonium nitrate on each consecutive year, divided into three applications.

2.3.2 Potassium fertilization

Before the experiment, the saline index of each blend of K fertilizer was determined by comparing a 10 g L^{-1} sodium nitrate solution with solutions prepared with the blends of KCl e K_2SO_4 at the same concentration (JACKSON, 1958). The electric conductivity of the solutions and the saline index were calculated according to the equation: $SI = [(ECa)/(ECb)] \times 100$, where SI is the saline index; ECa is the electric conductivity of the sample; ECb is the electric conductivity of the sodium nitrate solution. The SI found were 142, 130, 121, 112, and 104 %, for T1, T2, T3, T4, and T5, respectively.

The maintenance fertilization was done according to Guimarães et al., (1999) using the abovementioned blends. The doses of K_2O applied were 150, 200, and 300 kg ha^{-1} for the respective agricultural years of 2017/2018, 2018/2019, and 2019/2020. All K fertilizations were divided into three applications.

2.3.3 Agronomic variables assessed on the three harvests

K and Cl content in the leaves

The third and fourth pair of leaves on both sides of the plants were collected from the useful area 20 days after the second application of the cover fertilization. The leaves were washed in deionized water, dried at 65 °C, and grounded in a Willey mill. The plant material was digested in a solution of nitric-perchloric acid (4 parts of nitric acid to 1 part of perchloric acid) and K was determined by inductively coupled plasma (ICP). To determine Cl, 1 g of grounded material was added to 50 mL of ultrapure water under agitation for 15 min. (MALAVOLTA et al., 1997). After filtering the extract, the content of Cl (mg kg^{-1}) was determined by a selective electrode (Hanna®, model HI4107) coupled to a Hanna® device, model HI2221. The determination curve was built using the concentrations of 2, 20, 200, and 1000 mg L^{-1} of Cl.

Yield

The harvests were done when more than 70 % of the fruits were mature. For the chemical analyses, 4 L of beans at the cherry stage were collected two days before each harvest. Fruits were peeled with an electric peeler (Pinhalense®, model DPM-02) and submerged for 24 h to remove the mucilage. After removing the peels and the rotten beans, samples were air-dried until a 10.8 % to 11.2 % moisture level.

The yield was determined by harvesting all fruits in the useful area. After the harvest, 5 L of a mix of fruits in every stage of maturation were air-dried under sunlight for one day. When the samples reached around 12 % of moisture, beans were peeled and weighted. The moisture level was then adjusted to 12 %, which is considered adequate for commercialization. To estimate yield, the weight of the beans in the useful area was projected to the number of plants in one hectare (4524 plants).

K and Cl content and removal in the beans

K and Cl contents in the beans were determined at the cherry stage after air-drying (65 °C, until constant weight) and grounding the beans in a Willey mill. K content was determined after nitric-perchloric digestion with measures done by ICP. Cl content followed the same procedures to quantify Cl in the leaves. The amounts of these elements removed from the soil were obtained by multiplying their content in the beans by the yield on each treatment.

Stocks of Cl in the soil

Stocks of Cl in the 0-20 and 20-80 cm layers were checked during the experiment. Six soil samples were taken from the soil underneath the projection of the tree canopies (three from each side of the parcel). Extraction and determination of Cl followed the same procedure described for leaf Cl content, but with the proportion of 10 g of soil to 50 mL of ultrapure water. The stocks were determined by multiplying the element concentration by the mass of soil in each layer.

The analytical standard Tomato leaves (NIST 1573A), with 0.66 % of Cl, was used in both soil and plant material analysis. The mean recovery of Cl was higher than 92 %, assuring that the extraction and determination used for Cl were effective for both soil and plant material.

2.3.4 Chemical analysis of the beans and coffee sensorial analysis

After benefiting the coffee samples, the beans were stored in paper bags in a cold chamber until the chemical and sensorial analyses. The chemical analyses were performed at the Laboratory of Coffee Quality Analysis, in the Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG). Potassium leaching (KL, in µg g^{-1}) was determined after 5 h of soaking (PRETE, 1992) and electric conductivity (EC, in µS cm^{-1} g^{-1}) was determined according to Loeffler, Tekrony, and Egli (1988). The total titratable acidity (TTA, m mL NaOH 0.1 N 100 g^{-1}) was done according to Carvalho et al. (1994) in the adaptation of the methodology from the Association of Official Analytical Chemists (AOAC, 1990). The content of total sugars (TS, in

%) followed the anthrone method (DISCHE, 1962). The activity of the polyphenol oxidase enzyme (PPO, in u min^{-1} g^{-1}) was determined according to Carvalho et al. (1994). Total phenolic compounds (Pol, in %) were extracted according to Goldstein and Swain (1963) and determined by the Folin-Denis method, described by AOAC (1990). Caffeine content (Caf, in %) was determined by spectrophotometry at 273 nm (Li, Berger, and Hartland, 1990). The coffee beans were frozen in liquid nitrogen and grounded in an IKA mill for the analyses, except for the KL and EC determinations.

The sensorial analysis (cup quality) was performed at the Laboratory of Agricultural Products Processing, in the Universidade Federal de Lavras, following the Speciality Coffee Association of America (SCAA) protocol. Three professionals with skills to differentiate fragrances, characteristics, and flavors participated in the cup test. The evaluation was based on scores given to the following attributes: fragrance/aroma, uniformity, clean cup, sweetness, flavor, acidity, body, aftertaste, balance, defects, and overall. The coffees were classified as the SCAA (2009) according to their final scores (Table 2):

Table 2. Coffee beverage classification according to the cup quality.

Final score	Special description	Classification
95-100	Outstanding	Super premium specialty
94-90	Excepcional	Premium specialty
85-89	Excellent	Specialty
84-80	Very good	Especial
75-79	Good	Good quality – normal
74-70	Weak	Medium quality

Source: SCAA (2009).

2.3.5 Accumulation of polyphenols, total sugars and caffeine

The results obtained for the variables polyphenols, protein, total sugars and caffeine were used to calculate the accumulation of these compounds in cherry grains. For this, the values of the production of cherry grains (in kg ha^{-1}) and the values of these variables in percentage were used, with the final value transformed into kg ha^{-1} of polyphenols, protein, total sugars and caffeine.

2.4 Statistical analysis

After model validation and analysis of variance indicating differences among treatments ($P < 0.05$), the response variables were submitted to Tukey's test ($P < 0.05$) on the R 3.3.1

environment (R DEVELOPMENT CORE TEAM, 2018). Principal component analyses (PCA) were performed to correlate the agronomic variables with the coffee beverage variables and yield. In the PCA, two components (Dim1 and Dim2) were used to represent the total data variability. The package Facto MineR (version 1.42) was used in the R software.

3 RESULTS

3.1 Effects of the KCl and K_2SO_4 blends in the stocks of Cl in the soil, nutrition and yield of coffee plants

3.1.1 Harvest of 2017/2018

The initial content of K in the 0-20 and 20-80 cm layers were 91.5 and 58.6 mg dm^{-3}, while stocks of the element were 201 and 388 kg ha^{-1}, respectively. The content of Cl in the 0-20 and 20-80 cm layers were 153.8 and 203.8 mg dm^{-3}, while stocks were 386 and 1345 kg ha^{-1}, respectively (Table 3). K and Cl contents in the leaves were 19.2 g kg^{-1} and 2880 mg kg^{-1}, respectively.

Table 3. Initial content and stocks of K and Cl in the soil and in the leaves of coffee plants

Depth	BD	K^+	Cl^-	$S.K^+$	$S.Cl^-$	Leaf K^+	Leaf Cl^-
cm	g cm^{-3}	mg dm^{-3}		kg ha^{-1}		g kg^{-1}	mg kg^{-1}
0-20	1.1	91.5	153.8	201.3	338.3	19.2	2880
20-80	1.1	58.6	203.8	386.7	1345.0		

BD = Soil density by the volumetric ring method; $S.K^+$ = stocks of K; $S.Cl^-$ = stocks of Cl. Both stocks were calculated by multiplying the content of the element by the mass of soil in the layer.

Soil stocks of Cl were influenced ($P < 0.05$) by the different K blends (Fig. S1). Overall, the amount of Cl decreased along with the KCl proportion in the treatment. In the 0-20 cm layer, T1, T2, and T3 were similar and Cl stocks were 164, 205, and 198 kg ha^{-1}, respectively. The other treatments, T4, T5, and control, did not differ, and Cl respective stocks were 128, 126, and 114 kg ha^{-1}. In the 20-80 cm layer, the highest Cl stock was in the T1 treatment (622 kg ha^{-1}), followed by T2 (513 kg ha^{-1}), and T3 (409 kg ha^{-1}), which did not differ from T1 and T2. Other treatments showed similar Cl stocks, with 411, 459, and 333 kg ha^{-1} for T4, T5, and control, respectively.

On this harvest, the agronomic results were significantly different ($P < 0.05$) only for K and Cl content in the leaves (Fig. S2) and for K removal by the beans (Fig. S3). K content varied from 20.8 to 34.8 g kg^{-1} between T1 and T5. Other treatments had intermediary values and were similar. Cl content decreased with the increase in K_2SO_4 present in the treatments. The contents varied from 3644 to 5275 mg kg^{-1} between the control and T2 treatments. Mean results for Cl content in the beans, yield, and Cl removal were 1778 mg kg^{-1}, 3631 kg ha^{-1} (Fig. S3A), and 2.8 kg ha^{-1} (Fig. S3B), respectively. The lowest K removal by the beans was in treatment T3 (11.3 kg ha^{-1}). The K removal from other treatments had means close to 24 kg ha^{-1} (Fig. S3B).

3.1.2 Harvest of 2018/2019

Soil Stocks of Cl in this harvest were influenced by the blends ($P < 0.05$) only in the 0-20 cm layer. T1, T2, T3, and T5 treatments showed stocks near 65 kg ha^{-1}. The two lowest amounts of stocked Cl were in treatments T4 (50 kg ha^{-1}) and control (57 kg ha^{-1}). In the 20-80 cm layer, the average stock was 122 kg ha^{-1} (Fig. 2).

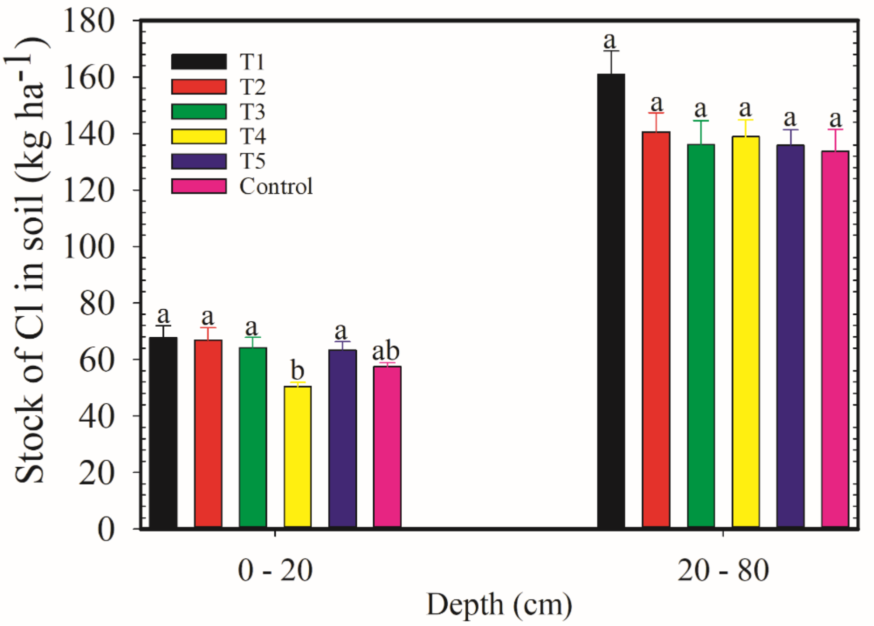


Figure 2: Stocks of Cl in the 0-20 and 20-80 cm layers after application of KCl and K_2SO_4 blends as cover fertilization on coffee plants. 2018/2019 harvest. Means followed by the same letter do not differ according to Tukey's test ($P < 0.05$). Vertical bars indicate the standard error of the mean (n = 4). T1: 100% KCl; T2: 75% KCl + 25% K_2SO_4; T3: 50% KCl + 50% K_2SO_4; T4: 25% KCl + 75% K_2SO_4; T5: 100% K_2SO_4; control did not receive K_2O.

The application of the blends influenced ($P < 0.05$) K and Cl content in the leaves (Fig. 3). K content varied from 17.3 to 21 g kg^{-1} and the lowest content was in the control treatment. Overall, Cl content in the leaves decreased with less KCl applied to the soil. Treatments T1 (6950 mg kg^{-1}) and T2 (7621 mg kg^{-1}) were far superior from T5 (2033 mg kg^{-1}). The Cl content in the control treatment (4825 mg kg^{-1}) was even superior to the contents of T4 (2693 mg kg^{-1}) and T5 treatments (Fig. 3).

The following means were registered: 693 mg kg^{-1} for the content of Cl in the beans, 1804 kg ha^{-1} to yield (Fig. 4A), and 6.0 and 0.21 kg ha^{-1} to K and Cl removal by the beans, respectively (Fig. 4B).

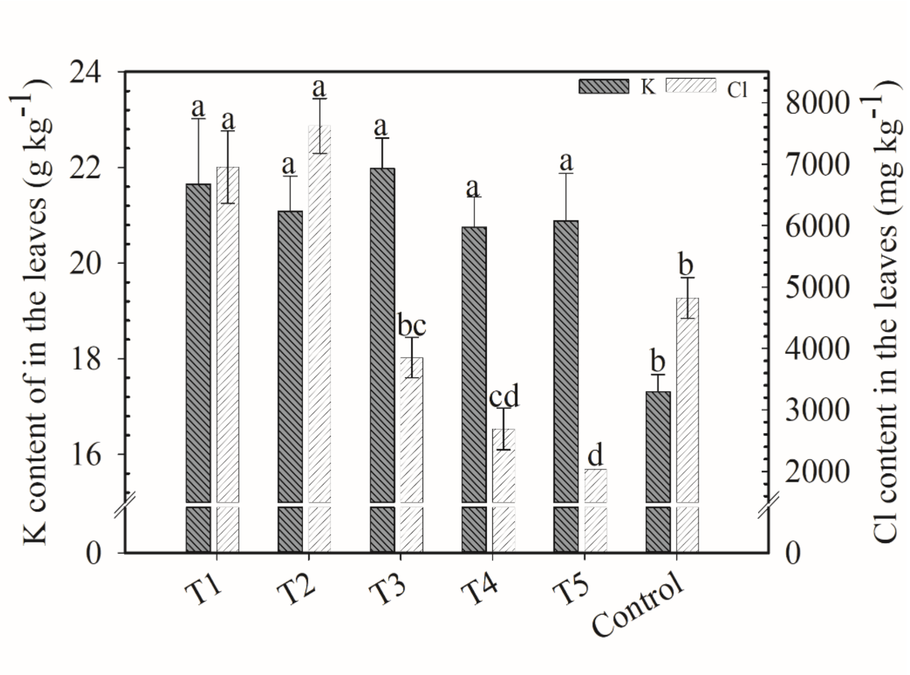


Figure 3: K and Cl contents in the leaves of coffee plants, 20 days after application of the second cover fertilization parcel. Harvest of 2018/2019. Means followed by the same letter do not differ according to Tukey's test (P < 0.05). Vertical bars indicate the standard error of the mean (n = 4). T1: 100% KCl; T2: 75% KCl + 25% K_2SO_4; T3: 50% KCl + 50% K_2SO_4; T4: 25% KCl + 75% K_2SO_4; T5: 100% K_2SO_4; control did not receive K_2O.

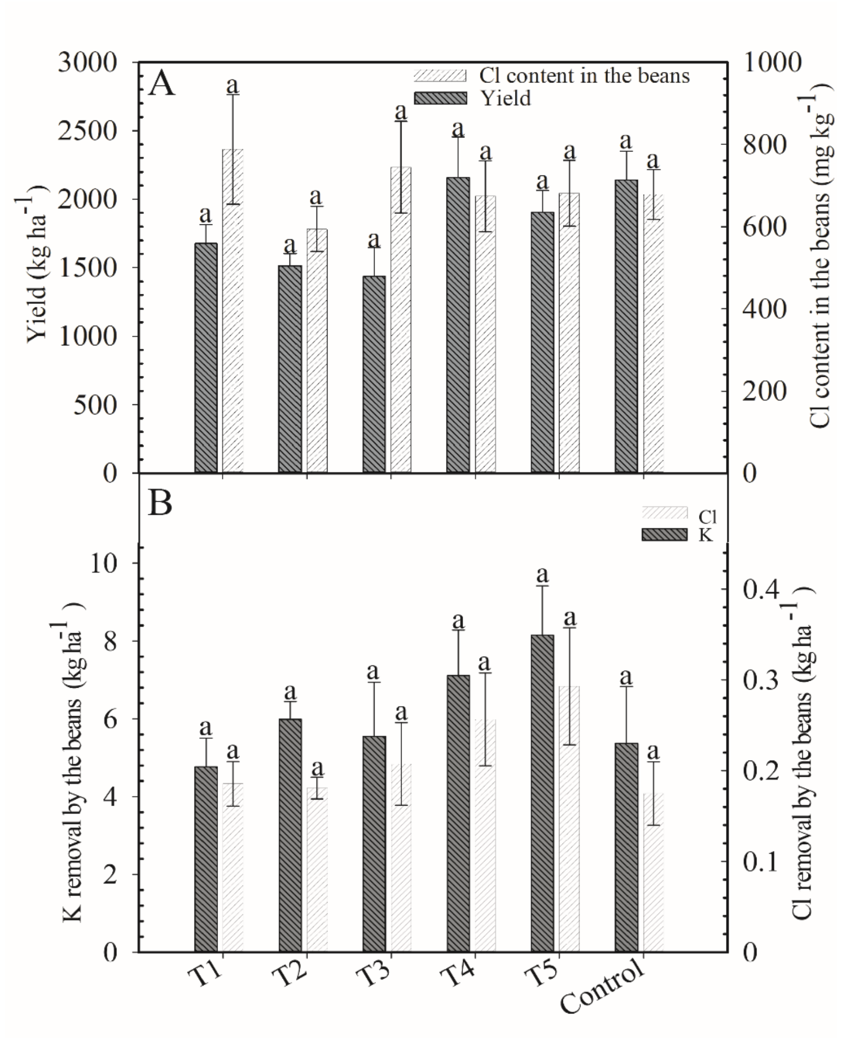


Figure 4: Yield of coffee plants and Cl content in the beans at cherry stage (A) and K and Cl removal by the beans (B) after application of blends of KCl e K_2SO_4 as cover fertilization. Harvest of 2018/2019. Means followed by the same letter do not differ according to Tukey's test ($P < 0.05$). Vertical bars indicate the standard error of the mean ($n = 4$). T1: 100% KCl; T2: 75% KCl + 25% K_2SO_4; T3: 50% KCl + 50% K_2SO_4; T4: 25% KCl + 75% K_2SO_4; T5: 100% K_2SO_4; control did not receive K_2O.

3.1.3 Harvest of 2019/2020

The same pattern of the first harvest was observed. Cl stocks in the soil decreased along with the proportion of KCl in the blend (Fig. 5). In the 0-20 cm layer, Cl stocks were higher for treatments T1 (119 kg ha^{-1}) and T3 (101 kg ha^{-1}) and lower for T5 (57 kg ha^{-1}) and the control (54 kg ha-1). In the 20-80 cm layer, T1, T2, T3, and T4 did not differ (mean of 122 kg ha^{-1}), surpassing treatments T5 and control (mean of 267 kg ha^{-1}) (Fig. 5).

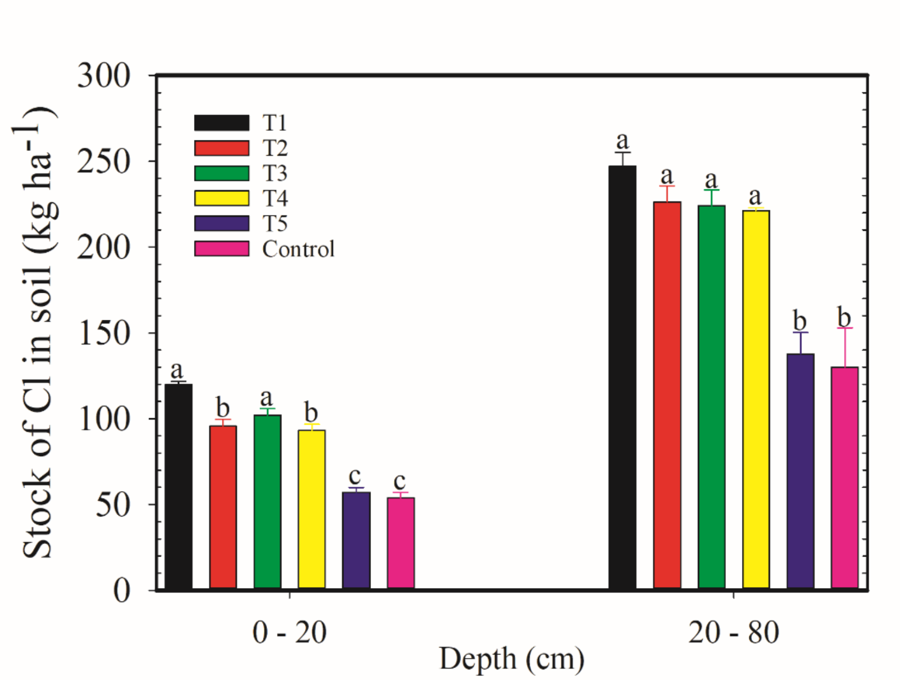


Figure 5: Stocks of Cl in the 0-20 and 20-80 cm layers after application of blends of KCl e K_2SO_4 as cover fertilization. Harvest of 2019/2020. Means followed by the same letter do not differ according to Tukey's test ($P < 0.05$). Vertical bars indicate the standard error of the mean (n = 4). T1: 100% KCl; T2: 75% KCl + 25% K_2SO_4; T3: 50% KCl + 50% K_2SO_4; T4: 25% KCl + 75% K_2SO_4; T5: 100% K_2SO_4; control did not receive K_2O.

Only the control treatment (16.3 g kg^{-1}) showed K content in the leaves below 22.4 g kg^{-1} (Fig. 6A). Cl content in the leaves decreased along with the amount of KCl applied in T1 to T5. The highest Cl content was in the T1 treatment (4919 mg kg^{-1}) and the lowest content was found in T5 (1762 mg kg^{-1}) and control (1819 mg kg^{-1}) treatments (Fig. 6A).

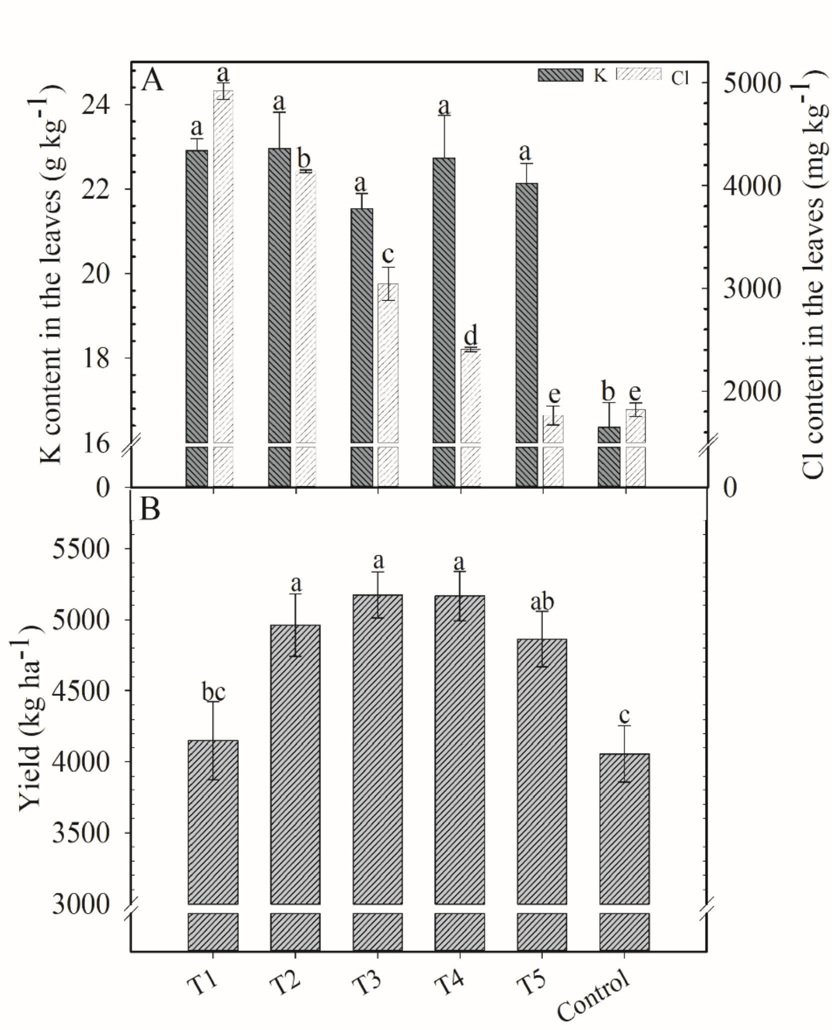


Figure 6: K and Cl content in the leaves (A) and yield (B) of coffee plants. Harvest of 2019/2020. Means followed by the same letter do not differ according to Tukey's test ($P < 0.05$). Vertical bars indicate the standard error of the mean (n = 4). T1: 100% KCl; T2: 75% KCl + 25% K_2SO_4; T3: 50% KCl + 50% K_2SO_4; T4: 25% KCl + 75% K_2SO_4; T5: 100% K_2SO_4; control did not receive K_2O.

There were differences in the yield of the coffee plants depending on the treatment, although they all received the same dose of K. High yields were found in treatments T2, T3, and T4. Yields of T1 and control treatments were the low, producing 4147 and 4055 kg ha^{-1}, respectively (Fig. 6B). The treatments that received K_2SO_4 up to 75 % of applied K had similar yields and with an average of 5100 kg ha^{-1} (Fig. 6B). Yields of treatments T2, T3, and T4 were 19, 24, and 24 % higher than the yield of T1. Treatment T5 yield was similar to the best yields but did not differ from the yield of T1 (Fig. 6B).

The other agronomic variables showed means of 883 mg kg^{-1} for the content of Cl in the beans (Fig. 7A), 45 kg ha^{-1} for the K removal, and 1.6 kg ha^{-1} for the Cl removal by the beans (Fig. 7B).

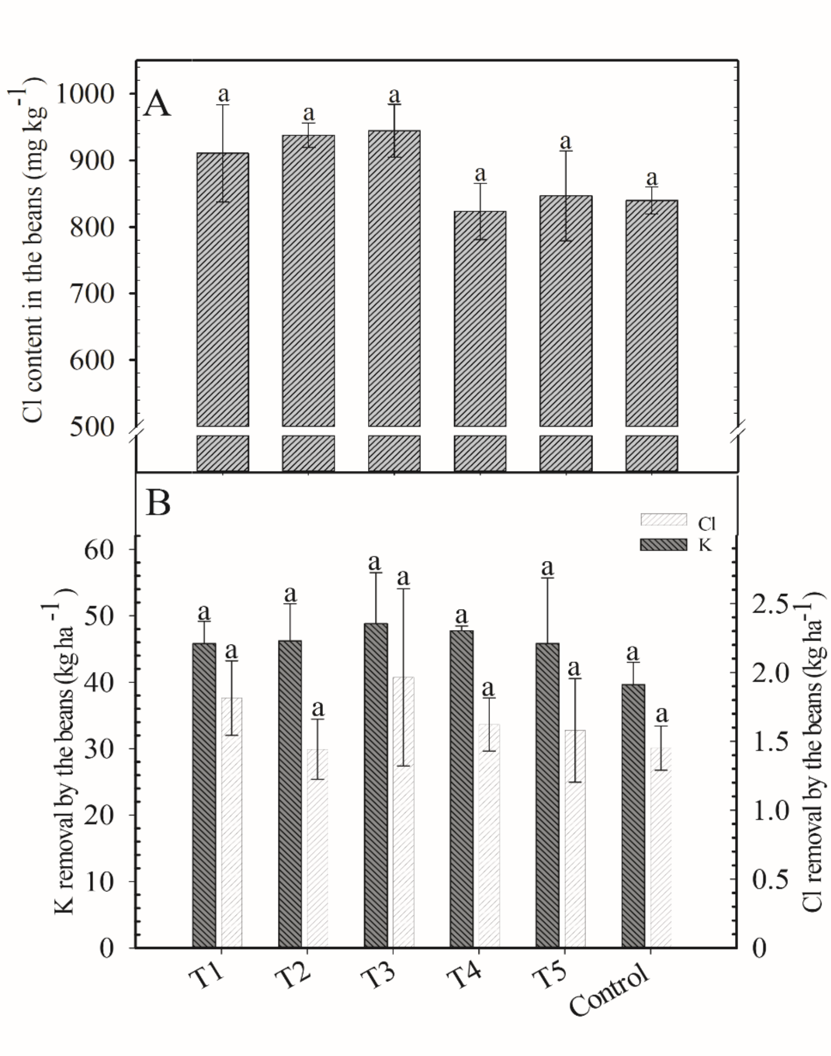


Figure 7: Cl content in the beans (A) and removal of K and Cl (B) by the beans of coffee at cherry stage after application of blends of KCl e K_2SO_4 as cover fertilization. Harvest of 2019/2020. Means followed by the same letter do not differ according to Tukey's test ($P < 0.05$). Vertical bars indicate the standard error of the mean (n = 4). T1: 100% KCl; T2: 75% KCl + 25% K_2SO_4; T3: 50% KCl + 50% K_2SO_4; T4: 25% KCl + 75% K_2SO_4; T5: 100% K_2SO_4; control did not receive K_2O.

3.2 Effect of KCl and K_2SO_4 blends on the chemical composition and quality of the coffee beverage

3.2.1 Harvest of 2017/2018

The treatments influenced ($P < 0.05$) the K leaching (KL) (Fig. S4). The highest KL was in T3 (36.7 $\mu g\ g^{-1}$) and the lowest was in T1 (30 $\mu g\ g^{-1}$). The other variables related to the quality of the coffee beverage had means of 81 points to the sensorial analysis; 91.8 $\mu S\ cm^{-1}\ g^{-1}$ to the electric conductivity (EC); 9.7 % to total sugars (TS); 1.04 % to caffeine content (Caf);

47.6 u min^{-1} g^{-1} to the activity of polyphenol oxidase (PPO); 186.5 mL NaOH 100 g^{-1} of sample to total titratable acidity (TTA); and 6.4 % to polyphenols (Pol) (Table S1).

For the accumulation of polyphenols, protein, total sugars and caffeine, there was no significant difference with the application of potassium fertilizer blends in coffee (Table S2). The mean values for these variables were 213, 104, 156 and 16.5 kg ha-1, for the variables protein, polyphenols, total sugars and caffeine, respectively.

3.2.2 Harvest of 2018/2019

KL, EC, and the cup quality showed significant differences ($P < 0.05$) among the treatments (Fig. 8). All treatments received over 80 points in the cup quality (Fig. 8A). The highest scores were achieved by treatments T3, T4, and T5, with 83, 84.5, and 83 points, respectively.

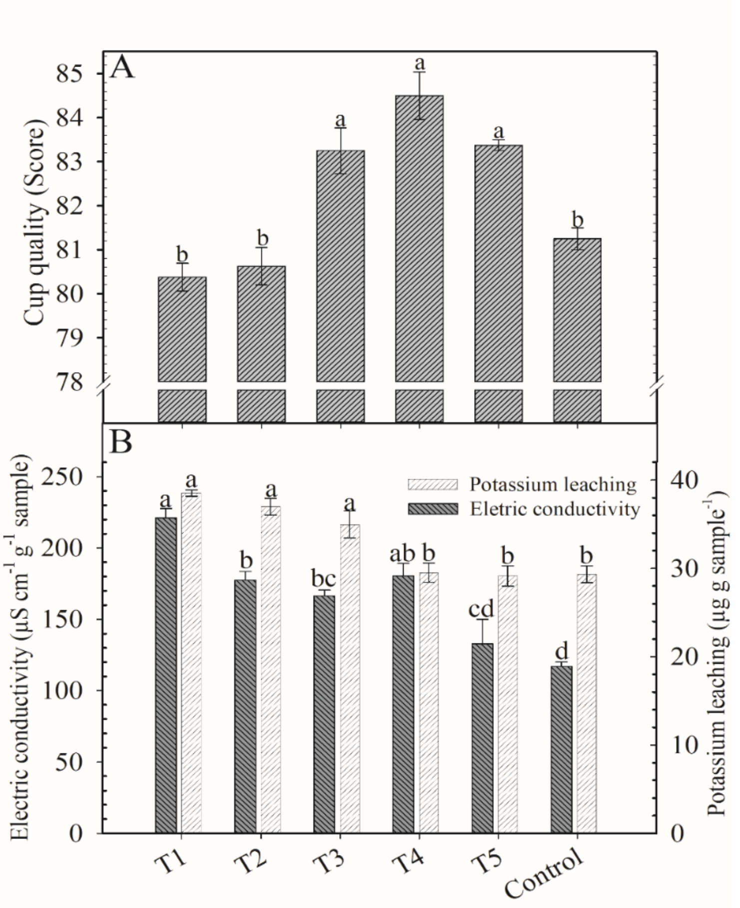


Figure 8: Scores of cup quality (A), electric conductivity and potassium leaching (B) in coffee beans at cherry stage after application of blends of KCl e K_2SO_4 as cover fertilization. Harvest of 2018/2019. Means followed by the same letter do not differ according to Tukey's test ($P < 0.05$). Vertical bars indicate the standard error of the mean (n = 4). T1: 100% KCl; T2: 75% KCl + 25% K_2SO_4; T3: 50% KCl + 50% K_2SO_4; T4: 25% KCl + 75% K_2SO_4; T5: 100% K_2SO_4; control did not receive K_2O.

The EC was higher in the T1 (221 µS cm^{-1} g^{-1}) treatment and lower in treatments T5 (132 µS cm^{-1} g^{-1}) and control (117 µS cm^{-1} g^{-1}). T4 and T1 were not different and the mean was 180 µS cm^{-1} g^{-1}. The T2 treatment was 24 % lower than T1, but it was not statistically different from treatment T4 (Fig. 8B).

Potassium leached (KL) more in treatments where the proportion of KCl was higher than K_2SO_4 (Fig. 8B). T1, T2, and T3 had similar KL (38.5, 37, and 35 µg g^{-1}, respectively), while other treatments were lower (29.5, 29, and 29.3 µg g^{-1}, for T4, T5, and control, respectively).

The means of the other variables analyzed in the beans were 9.1 % for TS, 1.03 % for Caf, 46 u min^{-1} g^{-1} for PPO, 190 mL NaOH 100 g^{-1} of sample for TTA, and 5.0 % for the content of Pol (Table 4).

Table 4. Variables analyzed in the coffee beans for the harvest of 2018/2019

Treatments	Pol	TS	Caf	PPO	TTA
T1	4.9a	9.4a	1.05a	47.0a	188.6a
T2	5.1a	9.0a	1.03a	45.7a	189.7a
T3	4.8a	8.9a	1.02a	47.8a	193.1a
T4	5.0a	9.5a	1.03a	45.9a	194.9a
T5	5.1a	9.0a	1.03a	47.7a	191.3a
Control	5.2a	8.9a	1.02a	44.5a	187.1a
CV (%)	6.9	5.3	2.8	8.5	2.9
Mean	5.0	9.1	1.03	46.4	190.8

*CV (%) = coefficient of variation; Pol = total phenolic compounds (%); TS = content of total sugars (%); Caf = content of caffeine (%); PPO = polyphenol oxidase activity (u min^{-1} g^{-1}); TTA = total tritable acidity (mL NaOH 0.1 N 100 g^{-1}). Means followed by the same letter do not differ according to Tukey's test ($P < 0.05$). T1: 100% KCl; T2: 75% KCl + 25% K_2SO_4; T3: 50% KCl + 50% K_2SO_4; T4: 25% KCl + 75% K_2SO_4; T5: 100% K_2SO_4; control did not receive K_2O.

For this crop, no significant differences were observed for the accumulation of polyphenols, protein, total sugars and caffeine (Table 7), however, the values for these variables were lower than those observed for the 2017/2018 crop (Table 5).

Table 5. Results for accumulation of polyphenols, protein, total sugars and caffeine in cherry beans for the harvest of 2018/2019.

Treatments	Protein	Polyphenols	Total sugars	Caffeine
	------------------------kg ha^{-1}------------------------			
T1	36a	12a	23a	2,6a
T2	46a	16a	27a	3,2a
T3	43a	14a	26a	3,0a
T4	56a	19a	36a	3,9a
T5	61a	21a	38a	4,3a
Control	40a	14a	24a	2,9a
CV (%)	21	22	19	23
Mean	47	16	29	3,3

CV (%) = coefficient of variation

The mean values for these variables were 47, 16, 29 and 3.3 kg ha^{-1}, for the variables protein, polyphenols, total sugars and caffeine, respectively.

3.2.3 Harvest of 2019/2020

The cup quality had different results ($P < 0.05$) among the treatments (Fig. 9) for the harvest of 2019/2020. The highest grade was achieved in T5 treatment (89 points), where K_2SO_4 was the only source of K_2O. The lowest grade was in the control treatment (84 points) that did not receive K. However, the T1 treatment was not different from the control (Fig. 9). Treatments T3 and T4 had similar scores (86 points), while T2 was similar to treatments T3 and T4, but not different from treatment T1 (Fig. 9).

The other variables were not influenced by the application of the different blends of K ($P < 0.05$). The following means were found: 124 µS cm^{-1} g^{-1} for EC; 9.6 % for TS; 1.02 % for Caf; 54 u min^{-1} g^{-1} for PPO; 70.9 µg g^{-1} for KL; 195 mL NaOH 100 g^{-1} of sample for TTA, and 5.0 % for Pol (Table 6).

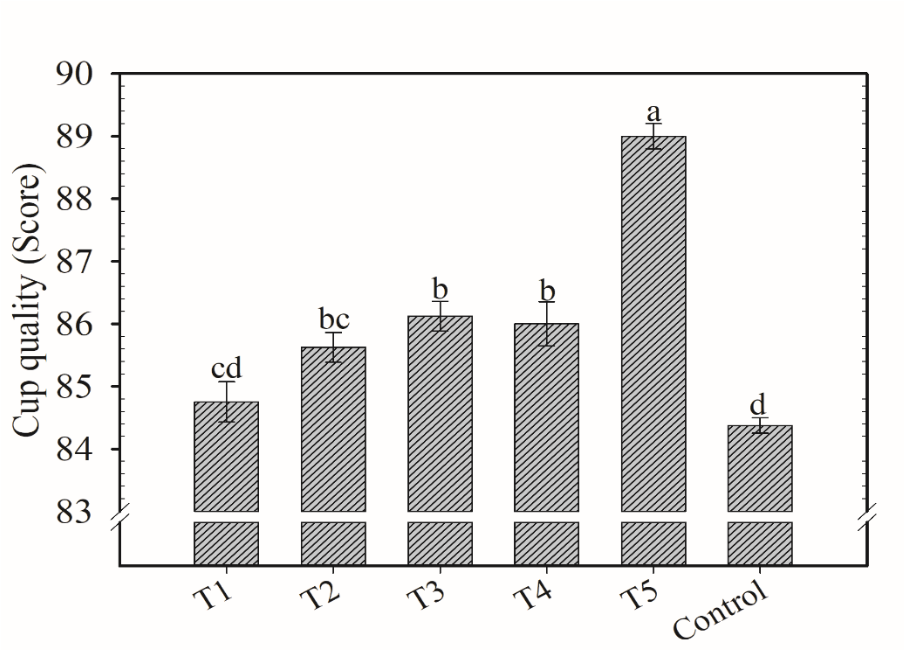


Figure 9: Scores of the cup quality of coffee beans at cherry stage after application of blends of KCl e K_2SO_4 as cover fertilization. Harvest of 2019/2020. Means followed by the same letter do not differ according to Tukey's test ($P < 0.05$). Vertical bars indicate the standard error of the mean (n = 4). T1: 100% KCl; T2: 75% KCl + 25% K_2SO_4; T3: 50% KCl + 50% K_2SO_4; T4: 25% KCl + 75% K_2SO_4; T5: 100% K_2SO_4; control did not receive K_2O.

Table 6. Variables analyzed in the coffee beans for the harvest of 2019/2020

Treatments	Pol	TS	Caf	PPO	KL	TTA	EC
T1	5.1a	9.7a	1.05a	47.4a	72.7a	194.0a	119.4a
T2	5.2a	9.7a	1.09a	57.8a	69.9a	195.5a	129.7a
T3	5.1a	9.5a	1.08a	57.5a	62.8a	194.8a	119.7a
T4	5.1a	9.7a	1.06a	52.1a	75.2a	196.4a	126.7a
T5	5.2a	9.6a	1.03a	55.6a	80.2a	197.3a	134.7a
Control	5.0a	9.4a	1.03a	54.0a	64.2a	192.5a	118.4a
CV (%)	3.5	3.5	6.1	11.6	13.8	3.7	12
Mean	5.1	9.6	1.06	54.1	70.9	195	124

*CV (%) = coefficient of variation; Pol = total phenolic compounds (%); TS = content of total sugars (%); Caf = content of caffeine (%); PPO = polyphenol oxidase activity (u min^{-1} g^{-1}); KL = K leaching (μg g^{-1}); TTA = total tritable acidity (mL NaOH 0.1 N 100 g^{-1}); EC = electric conductivity (μS cm^{-1} g^{-1}). Means followed by the same letter do not differ according to Tukey's test ($P < 0.05$). T1: 100% KCl; T2: 75% KCl + 25% K_2SO_4; T3: 50% KCl + 50% K_2SO_4; T4: 25% KCl + 75% K_2SO_4; T5: 100% K_2SO_4; control did not receive K_2O.

For this last crop, as for the first two, no significant differences were observed for the accumulation of polyphenols, protein, total sugars and caffeine (Table 7).

Table 7. Results for accumulation of polyphenols, protein, total sugars and caffeine in cherry beans for the harvest of 2018/2019.

Treatments	Protein	Polyphenols	Total sugars	Caffeine
	-----------------------kg ha^{-1}-----------------------			
T1	289a	119a	219a	24a
T2	302a	126a	220a	25a
T3	314a	126a	224a	25a
T4	293a	124a	229a	24a
T5	276a	119a	204a	23a
Control	260a	109a	193a	22a
CV (%)	22	22	19	21
Mean	289	120	215	24

CV (%) = coefficient of variation

The mean values for these variables were 289, 120, 215 and 24 kg ha^{-1}, for the variables protein, polyphenols, total sugars and caffeine, respectively. These values were very close to those observed for the harvest of 2017/2018.

3.2.4 Principal component analysis (PCA) for the agonomic variables, chemical composition of the beans and quality of the coffee beverage

The treatments in the PCA represent the proportions of KCl and K_2SO_4 as described before. Thus, the closer they are, the greater the correlation between the variables that constitute these treatment groups. The agronomic variables stocks of K and Cl in the 0-20 and 20-80 cm layers, K and Cl contents in the leaves, yield, K and Cl removal in the beans and Cl content in the beans are supplementary variables, that is, they do not contribute to explaining the variability of the data. These illustrative variables are represented as dashed arrows and they help to interpret the other data.

The PCA for the first harvest (2017/2018) indicates that the two components (Dim1 and Dim2) responded for 51.7 % of the total variability of the data. The variances explained by these two variables were 38.2 % and 18.9 %, respectively (Fig. S5).

The variables K and Cl removal by the beans, K content in the leaves, yield, TS, Pol, and Caf were highly correlated. The control treatment was more related to these variables. In addition, these variables were negatively correlated to the stock of K in the 20-80 cm layer, stock of Cl in the 0-20 cm layer, and cup quality. The EC, KL, and TTA variables were highly correlated. The variables Cl content in the beans, stock of K in the 0-20 cm layer, and Cl content in the leaves were low correlated with the stock of Cl in the 20-80 cm layer and with the PPO activity. Furthermore, these variables were negatively correlated with the variables EC, KL, and TTA.

In the harvest of 2018/2019, the two PCA components (Dim1 and Dim2) explained 54.9 % of the total variability. The variances of each component were 39.6 % and 15.3 %, respectively (Fig. 10).

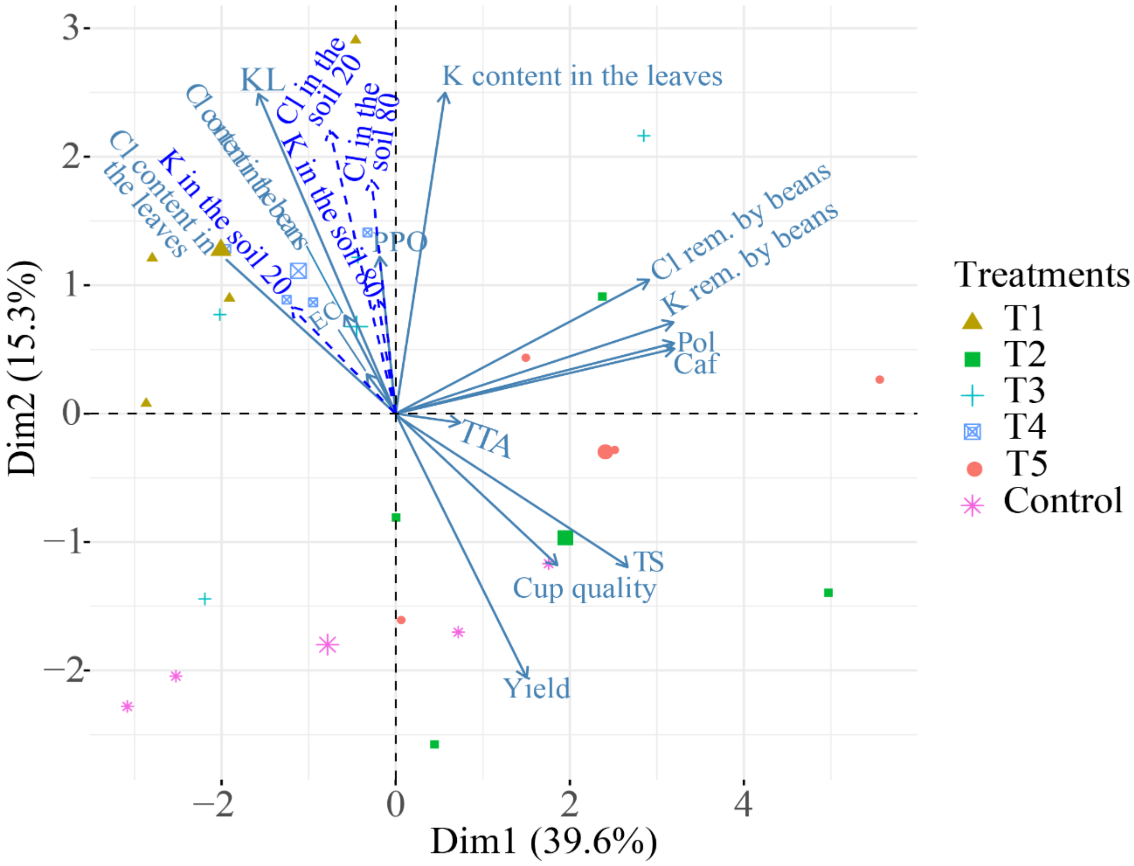


Figure 10: Principal component analysis for the harvest of 2018/2019. PPO = activity of the enzyme polyphenol oxidase; KL = K leaching; TTA = total titratable acidity; EC= electric conductivity; Pol = total phenolic compounds; Caf = content of caffeine; TS= content of total sugars; K in the soil 20 = stock of K in the 0-20 cm layer; K in the soil 80 = stock of K in the 20-80 cm layer; Cl in the soil 20 = stock of Cl in the 0-20 cm layer; Cl in the soil 80 = stock of Cl in the 20-80 cm layer, K rem. by beans: K removal by the beans, Cl rem. by beans: Cl removal by the beans. T1: 100% KCl, T2: 75% KCl + 25% K_2SO_4, T3: 50% KCl + 50% K_2SO_4, T4: 25% KCl + 75% K_2SO_4, T5: 100% K_2SO_4.

The cup quality was positively correlated with TS in the coffee beans and with yield. These variables are also correlated with the T2 treatment.

The KL variable was correlated to the stocks of Cl in both layers and with the Cl content in the leaves of the plants. T1 and T4 treatments were close to these variables. Pol, Caf, and K and Cl by the beans were strongly correlated. Overall, the PCA (Fig. 10) shows that the quality of the coffee beverage is negatively correlated with the content of Cl in the leaves and beans.

On the last evalulation harvest (2019/2020), the PCA explained 56.8% of the total variability. While the first component (Dim1) explained 36.8 % of the variance and the second component (Dim2) explained 20 % of the variance (Fig. 11).

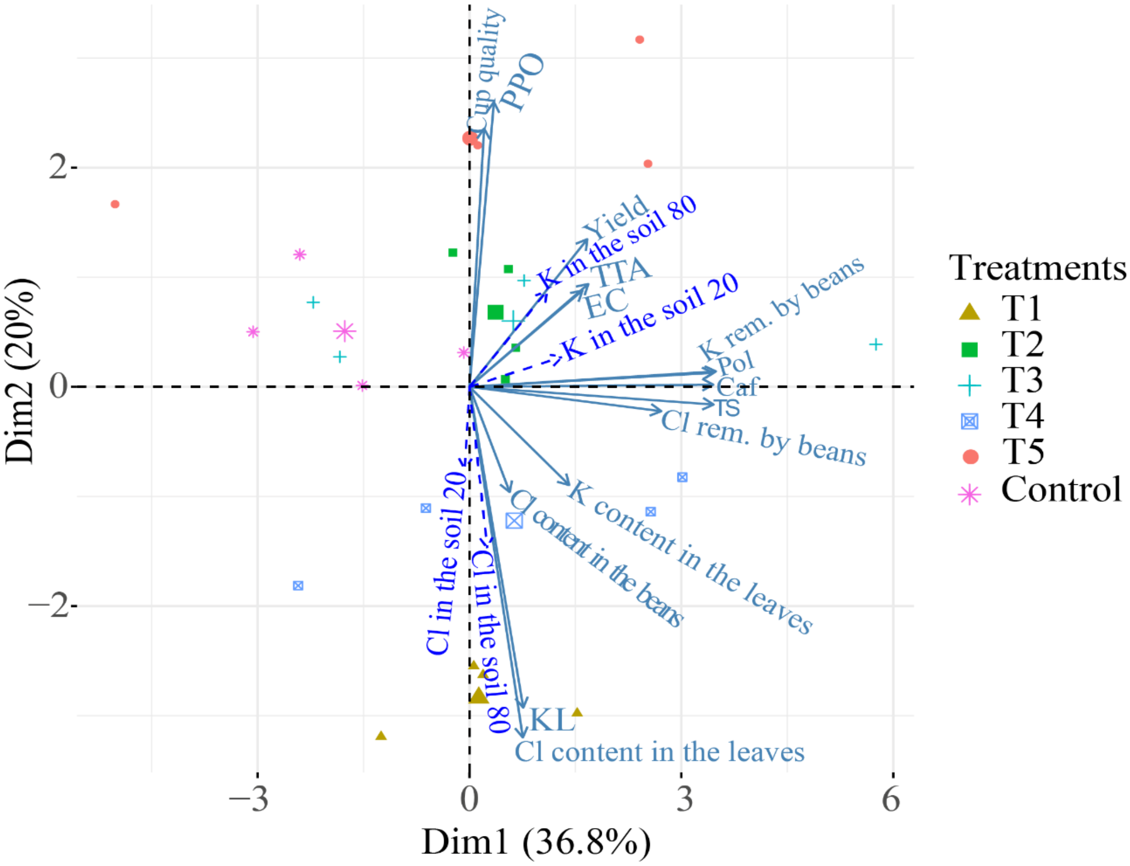


Figure 11: Principal component analysis for the harvest of 2019/2020. PPO = activity of the enzyme polyphenol oxidase; KL = K leaching; TTA = total titratable acidity; EC= electric conductivity; Pol = total phenolic compounds; Caf = content of caffeine; TS= content of total sugars; K in the soil 20 = stock of K in the 0-20 cm layer; K in the soil 80 = stock of K in the 20-80 cm layer; Cl in the soil 20 = stock of Cl in the 0-20 cm layer; Cl in the soil 80 = stock of Cl in the 20-80 cm layer, K rem. by beans: K removal by the beans, Cl rem. by beans: Cl removal by the beans. T1: 100% KCl, T2: 75% KCl + 25% K_2SO_4, T3: 50% KCl + 50% K_2SO_4, T4: 25% KCl + 75% K_2SO_4, T5: 100% K_2SO_4.

The cup quality and PPO activity were closely to treatment T5, where no KCl was applied. The stock of Cl in the 20-80 cm layer was strongly related to the T1 treatment, where only KCl was applied. KL and Cl content in the leaves were also correlated with the T1 treatment.

4 DISCUSSION

4.1 Effects of the aplication of KCl e K_2SO_4 blends in the soil Cl stocks, nutritional state, and yield of coffee plants

Despite the long-time fertilization with KCl in the area, the initial K stocks in the soil were considered a medium amount (GUIMARÃES et al., 1999) (Table 3). For the initial amount of Cl, however, there is no method of extraction and no reference values to relate to the needs of coffee plants. Cl is a micronutrient that is required in low amounts by plants. Under field conditions, Cl deficiency is uncommon, while the excess is frequently expressed.

The stocks of Cl reduced during the three years of study due to the leaching of the element to deeper layers in the soil (Fig. S1, 2, and 5). The Cl ion has low interaction with the soil solid phase (BORGGAARD, 1984), thus being easily leachable (GEILFUS, 2018).

There was a tendency to accumulate K in the leaves when plants received more KCl (Fig. S2, 3, and 6A). KCl fertilizer is more soluble than the K_2SO_4. Nonetheless, in all harvests, the foliar content of K remained adequate in the range of 19.7 e 31 g kg^{-1} (CLEMENTE et al., 2015; MARTINEZ et al., 2003), except for the low content in the control treatment in the last two harvests (Fig.S 3 e 6A).

The Cl content in the leaves in all harvests was reduced from T1 to T5 and control (Fig. S2, 3, and 6A). The content of Cl usually found in plant tissues ranges from 2000 to 30000 mg kg^{-1}, which is equivalent to the amount of macronutrients (MARSCHNER, 1995). However, plants vary in their tolerance to Cl (MARENCO and LOPES, 2009). According to MARSCHNER (1995), plants sensible to Cl show toxicity symptoms to concentrations higher than 3500 mg kg^{-1}. In tolerant plants, the symptoms appear when the concentration range from 20000 to 30000 mg kg^{-1}.

Under field conditions, toxicity symptoms caused by Cl excess are uncommon. Symptoms are characterized by the reduction of the width of the leaves, with possible curling, and the presence of wide necrosis with later leaf drying (DECHEN et al., 2018). In this study, despite the high content found when KCl was applied (over 2500 mg kg^{-1}) plants did not show toxicity symptoms. However, it is important to emphasize the damages to the metabolism, growth, and yield that can occur even in concentrations below the toxicity threshold. In fact, in the harvest of 2019/2020, when the foliar content of Cl reached 4919 mg kg^{-1} in treatment T1 (Fig. 6A), a lower yield was observed. An argument could be made for the higher availability of S in the treatments that received more K_2SO_4, but despite the source of K, all treatments received 2 t ha^{-1} of gypsum, which provided 340 kg ha^{-1} of S to the soil. The reduction in the yield is probably more related to the excess of Cl than the lack of S in the fertilization (Fig. 6B).

Conversely, it is notable the reduction in the yield, even with no clear statistical separation, between the treatment that received only K_2SO_4 and the treatment fertilized solely with KCl (Fig. 6B). It is possible that such difference may be related to the solubility of the K_2SO_4, 80 g L^{-1} at 25 °C, which is considerably lower than the solubility of the KCl, 279 g L^{-1} (MAGEN, 1996). This difference in the availability of K can compromise the yield in harvests of increased productivity as the harvest of 2019/2020. Another explanation is that the high solubility of KCl can benefit the absorption of cations, such as K, Ca, and Mg (MANCUSO et al., 2014), increasing plant nutrition, even though for a short period. A third possibility is the excess of SO_4^{2-}, limiting the availability and absorption of $H_2PO_4^-$ by the plant, since, besides the K_2SO_4 application, gypsum was also applied.

The results suggest advantages in providing the two sources of K (25 to 75 % of K_2SO_4) to increase yield (Fig. 6B). At the first two years of experiment, when the yields were lower, the exportation of K by the beans was less intense and plant production was not limited by the sources of K, once they were applied at the same dose.

The removal of K and Cl and the content of Cl in the beans were not different among the treatments (Fig. S3B, 4B, and &B) since these elements remain in high concentration in the mucilage and the bean peels (MORAES and CATANI, 1964). The exception is treatment T3 at the first harvest, but that might be related to the history of the area than to the treatments (Fig. S3B).

4.2 Effects of the aplication of KCl e K_2SO_4 blends in the chemical composition of the beans and in the quality of the coffee beverage

There was a response in the KL in the first and second harvests (Fig. S4 and 8B). This variable is related to the integrity of the cell wall and membrane and, consequently, to the coffee beverage quality. When these structures are less intact, the cell has a higher tendency to lose cytoplasmatic contents as a reflection of the reduced cell organization (CLEMENTE et al., 2015; MARTINEZ et al., 2014; PIMENTA et al., 1997). The KL results for the first year of the evaluation showed the opposite effect to what would be expected, but this lower value observed for the T1 treatment is due to the influence of frequent fertilization with KCl before the evaluation. (Fig. S4). In the second year of evaluation, after the establishment of a new K dynamic in the soil and the reduction of Cl levels (Fig. 2), less KL was found in treatments T4, T5, and control (Fig. 8B).

Another evidence of the reduction in the quality of the coffee beans and beverage with increasing doses of KCl is the high values of the EC observed in treatments T1, T2, and T3 (Fig. 8B). As KL, CE also has a direct relationship with the integrity of the cell membrane (AMORIM, 1978; GOULART et al., 2007; PRETE, 1992).

Despite being considered indicatives of the quality of the beverage, these variables should not be decisive to vouch for the quality of the coffee (GOULART et al., 2007). In fact, the results of the cup quality in the last harvest suggest the same tendency observed for these variables (Fig. 9). Notably, there was a response in the cup quality after the application of the treatments in the second harvest (Fig. 8A). As previously stated, in the first harvest, all response variables were very dependent on the previous fertilization in the area, thus the lack of response in the sensorial analysis.

However, from the second year of evaluation, some important facts should be emphasized about the K nutrition with the blends of fertilizers and the quality of the coffee beverage. Despite the lower scores for T1 and T2, the same behavior was observed for the control without K fertilization (Fig. 8A and 9). This result suggests that only reducing the application of Cl via KCl is not enough to improve the quality of the beverage, but also maintaining adequate levels of K is essential to produce a high-quality coffee (CLEMENTE et al., 2015; DIAS et al., 2018).

In the last harvest, treatment T5 achieved the highest score (89 points) in the sensorial analysis. T3 and T4, however, reached a few points less (86 points) than T5 (Fig. 9). Considering the higher cost of K_2SO_4 in relation to KCl, the choice for the composition of the K fertilizer should consider the economical cost that this difference of 3 points in the cup quality might return. Another important consideration is that there was a tendency for higher yield on T3 and T4 treatments, despite the lack of statistical differences among the treatments (Fig. 6B). The difference between both treatments in relation to treatment T5 yielded more five sacks of 60 kg of coffee beans, suggesting that yield should also be considered when choosing the best K fertilizer composition.

Regarding the values observed for the accumulation of polyphenols, protein, total sugars and caffeine, the aim was only to show how much of these compounds are produced per hectare, but in general, the variation in the values of these variables is more related to the total coffee productivity, more specifically, with the production of cherry beans per hectare. It is noted that, in the second harvest (2017/2018), where coffee productivity was lower, the values for these variables followed the same trend. In relation to the last crop (2019/2020), the values were very similar to those observed for the 2017/2018 crop, even though the productivity in the last crop was higher. In this sense, it is necessary to emphasize that the calculations are carried out in relation to the production of cherry grains, which in the 2017/2018 harvest was higher than the last harvest.

4.3 Principal component analyses for the agronomic variables and the quality of the coffee

The PCA results suggest that studies on how the fertilization of coffee plants affects the quality of the coffee beverage should be carried out for a long duration.

Overall, there were increased effects of the treatments after the second year of evaluation, probably due to the previous fertilizations with KCl, which is the most used source of K in Brazil (DIAS et al., 2018). However, some points should be considered in relation to the first harvest, such as the correlation among the variables Cl content in the beans, K content in the leaves, and yield (Fig. S5) showing the importance of the K fertilization to coffee plants. Nonetheless, the negative correlation of these variables with the cup quality suggests an unfavorable effect of one of these variables on the quality of the beverage, most probably the Cl content in the beans.

The correlation between EC and KL can be explained by the direct relationship shared by these two variables since they both indicate damages to the cell integrity of the beans (AMORIM, 1978; GOULART et al., 2007; PRETE, 1992) (Fig. S5). The PCA confirms these results. These damages can lead to the loss of compounds related to the quality of the beans and the cup quality, therefore, lower EC and KL indicate lower coffee quality (GOULART et al., 2007; REINATO et al., 2007). This lower bean quality is confirmed by the high negative correlation between PPO activity and the variables EC, PL, and TTA, showing that higher values of EC, KL, and TTA are associated with low PPO activity. Several studies found a positive correlation between the PPO activity and the sensorial quality of the coffee (CARVALHO et al., 1994, SILVA et al., 2002). Thus, it is possible to conclude that there is a reduction in the PPO activity and the quality of the beverage as EC, KL, and TTA increase. In fact, damages to the cell membrane lead to the loss of selective permeability, facilitating the reaction of PPO with the phenolic compounds (the specific substrate of this enzyme). That reaction produces quinones that inhibit the activity of PPO (CARVALHO et al., 1994; SILVA et al., 1999).

Noteworthy, the high positive correlation between cup quality and the content of TS in the beans indicates a direct relationship where the increase in TS also increases the cup quality (Fig. 10). Treatment T2 was close to this correlation, confirming the results for cup quality (Fig.

10) and indicating that increases in the K_2SO_4 proportion tend to increase cup quality. These results confirm the study of Silva et al., (1999), which also involved doses and sources of K, and add information about the sensorial quality of the coffee beverage. These fidings evidence the increase in the content of TS and better scores on the cup quality test as the proportion of K_2SO_4 in the blend also increases.

The reduction in the quality of the coffee beans is observed in the high correlation among KL, the stock of Cl in the soil, and the Cl content in the leaves. When the values of the variables related to Cl increase, Kl also increases, and the quality of the beans and the beverage reduces (Fig. 10).

In the last harvest (2019/2020), it is notable a direct effect of the Cl in the variables related to the quality of the beverage. Despite the PPO activity did not show differences for the treatments on both harvests, this variable behavior within the PCA is enough to indicate the direct relationship of this enzyme with the quality of the coffee beverage (Fig. 11).

In addition, the high negative correlations of cup quality and PPO in relation to the variables related to Cl (stocks in the soil and content in the leaves) confirm the negative influence of the Cl in the beverage. Besides, treatment T5, which received only K_2SO_4, is closely related to cup quality and PPO activity.

Previous reports state that Cl increases the water content of the coffee fruits with consequent microbial fermentation (GOUNY, 1973; LEITE and CARVALHO, 1994). We believe this explanation does not relate to this study since the beans were collected manually at the stage of cherry and benefited under controlled conditions, unlikely leading to an undesirable fermentation.

Although we did not perform physiological or morphological analyses of the coffee beans, the results allow us to infer that there is an effect of the Cl in the beans and that it might be related to the loss of quality of the beverage. The rationale is that the Cl can inhibit the activity of the PPO enzyme when reacting with the copper activator, thus reducing the enzymatic activity when KCl is applied (ROBINSON and ESKIN, 1991).

In conclusion, this study showed that the blends of K fertilizers responded positively to the quality of the coffee beverage when the proportion of K_2SO_4 relative to KCl was increased. There was a tendency to higher KL, EC, and TTA with the increase of the KCl proportion, which might lead to damages to the cell membrane caused by the Cl, and the consequent reduction of the PPO activity and quality of the beverage. However, the decision to use a determined blend of K should consider the improvement of the beverage and the economical return to the farm. Moreover, it should also consider the yield. For example, in this study, the blend that provided the best quality for the coffee beverage was not always the same responsible for the highest yield. And finally, it should also consider the economical costs of the fertilization with K_2SO_4, which is more expensive than KCl. Considering these aspects, a management strategy could be the separation of the farm into plots based on the tendency to produce better quality coffees in previous years. In this case, each plot would receive a determined blend of K and the highest proportions of K_2SO_4 should be applied to the plots with a tendency to produce higher quality coffees, while the higher proportion of KCl would fertilize plots of low-quality coffee.

5 CONCLUSIONS

The activity of the polyphenol oxidase enzyme and the cup quality indicate that the ion Cl^- reduces the quality of the coffee beverage.

The increased application of the Cl^- ion increases KL, EC, and TTA, indicators of the loss of coffee quality.

K content in the leaves was not influenced by the application of blends of K fertilizer, while Cl content increased linearly with KCl applied.

The application of KCl and K_2SO_4 blends influenced coffee yield and the optimum proportion was 25 % of KCl and 75 % of K_2SO_4

The highest score in the cup quality test was observed with 100 % K_2SO_4. However, other blends showed close scores. The decision for the fertilizer should consider the cost of the K source

KL and EC can indirectly show that the Cl can damage the coffee beans and reduce the selective permeability of the cell membrane, with possible negative consequences to the coffee beverage.

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SUPLEMENTARY MATERIAL

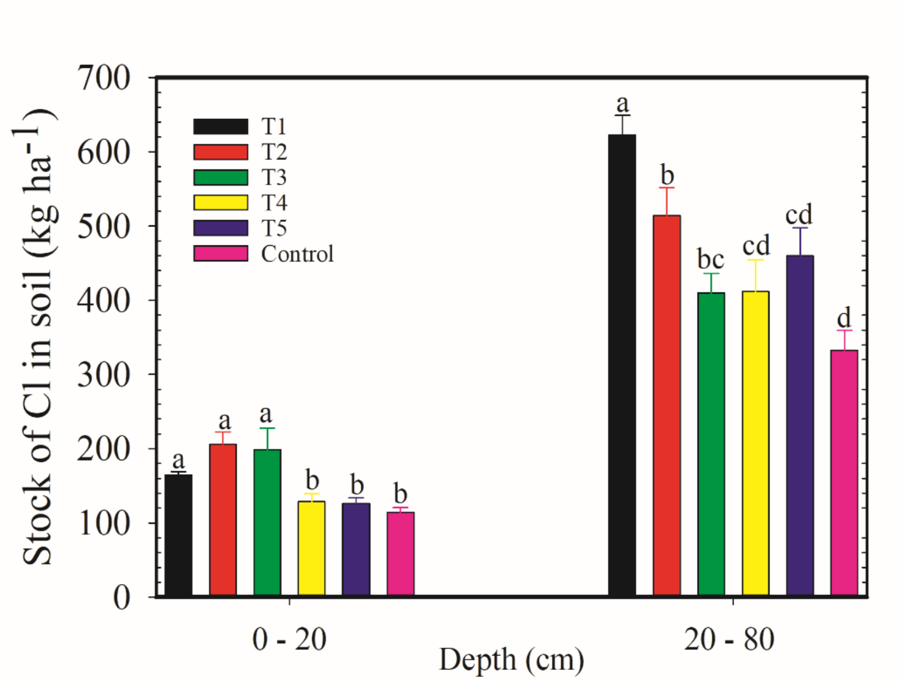

Figure S1: Stocks of Cl in the 0-20 and 20-80 cm layers after application of KCl and K_2SO_4 blends as cover fertilization on coffee plants. Harvest of 2017/2018. Means followed by the same letter do not differ according to Tukey's test ($P < 0.05$). Vertical bars indicate the standard error of the mean (n = 4). T1: 100% KCl; T2: 75% KCl + 25% K_2SO_4; T3: 50% KCl + 50% K_2SO_4; T4: 25% KCl + 75% K_2SO_4; T5: 100% K_2SO_4; control did not receive K_2O.

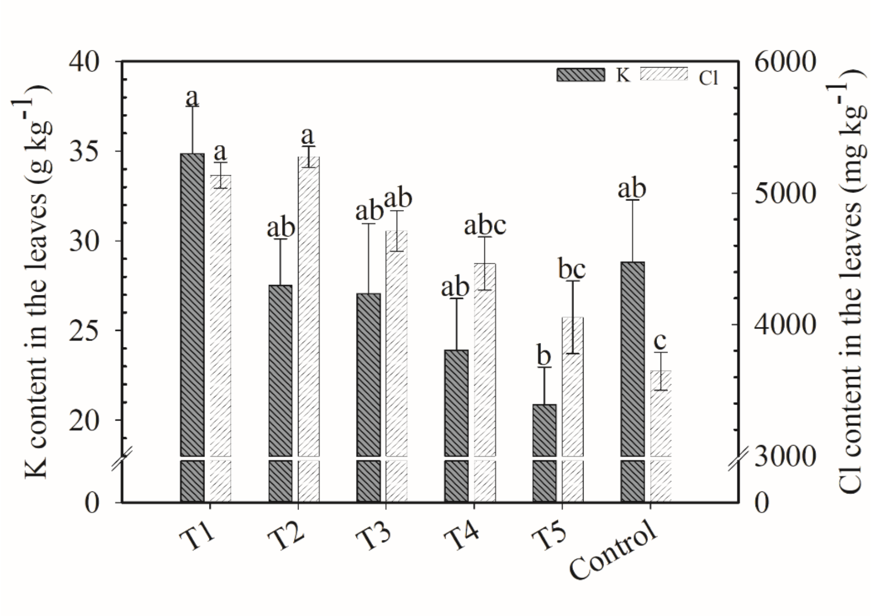

Figure S2: K and Cl content in the leaves of coffee plants, 20 days after application of the second cover fertilization parcel. Harvest of 2017/2018. Means followed by the same letter do not differ according to Tukey's test ($P < 0.05$). Vertical bars indicate the standard error of the mean (n = 4). T1: 100% KCl; T2: 75% KCl + 25% K_2SO_4; T3: 50% KCl + 50% K_2SO_4; T4: 25% KCl + 75% K_2SO_4; T5: 100% K_2SO_4; control did not receive K_2O.

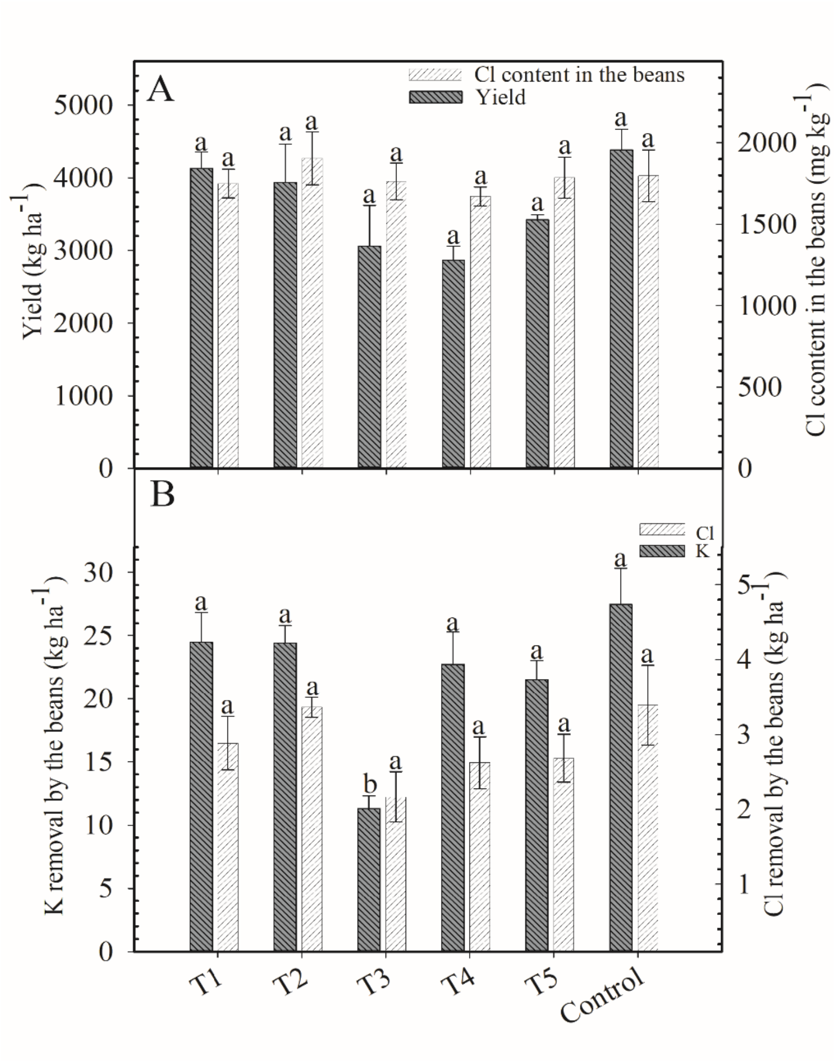

Figure S3: Yield of coffee plants and Cl content in the beans at cherry stage (A) and K and Cl removal by the beans (B) after application of blends of KCl e K_2SO_4 as cover fertilization. Harvest of 2017/2018. Means followed by the same letter do not differ according to Tukey's test ($P < 0.05$). Vertical bars indicate the standard error of the mean (n = 4). T1: 100% KCl; T2: 75% KCl + 25% K_2SO_4; T3: 50% KCl + 50% K_2SO_4; T4: 25% KCl + 75% K_2SO_4; T5: 100% K_2SO_4; control did not receive K_2O.

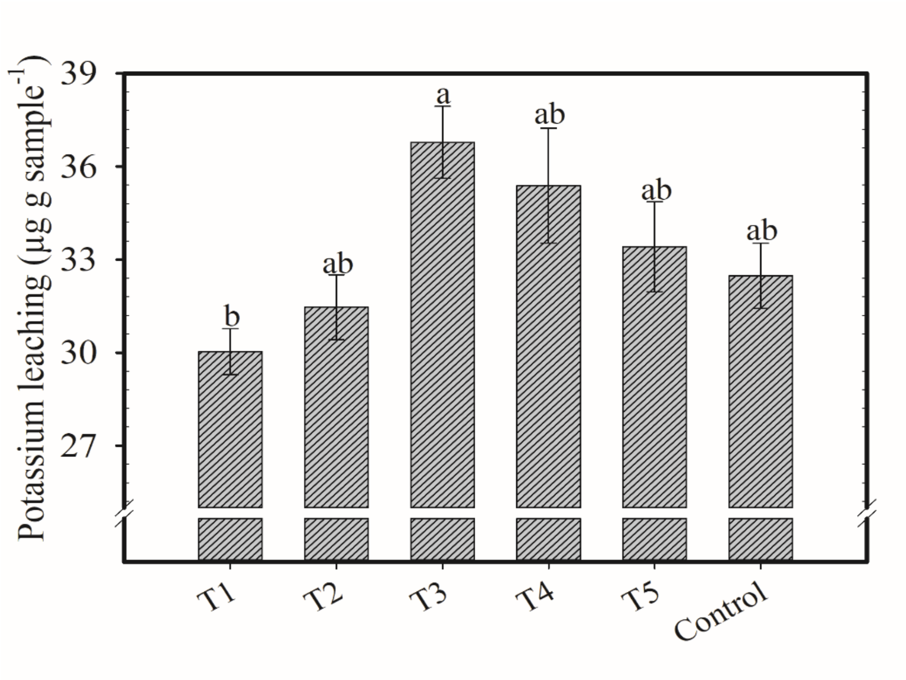

Figure S4: Potassium leaching in coffee beans at stage of cherry after application of blends of KCl e K_2SO_4 as cover fertilization. Harvest of 2017/2018. Means followed by the same letter do not differ according to Tukey's test ($P < 0.05$). Vertical bars indicate the standard error of the mean ($n = 4$). T1: 100% KCl; T2: 75% KCl + 25% K_2SO_4; T3: 50% KCl + 50% K_2SO_4; T4: 25% KCl + 75% K_2SO_4; T5: 100% K_2SO_4; control did not receive K_2O.

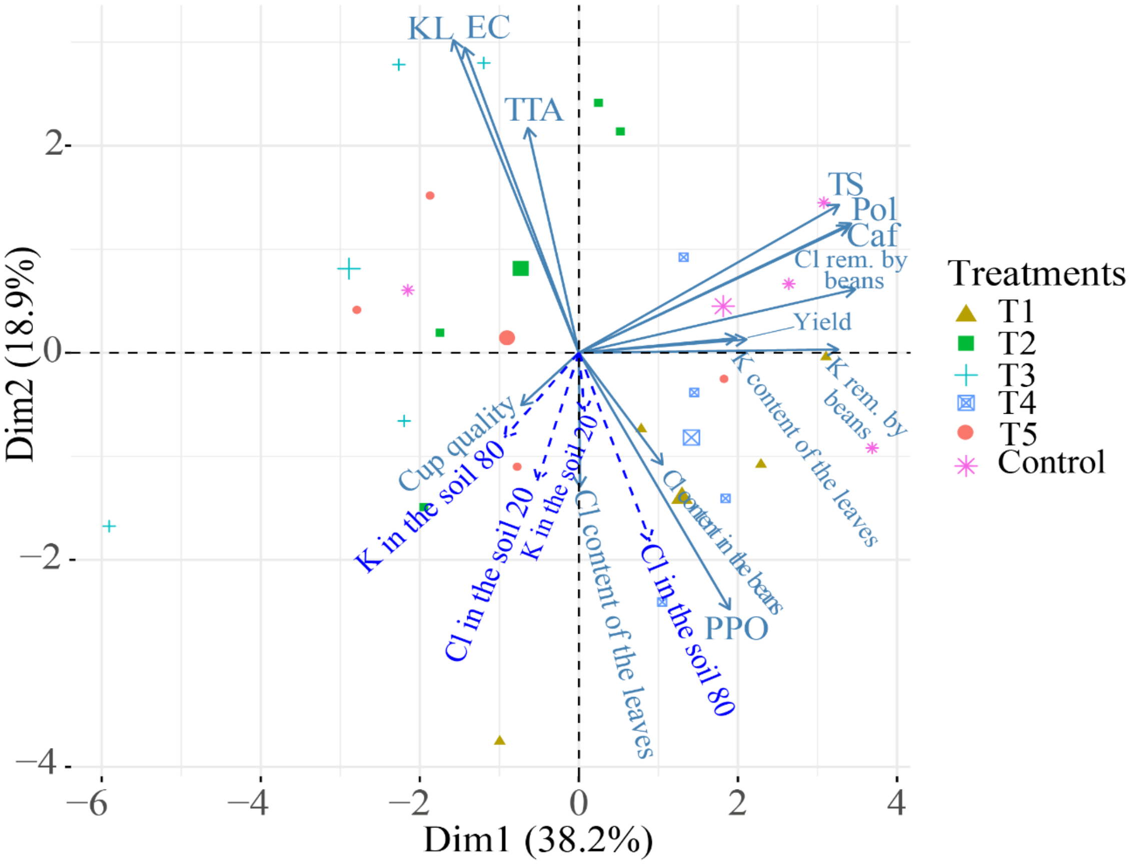

Figure S5: Principal component analysis for the harvest of 2017/2018. PPO = activity of the enzyme polyphenol oxidase; KL = K leaching; TTA = total titratable acidity; EC = electric conductivity; Pol = total phenolic compounds; Caf = content of caffeine; TS = content of total sugars; K in the soil 20 = stock of K in the 0-20 cm layer; K in the soil 80 = stock of K in the 20-80 cm layer; Cl in the soil 20 = stock of Cl in the 0-20 cm layer; Cl in the soil 80 = stock of Cl in the 20-80 cm layer, K rem. by beans: K removal by the beans, Cl rem. by beans: Cl removal by the beans. T1: 100% KCl, T2: 75% KCl + 25% K_2SO_4, T3: 50% KCl + 50% K_2SO_4, T4: 25% KCl + 75% K_2SO_4, T5: 100% K_2SO_4.

Table S1. Variables analyzed in the coffee beans for the harvest of 2017/2018

Treatments	CQ	Pol	TS	Caf	PPO	TTA	EC
T1	80.5a	6.3a	9.9a	1.06a	48.2a	183.2a	87.5a
T2	81.2a	6.4a	9.5a	1.04a	45.8a	182.8a	89.1a
T3	81.2a	6.3a	10.0a	1.03a	49.3a	191.5a	96.1a
T4	81.0a	6.5a	10.2a	1.08a	47.0a	193.3a	92.1a
T5	81.2a	6.4a	9.2a	1.05a	46.9a	185.2a	93.3a
Control	81.0a	6.7a	9.6a	1.00a	48.2a	181.7a	92.6a
CV (%)	1.7	2.3	5.5	6.5	3.3	4.3	4.6
Mean	81.0	6.4	9.7	1.04	47.6	186.5	91.8

CV (%) = coefficient of variation; CQ = cup quality score; Pol = total phenolic compounds (%); TS = content of total sugars (%); Caf = content of caffeine (%); PPO = polyphenol oxidase activity (u min^{-1} g^{-1}); TTA = total tritable acidity (mL NaOH 0.1 N 100 g^{-1}). Means followed by the same letter do not differ according to Tukey's test ($P < 0.05$). T1: 100% KCl; T2: 75% KCl + 25% K2SO4; T3: 50% KCl + 50% K2SO4; T4: 25% KCl + 75% K2SO4; T5: 100% K2SO4; control did not receive K_2O.

Table S2. Results for accumulation of polyphenols, protein, total sugars and caffeine in cherry beans for the harvest of 2017/2018

Treatments	Protein	Polyphenols	Total sugars	Caffeine
	------------------------kg ha^{-1}------------------------			
T1	217a	104a	163a	17a
T2	244a	115a	171a	18a
T3	165a	80a	127a	13a
T4	207a	101a	157a	17a
T5	200a	97a	139a	16a
Control	244a	125a	178a	18a
CV (%)	19	20	17	18
Mean	213	104	156	16,5

CV (%) = coefficient of variation;

ARTIGO 2 – CORN CROPPING SYSTEM AND NITROGEN FERTILIZERS TECHNOLOGIES AFFECT AMMONIA VOLATILIZATION IN BRAZILIAN TROPICAL SOILS

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Corn Cropping System and Nitrogen Fertilizers Technologies Affect Ammonia Volatilization in Brazilian Tropical Soils

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Abstract

With the advance of the no-tillage system (NT) in Brazil, the adoption of technologies for nitrogen fertilization in these soils become essential for increasing the efficiency of N use in the system. In this sense, the objective of this study was to quantify ammonia losses, N removal in grains, and with 2nd crop yield in no-tillage and conventional (T) planting areas that received application of different N fertilizers and their technologies. Ammonia volatilization, N extraction in grains and corn yield in response to the application of conventional fertilizers were compared to urease inhibitors treated urea in NT and T systems. The treatments were: no-N (Control); Prilled urea (PU); urea + NBPT (U_{NBPT}); urea + Cu + B (U_{CuB}); ammonium nitrate (AN), and ammonium sulfate (AS). In the NT, the N-NH_3 losses were 49% greater than in the T; without differences for corn yield. The fertilizers as AN, and AS had the lowest N-NH_3 losses, regardless of tillage system. U_{NBPT} reduced the mean N-NH_3 loss by 33% compared to PU. U_{NBPT} (1,200 mg kg^{-1}) and U_{NBPT} (180 mg kg^{-1}) reduced by 72% and 22% the N-NH_3 losses compared to PU in the NT.

Keywords: no-till; urea technologies; nitrogen use efficiency; urease inhibitors

Declarations

Conflicts of interest: The authors declare that there is no conflict of interest.

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1 Introduction

Among the nutrients most used in the fertilization of corn, nitrogen (N) stands out as it is required in large amounts. It is estimated that 286 kg N are required for a corn yield of 12 Mg ha^{-1} [1], which is the average yield of corn in areas of productive potential in Brazil. Urea is the N-fertilizer source most used in corn production systems in Brazil, with values corresponding to 60% of the N used in Brazilian agriculture. However, the agronomic efficiency of conventional urea applied without incorporation is low owing to the losses of N-NH_3 by volatilization with negative impacts to the environment[2,3,4]. Aligned with worldwide trends, initiatives or guidelines related to the mitigation of N-NH_3 losses from N-fertilizers, such as conventional urea, may also increase in Brazil[5,6].

The N-NH_3 losses are higher with the use of urea without any kind of technologies, and are intensified by soil and climate conditions such as pH, CEC, humidity, temperature and level of urease activity in the soil, relative air humidity, rainfall, and presence of crop residues on the soil[7,8,9]. Considering the typical tropical conditions in Brazil, these losses can be aggravated with average losses of N-NH_3 around 30% in the varied cultivation systems[4,10,11].

Besides the propitious conditions of the tropical climate, the increase in the number of areas cultivated with corn under no-till (NT) is another key factor that favors the N-NH_3 losses. The areas under NT have increased in Brazil due to the NT advantages, such as increased organic matter content, reduced losses of soil and nutrients by erosion, and increased maintenance of soil moisture[12,13]. On the other side, in NT systems the presence of straw on the soil surface intensifies the N-NH_3 losses, particularly with the application of urea[14], being 25% greater than the N-NH_3 losses in conventional tillage systems[3]. The increase in soil organic matter content enhances the activity of urease, enzyme that operates in the hydrolysis of urea into N-NH_3 and CO_2[15,14]. Moreover, the straw prevents the direct contact between the fertilizer and the soil, reducing its incorporation by the rainwater[2,7].

Ammonia volatilization cause decline in N retention in soils or soil fertility, grain yield and N use efficiency, and in some regions in the world, can lead to environment pollution, directly reflecting in the production costs and social costs of N[16]. Thereby, in the last years, the search for technologies that reduce nitrogen losses from urea has expanded. One of the ways to prevent N-NH_3 losses consists of its mechanical incorporation to the soil[17,3,9] or by the rainwater and irrigation[2]. However, the mechanical incorporation is not a recommended practice in the NT system due to the presence of straw and the fact that soil disruption should be avoided. Besides, mechanical incorporation is rarely adopted by Brazilian farmers, even in conventional systems. Thus, the use of conventional urea without incorporation is an option that is getting less common in agricultural regions of the world, in which there are initiatives and guidelines to mitigate ammonia emissions[5].

Considering this challenge, the fertilizer industry and researchers around the world have turned their attention to the production of slow-release, controlled released or stabilized fertilizers in order to improve the N use efficiency in agriculture[3,14].

Stabilized fertilizers have additives that delay or inhibit some transformation process of N in the soil, such as the urease activity or the nitrification reaction. Several compounds such as NBPT (N-(n-Butyl) thiophosphoric triamide), NPPT (N-(n-Propyl) thiophosphoric triamide), metallic cations, boron, and organic N compounds have been studied with the aim of reducing urease activity in the soil and minimizing N losses to the atmosphere[2,18,19,5].

Thus, it is increasingly important to adopt fertilizer technologies that are able to ensure greater N use efficiency towards 4R's stewardship. The use of slow-release, controlled and stabilized N fertilizers in order to mitigate N losses in corn production systems is very relevant in an agronomic and environmental scenarios.

Thus, the hypotheses of this study were: 1) the fertilizers: ammonium nitrate (AN), ammonium sulfate (AS), urea treated with NBPT (U_{NBPT}), and urea treated with Cu and B (U_{CuB}) reduce N-NH_3 losses compared to conventional urea (PU) under no-tillage and conventional systems; 2) N-NH_3 losses from conventional urea are higher in the no-tillage system, but urea technologies or the use of ammonium nitrate and ammonium sulfate can reduce such losses. To test these hypotheses, the present study was performed in two cropping systems (conventional and no-tillage) for two years under field conditions in southeastern Brazil, aiming to evaluate technologies for N-fertilizers and their efficiency to mitigate N-NH_3 losses and improve corn nutrition and yield in the second crop season.

2 Results

2.1 Ammonia Volatilization

The daily losses of N-NH_3 in the 2017/2018 crop season varied according to the N fertilizers applied in the NT system in relation to the conventional tillage (Fig. 1).

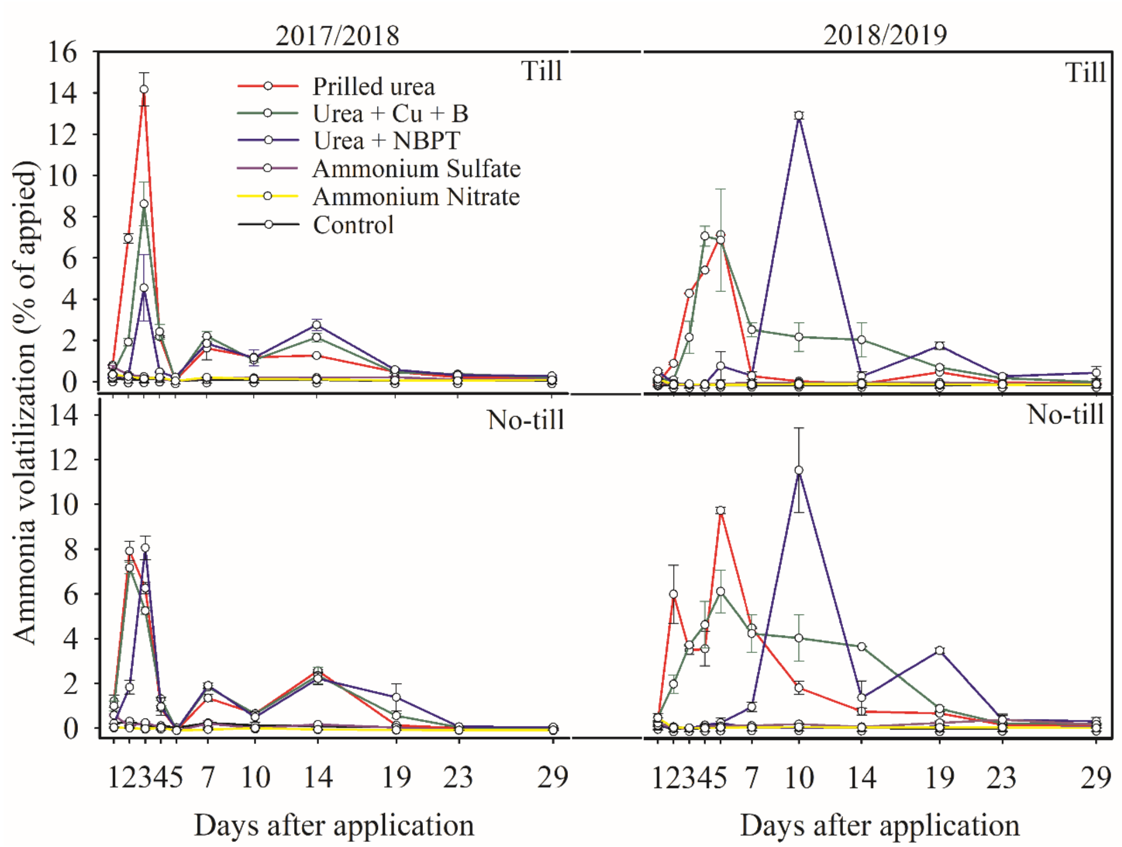

Figure 1 – Daily losses of N-NH_3 of the fertilizer sources applied as tops dressing during corn cultivation in the 2017/2018 and 2018/2019 crop seasons, in conventional tillage (T) and no-till (NT) systems.

In the conventional system, the maximum loss of N-NH_3 was 21 kg ha^{-1} at 2.4 days for PU (Table 1, Fig. 1). For the UCuB and UNBPT (180 mg kg^{-1}) treatments, the maximum losses were 6.8 and 1.6 kg N at 3.1 and 5.7 days after fertilization, respectively. The efficiency of these treatments to reduce N-NH_3 losses is evidenced when we observe the time in days for these treatments to reach 10, 20, and 50% of the maximum losses for PU, which were 1.6, 2.4, and 4.2 days for UCuB and 2.2, 5.1 for UNBPT. For 50% of the losses, UNBPT did not reach 50% of the maximum loss of PU (Table 1).

In the NT system, the maximum loss for PU was 13.6 kg ha^{-1} at 2.3 days after fertilization. The maximum loss values for the U_{CuB} and U_{NBPT} (180 mg kg^{-1}) treatments were 9.4 and 7.4 kg ha^{-1} at 2.6 and 3.3 days after fertilization, respectively (Table 1, Fig. 1). In this case, the time in days for the occurrence of 10, 20, and 50% of the maximum losses for urea were 0.1, 1.1, and 2.6 days for U_{CuB} and 0.9, 2, and 4 days for U_{NBPT} (Table 1). The AS and AN treatments had maximum losses between 2 and 7 days in both systems but with values lower than 0.5 kg N ha^{-1}.

The percentages of N-NH_3 losses that occurred in the first seven days under the conventional tillage system were 89, 79 and 60% for the PU, U_{CuB} and U_{NBPT} treatments, respectively. As for NT, these values were 82, 80 and 74 %, for the PU, U_{CuB} and U_{NBPT} treatments, respectively. Thus, more than 80% of the N-NH_3 losses occurred during the first seven days after the application of PU.

In the 2018/2019 crop season, the N-NH_3 losses were affected by the N sources applied and cropping systems (Table 1, Fig. 1). In the conventional system, the maximum N-NH_3 loss was 11.4 kg ha for PU at 3.6 days (Table 1, Fig. 1). The U_{CuB} and U_{NBPT} treatments had losses of 10.6 and 8 kg N ha^{-1} at 4.3 and 8.6 days after application, respectively. The time needed to reach 10, 20, and 50% of the maximum losses for PU were 2.3, 3, and 4 days for U_{CuB} and 7, 7.6, and 9 days for U_{NBPT}. As for the losses in the NT system, the day of maximum PU loss was similar to the conventional system (9.6 kg ha^{-1} at 3.9 days). In the U_{CuB} and U_{NBPT} treatments, the maximum losses occurred at 5 and 9 days, with values of 6.6 and 6.3 kg of N ha^{-1}, respectively. However, the time needed to reach 10, 20, and 50% of the maximum loss for PU was higher than the conventional system, with values of 1.7, 2.7, and 5 days for U_{CuB} and 7.5, 8.3, and 11 days for U_{NBPT} (Table 1). As for the 2017/2018 crop season, the AS and AN treatments had maximum loss between 1 and 14 days, but with values below 0.2 kg of N ha^{-1} (Table 1, Fig. 1).

In the conventional tillage system, PU had the higher percentage of volatilized N-NH_3 in the first seven days (92%), followed by U_{CuB} and U_{NBPT}, with 67% and 8%, respectively. As for the NT system, the accumulated losses in the first seven days were 88% (PU), 70% (U_{CuB}) and 10% (U_{NBPT}). Similarly, to the 2017/2018 crop season, the losses for PU reached approximately 90% until the 7th day relatively to the 29 days of collection.

Table 1 – Regression parameters adjusted to the accumulated losses of $N\text{-}NH_3$ by volatilization and maximum daily losses of the fertilizers under conventional and NT system

Treatment	System	Parameters				MDL (kg)	Time for volatilization of 10, 20 and 50% of the maximum losses observed for PU.		
		α	b	k	R^2		10% (day)	20% (day)	50% (day)
--Crop season 2017/2018--									
PU	NT	30.27	2.30	1.20	0.91	13.62	0.5	1.2	2.3
	T	27.17	2.41	2.10	0.97	21.39	1.3	1.7	2.4
U_{CuB}	NT	29.34	2.59	0.86	0.91	9.46	0.1	1.1	2.6
	T	18.16	3.17	1.01	0.93	6.87	1.6	2.4	4.2
U_{NBPT}	NT	24.78	3.31	0.80	0.89	7.43	0.9	2.0	4.0
	T	12.13	5.71	0.36	0.93	1.63	2,2	5.1	**
AS	NT	3.64	3.33	0.26	0.97	0.35	-	-	-
	T	2.33	2.90	0.19	0.96	0.16	-	-	-
AN	NT	1.38	3.97	0.33	0.96	0.17	-	-	-
	T	1.49	3.34	0.36	0.97	0.20	-	-	-
Control	NT	1.26	3.61	0.38	0.97	0.17	-	-	-
	T	0.84	4.03	0.35	0.97	0.11	-	-	-
--Crop season 2018/2019--									
PU	NT	30.50	3.98	0.84	0.98	9.60	1.5	2,3	4.0
	T	19.52	3.69	1.56	0.99	11.41	2.3	2.8	3.7
U_{CuB}	NT	29.08	4.98	0.61	0.98	6.65	1.7	2.7	5.0
	T	23.98	4.37	1.18	0.97	10.61	2.3	3.0	4.0
U_{NBPT}	NT	17.70	8.94	0.95	0.98	6.30	7.5	8.3	11
	T	17.02	8.57	1.26	0.99	8.04	7.0	7.6	9.0
AS	NT	0.92	4.78	0.36	0.94	0.12	-	-	-
	T	1.50	1.09	0.13	0.97	0.07	-	-	-
AN	NT	1.18	2.27	0.15	0.99	0.06	-	-	-
	T	0.89	3.90	0.16	0.98	0.05	-	-	-
Control	NT	0.62	5.07	0.25	0.98	0.05	-	-	-
	T	0.30	6.43	0.22	0.90	0.02	-	-	-

α: Asymptotic value (percentage of maximum volatilization); b: Day when the maximum $N\text{-}NH_3$ loss occurs; k: relative index and MDL (maximum daily loss); ** The maximum loss of this treatment did not reach 50% of the maximum loss for PU.

The accumulated N-NH_3 losses were affected ($P<0.05$) by the interaction between the applied fertilizers and the cropping systems in the 2017/2018 crop season. The mean accumulated losses of N-NH_3 by volatilization under NT (21.6 kg N ha^{-1}) were 49% greater relatively to the conventional tillage (14.5 kg N ha^{-1}) (Fig. 2).

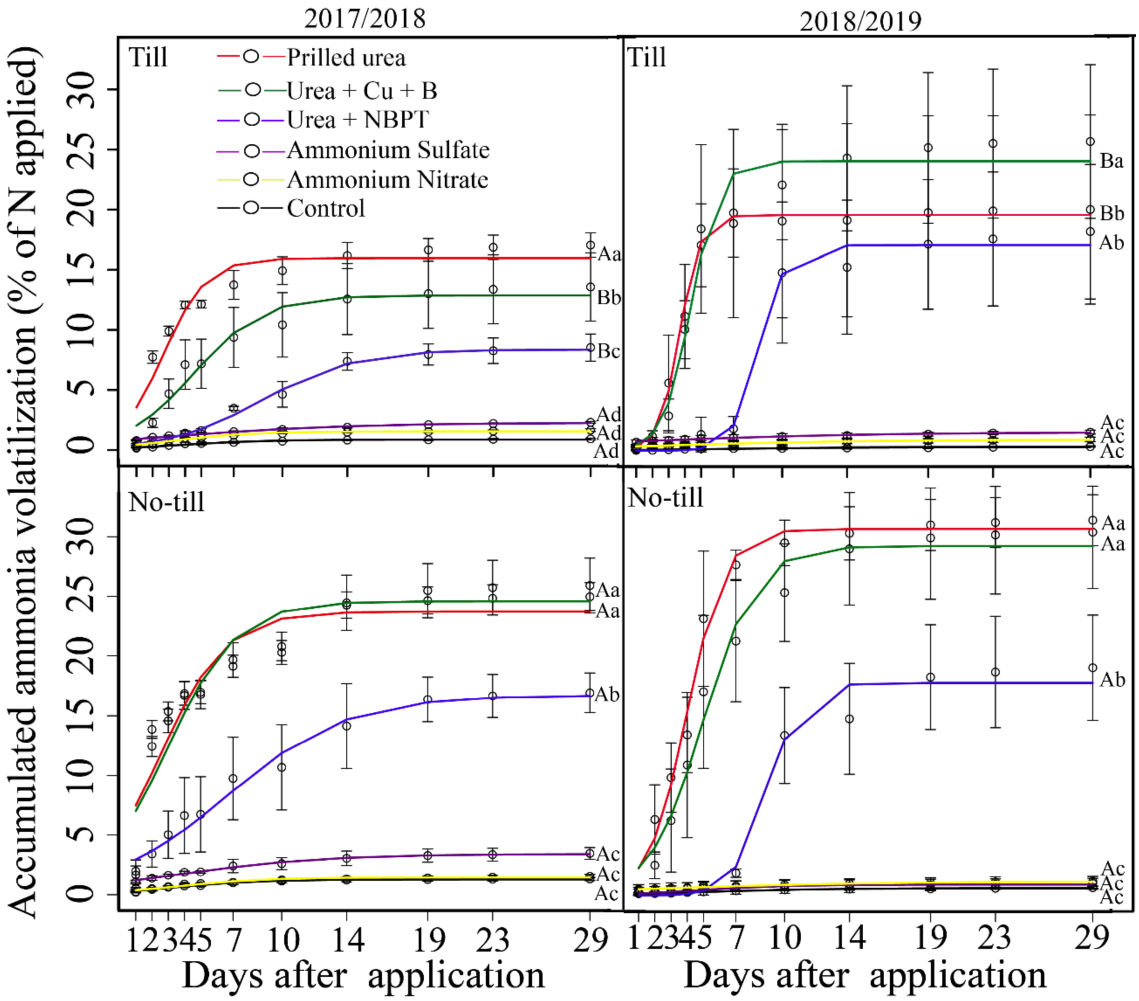

Figure 2 – Accumulated losses of N-NH_3 by volatilization per fertilizers applied as top-dressing fertilization of corn in the 2017/2018 and 2018/2019 crop seasons, under conventional tillage (T) and no-till (NT) systems. Means followed by the same upper letter (tillage system) and lower letter (sources of N fertilizer) do not differ at 5% significance level by the Scott-Knott test.

In the 2017/2018 season, the accumulated N-NH_3 losses presented the following decreasing order for the NT system: PU (33 kg ha^{-1}) = U_{CuB} (31 kg ha^{-1}) > U_{NBPT} (27 kg ha^{-1}) > AS (3.7 kg ha^{-1}) = NA (1.5 kg ha^{-1}). In the conventional system, the accumulated losses decreased as follows: PU (29 kg ha^{-1}) > U_{CuB} (19.7 kg ha^{-1}) > U_{NBPT} (12.6 kg ha^{-1}) > AS (2.4 kg ha^{-1}) = AN (1.6 kg ha^{-1}) (Table 2, Fig. 2). Observing the influence of the cropping systems on the accumulated N-NH_3 losses, only the U_{NBPT} and U_{CuB} treatments showed losses 53 and 37 % higher in the NT system than the conventional planting system.

In the 2018/2019 season, accumulated N-NH_3 losses were also affected ($P<0.05$) by the interaction between the applied fertilizers and the cropping systems. The mean accumulated N-NH_3 losses under NT were 26.2% greater than the losses observed under conventional tillage (Fig. 2).

The accumulated N-NH_3 losses for NT decreased as follows: PU (30.2 kg ha^{-1}) = U_{CuB} (31.2 kg ha^{-1}) > U_{NBPT} (19 kg ha^{-1}) > AN (1.0 kg ha^{-1}) -1) = AS (1.2 kg ha^{-1}). As for the conventional system, the accumulated N-NH_3 losses decreased in the following sequence: U_{CuB} (25.6 kg ha^{-1}) > PU (20 kg ha^{-1}) = U_{NBPT} (18 kg ha^{-1}) > AS (1.5 kg ha^{-1}) = AN (0.9 kg ha^{-1}) (Table 2, Fig. 2). The accumulated N-NH_3 losses were 15 and 36% higher in the NT system than in the conventional system for the U_{CuB} and PU treatments, whereas the other treatments did not differ. In both crop seasons and cropping systems, the accumulated losses of N-NH_3 in the AS and NA treatments were equal to the control treatment, without N application.

For a better understanding of the studied technologies within cropping systems, the losses from both crop seasons were added, and using these values, we calculated the percentage increase of the losses under NT relatively to conventional tillage (Table 2). When interpreting these results, we notice that the percentage of increase in the losses NT > T varied between 7 and 34% (Table 2).

Table 2 – Accumulated losses under NT and conventional tillage systems, accumulated losses, and percentage of increase in the losses under NT relatively to conventional tillage.

Treatment	-----2017/2018-----		-----2018/2019-----		---------Sum---------		
	NH_3 (kg ha^{-1})	% Red.	NH_3 (kg ha^{-1})	% Red.	NH_3 (kg ha^{-1})	% Red.	% >PD/PC
---------------No-till---------------							
UNBPT	27.4Ab	- 17	18.9 Ab	- 39	46.3 Ab	- 28	+ 34
UCuB	31.7 Aa	- 4	30.2Aa	- 3	61.9 Aa	- 3	+ 27
AN	1.5 Ac	- 95	1.0 Ac	- 96	2.7 Ac	- 96	+ 7
AS	3.7 Ac	- 89	1.2 Ac	- 97	4.8 Ac	- 92	+ 17
PU	32.9 Aa	------	31.2 Aa	------	64.1 Aa	------	+ 23
CV(%)	11	------	18	------	13	------	------
---------------Till---------------							
UNBPT	12.6 Bc	- 57	18.5 Ab	- 6	30.7 Bb	- 37	------
UCuB	19.8 Bb	- 32	25.6 Ba	+ 28	45.3 Ba	- 7	------
AN	1.6 Ad	- 94	0.9 Ac	- 95	2.5 Ac	- 95	------
AS	2.4 Ad	- 92	1.5 Ac	- 92	4.0 Ac	- 92	------
PU	29.0 Aa	------	20.0 Bb	------	49.0 Ba	------	------
CV(%)	11	------	18	------	13	------	

*NH_3 = losses of N-NH_3 by volatilization, % Red = percentage of reduction in the N-NH_3 losses in relation to PU, >PD/PC = percentage of increase in the N-NH_3 losses under NT relatively to conventional tillage. Means followed by the same upper letter do not differ between tillage systems, and lower letters do not differ between the studied sources.

2.2 Effects of Fertilizers and Soil Nitrogen Stocks on Nutrient Accumulation and Corn Yield

For the 2017/2018 crop season, the N extraction by the grains (Fig. S1), corn straw (straw), and total extraction (grains + straw) were not influenced ($p \geq 0.05$) by the interaction between N fertilizer and tillage system. When evaluating these factors separately, there was effect ($p \leq 0.05$) on the N extraction by the corn straw (straw) and total extraction (grains + straw) as a function of the N sources. It was observed a difference in the N extraction values only between the N sources applied in relation to the control, without N application (Fig. S1).

The extraction of N by corn grains in the 2018/2019 crop season was not affected ($p \geq 0.05$) by the interaction between sources and cropping systems, nor by the isolated effect of these factors. The mean N extraction by the grains was 182 kg of N ha^{-1} (Fig. S2 A). The N extraction by straw was not influenced by the interaction between fertilizers sources and cropping systems ($p \geq 0.05$), only by the effect of fertilizer sources (Fig. S2 A). The lowest N extraction by the straw occurred in the control treatment (44 kg ha^{-1}) (Fig. S2 A). The total N extraction (grains + straw) was influenced by the interaction between tillage system and N source ($p \leq 0.05$). The lowest total N extraction was observed in the treatments control (199 kg ha^{-1}) and UCuB (224 kg ha^{-1}) under NT; the other treatments did not differ from each other (Fig.

S2 B). Regarding the tillage systems, there was a difference only for U_{CuB}, with greater N extraction (318 kg ha^{-1}) under NT (Fig. S2 B).

Corn grain yield and production of straw were not affected by the N sources applied and the tillage system (T and NT) in both crop seasons ($p \geq 0.05$) (Fig. S3).

In the 2017/2018 crop season, the average grain yield of the N sources varied between 9,532 and 10,982 kg ha^{-1} under conventional tillage, and between 8,914 and 10,895 kg ha^{-1} under NT. The straw production varied between 6,431 e 7,513 kg ha^{-1} under conventional tillage, and between 6,124 and 6,988 kg ha^{-1} under NT (Fig. S3 B).

In the 2018/2019 crop season, the averages observed in the studied N sources ranged between 11,622 and 15,795 kg ha^{-1} under conventional tillage, and between 11,533 and 15,799 kg ha^{-1} under NT (Fig. S3 C). The average straw production in the 2018/2019 crop season ranged between 8,634 and 11,600 kg ha^{-1} under conventional tillage, and between 7,848 and 10,948 kg ha^{-1} under NT (Fig. S3 D).

3 Discussion

Observing the behavior of the evaluated N fertilizers regarding the daily losses, we noted that the highest values in both crop seasons occurred with the application of PU, approximately 2.5 days after the application in both crop seasons and cropping systems (Table 1, Fig. 1). That is because when urea is applied to the soil, without any additive or technology that reduces its solubility or the hydrolysis rate, ammonia is rapidly created in the solution and catalyzed by urease into NH_3 and CO_2[14,10]. If not incorporated, this ammonia becomes susceptible to losses by volatilization.

In our study, rainfall up to the second day after fertilization was 85 and 28 mm in the first and second year, respectively (Fig. S6). However, it is complicated to accurately inform the amount and intensity of rainfall needed to incorporate urea into the soil since the values obtained were insufficient. Similar to what was observed in the application of PU, several studies also demonstrate that the maximum daily loss of N-NH_3 occurs in the first days following the application of the fertilizers[20,21,11]. Thus, we can argue that these losses will occur in the first days for urea without any treatment or technology, as long as the moisture conditions allow the hydrolysis process. The moisture conditions do not rely only on rainfall, since a value of relative air humidity above 74.3% (critical humidity of urea at 30°C) can already start the hydrolysis process4. In our study, in the first crop season, the mean temperature was higher than 30°C, and the relative air humidity was higher than the critical humidity of urea in both crop seasons (Fig. S6). Such increased air humidity can promote increased N-NH_3 losses, even without rainfall.

The delay in the day of maximum loss observed in both systems for the stabilized fertilizers (U_{CuB} and U_{NBPT}) compared to PU is due to the inhibition mechanism of each technology. For U_{NBPT}, this reduction in urease activity is due to the ability of NBPT to be oxidized into its analog compound NBPTO, which can inhibit the urease activity by forming stable complexes with the enzyme[22]. As for U_{CuB}, urease activity is inhibited due to the binding of Cu to the sulfhydryl group. Such binding blocks the active site of the enzyme, and the urea molecule cannot bind to the sulfhydryl group. Thus, the urea hydrolysis process cannot occur[23]. The effect of B on urease inhibition diverges among different authors, but according to Santos et al.[11], the study by Benini et al.[24] provides a better explanation. These authors attribute the efficiency of B to its competitive inhibition when binding between the Ni ions of the enzyme, where the urea molecule would bind, which prevents the hydrolysis process from occurring.

The delayed urea hydrolysis when using these two technologies may favor the incorporation of fertilizers into the soil after subsequent precipitation events, which may reduce N-NH_3 losses. This effect occurred for both sources in the 2017/2018 crop season. After the 5th day, which had a 42-mm rainfall (Fig. S6), fertilizers were probably incorporated into the soil. Then, the daily N-NH_3 losses decreased from that point (Fig. 1).

The inhibition of urease by NBPT, indirectly observed by the N-NH_3 losses, occurs in varying intensities between the NT and conventional systems (Fig. 1). In our study, such behavior is evidenced by increasing the NBPT concentration in urea from 180 mg kg^{-1} to 1200 mg kg^{-1} (2017/2018) in the second year of the experiment. We noticed that the day of the maximum loss for the U_{NBPT} treatment (1200 mg kg^{-1}) under conventional tillage was delayed, occurring at the 8.5th day after application. Although it is not possible to compare the two crops under study, this represents a delay of 67% relatively to the previous crop season (Table 1, Fig. 1). When observing the day when the maximum loss occurred under NT, we noticed that there was no differences between the tillage systems (Table 1), thus we can deduce that this concentration was efficient for both cropping systems.

However, we cannot interpret this concentration (1200 mg kg^{-1}) as adequate for the treatment of urea to be used in both systems, and that is because, theoretically in the conventional tillage this concentration can be lower, which also reflects the efficient use of the inhibitor. These results show that the amount of NBPT used in the treatment of urea may need an adjustment as a function of the soil and crops conditions, that is, it is needed to generate more precise information about the relationship between the NBPT concentration and the values of urease activity.

Despite the positive results reported on the use of these metals, in the 2017/2018 crop season we observed that the maximum loss of U_{CuB} occurred in a time frame (days) similar to PU under conventional tillage (Table 1). The lower efficiency of U_{CuB} can be explained by the low concentration of micronutrients (Cu and B) in the fertilizer. Furthermore, it should be emphasized that the amount of metallic cations and compounds containing mostly B added to urea aiming the inhibition of urease should be carefully evaluated. In this study 0.3% of Cu (copper sulfate) and 0.3% of B (boric acid) were added to urea, which, considering the dose of 150 kg ha^{-1}, correspond to 450 g of Cu and B in the region of the dissolution of urea.

The fact that the other sources used in this study (AS, AN) did not promote significant daily losses is due to the N form present in the AS and AN fertilizers, and also due to their acidic reaction, which creates a less favorable environment to the N-NH_3 losses by volatilization, as previously reported in several studies[25,26,4].

The accumulated N-NH_3 losses were higher under NT than under conventional tillage in both crop seasons (Fig. 2). This occurred as a result of the greater presence of crop residues (straw) in this system, which favors the rapid hydrolysis of the fertilizer due to the increased urease activity[27,28]. Moreover, the crop residues present in the NT system reduce the diffusion of urea in the soil by reducing the contact urea/soil and preserving soil moisture[15,2,7].

Our findings demonstrate alternatives to reduce urease activity and N-NH_3 losses in NT systems, which would be the use of technologies that enhance urea use efficiency. Thus, U_{NBPT} stands out as the best technology as it reduced, on average, 28% of losses relatively to PU in NT for both crop seasons (Table 2). Another technology that may be used is the coating with metallic ions and compounds containing B. However, Cu and B concentrations deserve further investigation since, in our study, the reduction of N-NH_3 losses was only 3% on average for both crop seasons (Table 2).

Owing to their acidic reaction, the AS and AN sources presented the lowest accumulated losses. The accumulated losses observed for these sources (lower than 0,5%) are already reported in several studies conducted in soils cultivated under this pH range[29,2,4]. These values of N-NH_3 losses quantified for AS and AN are not from these fertilizers, since they are equal to the values observed in the soil without N application. This shows that these losses occur naturally even in the control treatment without N fertilization.

The reductions in N-NH_3 losses by volatilization were not followed by expressive increases in N extraction by corn. In both crop seasons, the low extraction by corn straw in the control treatment (Fig. S1, S2) is due to the absence of N fertilization.

The grain yield in both crop seasons (Fig. S3) was twice the average Brazilian yield (5029 kg ha^{-1})[30]. The absence of responses regarding treatments and cropping systems is related to the high N supply by the soil (Tables 5 and S2). This is due to the increased N stock in the soil (67 kg ha^{-1} on average) and also to the potential of N mineralization in the soil (60 kg ha^{-1} $year^{-1}$ on average), which has been under NT for at least 15 years (Table S1).

Other results on grain yield, N extraction by the grains and straw show that there is reduction of ammonia losses with the use of inhibitors and other technologies for fertilizers, but these losses were not followed by increased N extraction by the grains, straw and yield[31,14,32], although they did not estimate the potential supply of N by the soil organic matter.

In order to explain this absence of response to the N fertilization, interpreting the data on table S2, we can observe that this soil had the potential supply of approximately 100 kg N ha^{-1} in the 2017/2018 crop season and proximately 156 kg N ha^{-1} in 2018/2019; thus, the application of 150 kg N ha^{-1} will hardly present a response in yield. These results show that, from an economic point of view, the reduction of losses has little effect on crop productivity, however, the maintenance of N in the soil organic matter is as important as the increase in crop productivity. In addition to N being stored in the soil for future crops, this maintenance of N in soil organic matter mitigates the emission of greenhouse gases into the atmosphere.

3 Conclusions and future perspectives

The technologies for urea reduce the N-NH_3 losses compared to PU in both studied systems, and the losses under NT are higher than in conventional systems. Urea treated with NBPT (1200 mg kg^{-1}) is an option of technology for the efficient N use in grain production systems under NT, as it causes a 5-day delay in the day of maximum loss compared to urea treated with NBPT (180 mg kg^{-1}). The use of ammonium nitrate and sulfate also represent adequate choices to reducing the N-NH_3 losses in grain production systems. In the present study, a reduction of N-NH_3 losses does not directly reflect an increase in yield and N extraction by corn.

Based on the results observed in this study, we noticed that the NBPT concentration to be used in soils under NT should be adjusted. Thus, studies that evaluate increasing NBPT concentrations in NT systems will be performed by our research group in order to better define the NBPT dose in formulations according to the varying conditions of grain production in tropical regions.

4 Methods

4.1 Preparation and characterization of the used fertilizers

In the 2017/2018 crop, the N sources were: Urea treated with NBPT, with 46% N and 180 mg NBPT kg^{-1} (U_{NBPT}); 2) Prilled Urea, with 46% N (PU); 3) Urea treated with Cu and B, with 43% N, 0.3% Cu, and 0.3% B (U_{CuB}); 4) Ammonium nitrate, with 33% N (AN), and 5) Ammonium sulfate, with 19% N and 22% S (AS). All fertilizers were purchased from a fertilizer store. As for the 2018/2019 crop season, the U_{NBPT} was treated in the laboratory since the NBPT concentration in the fertilizer obtained in the 2017/2018 season was lower than that described in the commercial fertilizer (530 mg kg^{-1}). The other fertilizers were obtained from a fertilizer store.

The treatment of urea with NBPT used in the 2018/2019 crop season was performed at the Laboratory of Technologies for Fertilizers at the Federal University of Lavras. For that, a solution including diethanolamine (CAS number 111-42-2) (70 %) and NBPT (30%) was prepared. From this solution, 8.6 g were taken and homogenized with 2 kg of granular urea in a bench top mixer. Afterwards, the NBPT concentration was determined by high-performance liquid chromatography (HPLC), model HP1100 Agilent with diode-array detection (DAD)[33], which was 1,200 mg kg^{-1}.

4.2 Site Description and Management Practices

Two experiments with corn (*Zea mays*), hybrid 2B-512PW of the Dowscience® company were performed during the second crop season of 2017/2018 and 2018/2019, after the cultivation of soybean (*Glycine max*), in Medeiros and Bambuí, Minas Gerais state, Brazil (20°07’00” S, 46°09’55” W and 20°06’47” S, 46°10’00”W, respectively (Fig. 3).

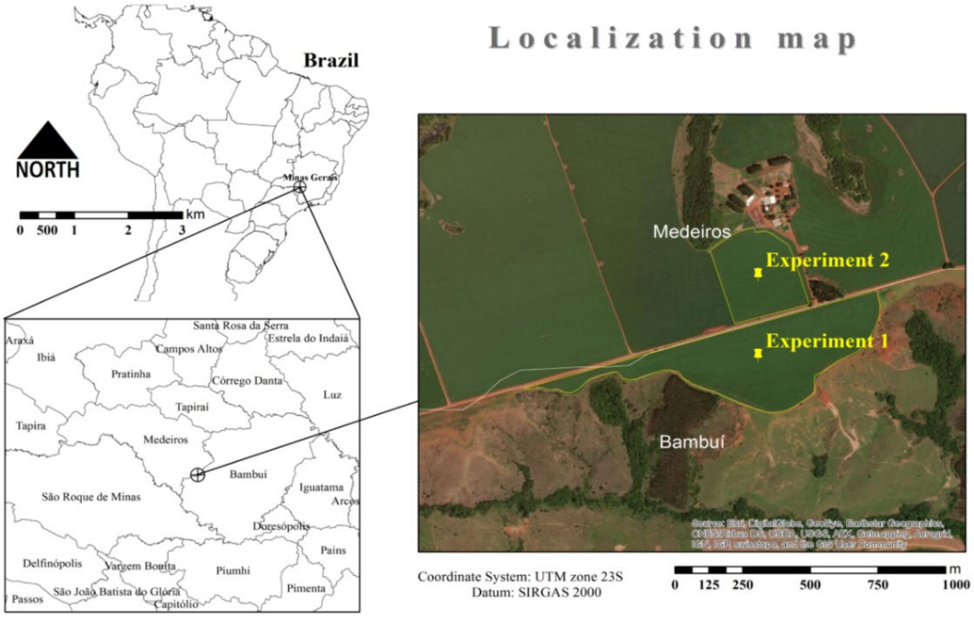

Figure 3 – Location of the experimental areas, Experiment 1 (crop season 2017/2018) and Experiment 2 (crop season 2018/2019).

The experiments were installed in a slope within a hilly region, in a soil classified Acrudox[34].

The information regarding the main characteristics of the sites and crop seasons are summarized on table 3.

Table 3 – Characteristics of the experiments performed in the 2017/2018 and 2018/2019 crop seasons

Characteristics	**2017/2018** Medeiros, Minas Gerais State, Brazil.	**2018/2019** Bambuí, Minas Gerais State, Brazil.
Soil type	Acrudox	Acrudox
Latitude	20°07'00" S	20°06'47" S
Longitude	46°09'55" W	46°10'00"W
Annual average temperature (°C)	20.3	21.3
Average annual precipitation (mm)	1,457	1,369
Acumulated precipitation (mm)$^{(a)}$	134.5	155.5
Total N (kg ha^{-1}, 0 – 0.20 m)	2,330 (T); 2,024 (NT)	2,250 (T); 1,765 (NT)
NO_3^-(kg ha^{-1}, 0 – 0.20 m)	24.6 (T), 18.7 (NT)	55.75 (T), 62 (NT)
NH_4^+ (kg ha^{-1}, 0 – 0.20 m)	8.2 (T), 27.7 (NT)	44 (T), 36.5 (NT)
pH (0 – 0.20 m)$^{(b)}$	5.5 (T), 5.6 9 (NT)	5.9 (T), 5.8 (NT)

$^{(a)}$Accumulated after 29 days of fertilization $^{(b)}$ pH in water 1:2.5 (v/v).

4.3 Cropping Systems and Field Management

The rationale of this study emerged after reading some papers previously published in the scientific literature. Table 4 lists the main results on the subject found in the scientific literature.

Table 4 – Ammonia (N-NH_3) losses in no-till (NT) and till (T) till systems

Crops	Fertilizers and N rates	NT	T	References
		N-NH_3		
Corn	Urea (60 kg N ha^{-1})	3	2.3	34
Rice	Urea	24.8	0.63	45
	Coated urea Cu + B (120 kg N ha^{-1})	11.6	0.01	
Corn 28 years	Urea (160 kg N ha$^{-1)}$	12.7	2.1	15
Camelina sativa	Urea	0.51	0.51	21
L. 20 years	Urea + NBPT (90 kg N ha^{-1})	0.28	0.29	
Wheat/Wheat	Diammonium phosphate (80 kg N ha^{-1})	16.8	16	46
	Urea + ammonium nitrate (120 kg N ha^{-}	10.4	10	
Corn	Urea	18	-	31
	Urea + Cu + B (100 kg ha^{-1})	11		
Corn	Urea	21.1	-	2
	Urea + Cu + B (150 kg ha^{-1})	17.3		
Corn 20 years	Urea	22.0	-	14
	Urea + NBPT (200 kg ha^{-1})	4.4		
Corn 20 years	Urea	26.0	-	4
	Urea + NBPT (150 kg ha^{-1})	5.4		

Since the aim of this study is the comparison between the N fertilizers and their technologies, and also the influence of the tillage systems on the N-NH_3 losses by volatilization, we decided to simulate the conventional tillage within a NT area that had approximately 15 years of implantation. To simulate the conventional tillage in the NT area, the straw was manually removed from the plots designed to represent the conventional tillage, and the soil was plowed up to 20 cm depth in the 2016/2017 and 2017/2018 summer crop seasons (Fig. S4).

Thus, corn sowing (second crop season) was performed after soybean cultivation in the summer for both years. Before the sowing, soil samples were collected for chemical and physical characterization. Six composite samples were collected, obtained from a homogenous mixture of ten simple soil samples collected at the 0-0.05, 0.05-0.10 and 0.10-0.20 m soil depths. The clay, silt, and sand content values were 40, 31, 29% and 44; 36; 20% for the 2017/2018 and 2018/2019 crop seasons, respectively, and the results of the chemical analysis are presented on tables 5 and S1.

Furthermore, soil samples were collected to determine bulk density, and stocks of total N (Ntotal), total C (Ctotal), and mineral N (Nmineral). Soil bulk density was determined by the core method[35]. Total N was determined by the Kjeldhal method[36]. The mineral N was determined by extraction with 1 mol L^{-1} KCl and magnesium oxides and devarda's alloy[37]. The stocks of total N, mineral N, and total carbon from each soil depth were calculated according as described in Santos et al.[11] (Table 5).

Table 5 – Soil organic carbon and nitrogen contents and carbon and nitrogen stocks at different soil depths in conventional tillage (T) and no-till (NT) systems in the 2017/2018 and 2018/2019 crop seasons.

Sist.	Depth	OC	TN	C/N	$N\text{-}NH_4^+$	$N\text{-}NO_3$	BD	$E_{N.T.}$	$E_{C.O.}$	E_{NH4}^+	E_{NO3}^-	E_{NM}
						2017/2018 Crop season						
	cm	--g kg^{-1}--			---mg dm^{-3}------		kg dm^{-3}	------------------kg ha^{-1}-----------------				
T	0-5	17	2.5	6.8	13.9	29.0	0.9	1,125	7,650	6.6	13.8	20.4
	5-10	18	3.3	5,4	32.3	92.6	1.1	1,849	9,900	18.1	51.8	70.0
	10-20	18	2.8	6,4	6.6	14.4	1.1	3,139	19,800	3.8	16.4	20.2
	0-20	18	2.8	6,4	29.8	75.2	-----	**2,330**	**14,287**	8.0	24.5	**32.5**
NT	0-5	25	2.8	9,0	45.1	38.9	1.2	1,656	15,000	26.5	22.8	49.3
	5-10	22	2.1	10.4	50.8	13.6	1.2	1,231	13,200	29.5	7.9	37.4
	10-20	19	2.3	8.2	50.1	6.1	1.1	2,606	20,900	27.4	6.7	34.1
	0-20	21	2.4	9.0	98.1	32.3	-----	**2,024**	**17,500**	27.7	11.0	**38.7**
						2018/2019 Crop season						
T	0-5	14	3.9	3,6	40.9	39	1.0	1,955	7,000	21	19	40
	5-10	16	2.9	5,5	141	81	1.0	1,465	8,000	69	40	109
	10-20	14	2.8	5,0	42.2	80	1.0	2,790	7,000	43	82	125
	0-20	14	3.1	4,5	66.6	71	-----	**2,250**	**7,250**	44	55.7	**99.7**
NT	0-5	21	2.2	9,5	35.8	67	1.0	1,450	10,500	18	34	52
	5-10	16	1.9	8,4	75.8	80	1.1	1,050	8,800	42	44	85
	10-20	11	1.9	6,0	36.5	72	1.2	2,280	13,200	43	85	128
	0-20	15	2.0	7,5	51.2	81	-----	**1,765**	**11,425**	36.5	62	**98.5**

Sist.: tillage system, OC = organic carbon, TN = total nitrogen, C/N = carbon to nitrogen ratio, BD = bulk density determined by the core method; E_{NT} = total nitrogen stock, E_{CO} = organic carbon stock, E_{NH4}^+ = nitrogen stock as ammonium, E_{NO3}^-= nitrogen stock as nitrate, E_{NM} = mineral nitrogen (E_{NH4+} + E_{NO3})

4.4 Estimate of N Mineralization in the Soil

We were interested in monitoring the behavior of N and their Technologies when applied in both tillage systems. For that, we decided to estimate the mineralization of N in both tillage systems. The objective was to perform a complete characterization of the studied areas and also explain some behaviors of the tillage systems in relation to the evaluated agronomic parameters. For this, the estimation of N mineralization was performed as proposed by Brady and Weil[38], with adaptations described in Santos et al.[11].

The data used on this estimate can be found on table 5, and since both experiments were conducted in soils with clayey texture under tropical conditions, we adopted the value of 3% of annual N mineralization, as proposed by Brady and Weil[38]. The results of the estimate of N mineralization in the studied soils are presented on table S2.

4.5 Treatments and Experimental Design

The experiments consisted of fourteen (12) treatments setup in a 6 x 2 factorial scheme (N fertilizers and their technologies applied in the soil as top-dressing fertilization: 1) prilled urea (PU), 2) urea treated with NBPT (N-(n-butyl) thiophosphoric triamide), 3) urea + Cu + B (U_{CuB}), 4) ammonium nitrate (AN), 5) ammonium sulfate (AS), and 6) without N application – control; and tillage systems management for corn cultivation: conventional tillage (T) and no-till (NT) (Fig. S5).

The sowing of corn in the 2017/2018 crop season was performed along with the application of 18 kg N ha^{-1} and 11.4 kg P_2O_5 ha^{-1} (formula 27-17-00). In the 2018/2019 crop season, the sowing was performed along with 18 kg N ha^{-1} and 32 kg P_2O_5 ha^{-1} (Bulk blend of fertilizers 14-25-00).

The spacing between rows was 0.75 m, totaling 55,000 plants per hectare. Each experimental plot consisted of six sowing rows with 5 m length each. 150 kg N ha^{-1} were applied via top dressing fertilization. Fertilizers were applied in the sowing lines at a distance of approximately 10 cm from the plant collar. The three central meters and three central lines of each plot (6.75 m^2) were considered the useful plot.

4.5.1 Ammonia Volatilization

To quantify the N-NH_3 losses, PVC collectors were used as described by Nönmik[39], and adapted by Lara-Cabezas et al.[40]. As a support of the collectors, three bases of PVC tubes were installed in each experimental plot at a distance of 10 cm from the corn sowing row. The bases had 12 x 20 x 5 cm (diameter, height, and depth in the soil).

After the application of the treatments in the bases, $N\text{-}NH_3$ collectors with dimensions 50 x 12 cm (height and diameter, respectively) were installed. Two sponges (0.02 g cm^{-3} density) soaked with phosphoric acid solution (60 ml L^{-1}) and glycerin (50 ml L^{-1}) were placed inside each collector. The sponge located in the upper part of the collector meant to prevent the contamination of the lower sponge with gases from the atmosphere, whereas the sponge at the lower part was used to absorb the ammonia volatilized. In order to reduce the spatial variability of the $N\text{-}NH_3$ losses, and to simulate the field conditions, such as temperature and precipitation, the collectors were alternated between the three bases. Thus, after each collection of sponges, the collector was changed from its base.

The $N\text{-}NH_3$ collections were carried 1, 2, 3, 4, 5, 7, 10, 14, 19, 23 and 29 days after the application of the treatments in the top-dressing fertilization of corn. The solution in sponges collected in the field was extracted and analyzed as described in Santos et al.[11].

After calculating the N levels in the samples, the obtained value (corresponding to the area occupied by the base with the chambers installed in the field) was extrapolated to the percentage of $N\text{-}NH_3$ loss per hectare. To calculate the accumulated losses during the 29 days, losses from the 1st and the 2nd day were added; the sum of these added to the 3rd day and so on. During the period of evaluation of $N\text{-}NH_3$ losses by volatilization, the climate data were collected by the automatic weather station from the Ministry of Agriculture (MAPA), located in Bambuí, Minas Gerais State, Brazil.

4.5.2 Weather Conditions

Data on rainfall, relative air humidity, and maximum and minimum temperature were recorded by the meteorological station of the farm. Data were collected throughout the entire period of evaluation of $N\text{-}NH_3$ losses by volatilization. Rainfall, maximum and minimum temperature values, and relative air humidity after 29 days of the application of top-dressing N fertilization in both experiments in Medeiros e Bambuí in the 2017/2018 and 2018/2019 crop season are presented on Figure S6.

In the 2017/2018 crop season, precipitations of 45, 18, 10 e 42.5 mm occurred in the first seven days after the application of the N fertilizers, totaling 115.5 mm of precipitation; the average temperature was 23.5 ºC. As for the 2018/2019 crop season, precipitations of 7.5, 24, 11, 41 e 5 mm occurred in the first seven days after the application of the treatments, totaling 80.5 mm; the average temperature was 23 ºC. During the entire growth cycle of the corn, the precipitation was 435 mm and 372 in the 2017/2018 and 2018/2019 crop seasons, respectively.

4.5.3 Nitrogen Accumulation and Corn Yield

When the corn grains reached the physiological maturity, the corn cobs were harvested and separated from the culm and leaves (which correspond to the straw). The grains were removed from the cobs using a thresher and afterwards, the grain moisture was quantified using a Gehaka® equipment G600 for subsequent correction of moisture to 13%. Then, this value was extrapolated to represent the grain yield in kg ha^{-1}. From this sample of grains, a subsample was taken and oven dried at 65°C for subsequent analysis of N content in the grains.

To estimate the straw production, the samples were weighed, grinded in a forage harvester, and had subsamples taken for determination of moisture content. Afterwards, the results were extrapolated to production of straw per hectare, and the values were given in kg ha^{-1}. Similar to the grains, subsamples of straw were dried and grinded in a Willey mill for analysis of N content by the Kjeldahl method[36] and following the methodology described by Tedesco et al.[37].

4.6 Statistical Analysis

The treatments were submitted to a non-linear regression analysis using a logistic model to evaluate the losses of ammonia by volatilization, equation (1):

$$Yi = \left[\frac{\alpha}{1 + e^{k}(b - daai)} \right] + Ei$$

Equation 1

in which, Yi is the i-th observation of the accumulated loss of $N\text{-}NH_3$ in %, being $i = 1, 2, \ldots, n$; daa_i is the i-th day after the application of the treatment; α is the asymptotic value that can be interpreted as the maximum amount of accumulated loss of $N\text{-}NH_3$; *b* is the abscissa of the inflection point and indicates the day when the maximum loss by volatilization occurs; *k* is the value that represents the precocity index, and the higher its value, the lower the time needed to reach the maximum loss by volatilization (α); E_i is the error associated to the i-th observation, which is assumed to be independent and equally distributed according to a zero average standard and constant variance, $E \sim N(0, I\sigma^2)$.

This model has been largely applied to estimate plant growth, and recently, has been used to estimate the accumulated loss of $N\text{-}NH_3$[40,19,42].

To estimate the maximum daily loss (day when the highest loss of $N\text{-}NH_3$ occurred), that is, to determine the inflection point of the curve, it was used the following equation (2):

PMD = *k* x (α/4) Equation 2

in which, k is a relative index used to obtain to maximum daily loss (MDL), and α is the asymptotic value that can be interpreted as the maximum amount of accumulated loss of N-NH_3.

Analysis of variance was applied to test the influence of the fertilizers in the parameters: accumulated losses of ammonia by volatilization at the end of the evaluation days, grain yield, straw production, and N removal. The significance of the differences was evaluated in $P \leq 0.05$, and after validating the statistic model, the mean values were grouped by the Scott-Knott algorithm using the R software 3.3.1[43].

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Supplementary material

Table S1 – Soil chemical attributes before the installation of the experiment with 2017/2018 (1º) and 2018/2019 (2º) crop seasons.

Attributes	**Till**						**No-till**					
	Soil depth (cm)											
	0-5		**5-10**		**10-20**		**0-5**		**5-10**		**10-20**	
	1º	2º	1º	2º	1º	2º	1º	2º	1º	2º	1º	2º
pH (H_20)	5.4	6.2	5.4	6.1	5.1	5.7	6.0	6.9	5.8	5.1	5.3	5.7
P mg dm^{-3}	9.9	6.3	10.9	8.1	11.3	9.9	7.9	4.1	13.6	4.9	10.3	1.2
K mg dm^{-3}	175	88	172	100	152	110	225	154	225	112	170	55
Ca^{2+} cmol$_c$dm^{-3}	3.6	2.7	3.2	3.0	3.1	2.5	5.3	4.7	4.7	2.9	3.5	1.4
Mg^{2+} cmol$_c$ dm^{-}	1.0	0.8	1.0	0.8	0.8	0.7	1.6	1.5	1.4	0.8	1.0	0.3
CEC cmol$_c$dm^{-3}	7.7	6.3	7.5	6.9	7.5	6.7	9.3	8.4	9.0	6.7	7.8	1.8
OM dag kg^{-1}	3.0	2.6	3.1	2.7	3.1	2.6	4.4	3.7	3.8	2.8	3.3	2.0
NOM dag kg^{-1}	8.3	15.0	10.6	9.3	9.0	10.7	6.3	5.9	5.5	6.8	7.0	9.5
O.C.	1,7	1,4	1,8	1,6	1,8	1,4	2,5	2,1	2,2	1,6	1,9	1,1
P-rem mg L^{-1}	11.6	13	11.1	12	10.4	11.7	11.6	10.5	12.8	8.9	11	5.8

pH in water 1: 2.5 (v/v); Soil available K and P contents extracted by the Mehlich-1 solution; Exchangeable Ca^{2+}, Mg^{2+}; OM: Organic matter determined by the modified Walkley–Black method; NOM: Nitrogen in soil organic matter OC: organic carbon determined by the modified Walkley–Black method, P-rem: Remaining P; CEC: Cation exchange capacity at pH 7.

Table S2 – Estimate of the annual mineralization and total availability of N in the studied areas.

Crop Season	System	Depth	Mineralized N[a] (kg ha^{-1} year^{-1})	Mineral N[b] (kg ha^{-1})	Available N[c] (kg ha^{-1})
2017/2018	NT	0-5	50	49	99
		5-10	37	37	74
		10-20	76	34	110
		Total	**59.7**	**38.5**	**98.2**
	T	0-5	33	20	53
		5-10	54	70	124
		10-20	92	20	112
		Total	**67.7**	**32.5**	**100,2**
2018/2019	NT	0-5	32	52	84
		5-10	31	85	116
		10-20	68	128	196
		Total	**49.7**	**98.2**	**147.9**
	T	0-5	58	40	98
		5-10	37	109	146
		10-20	83	125	208
		Total	**65.2**	**99.7**	**164.9**

[a] Estimate of the annual N mineralization, [b] Data compiled from table 2, referring to the sum N-NH_4^+ and N-NO_3^-, [c] Potentially available nitrogen, since it will depend on the mineralization rate.

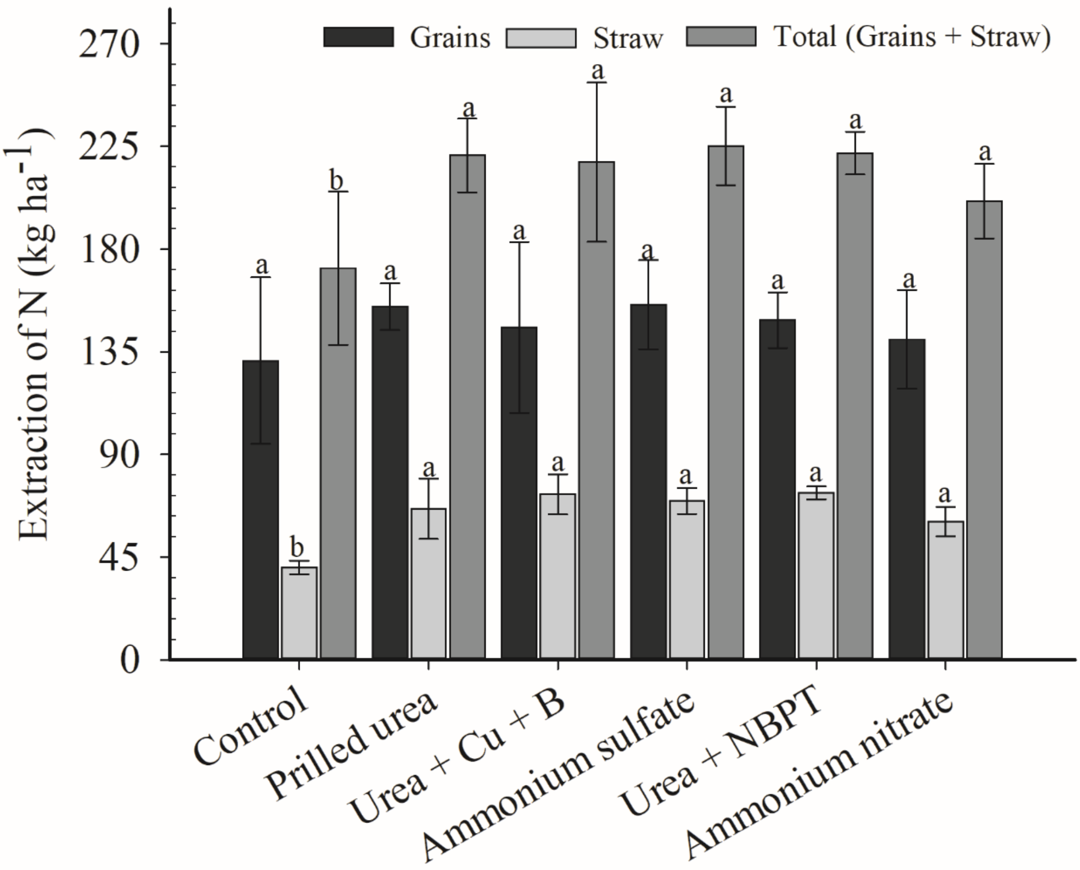

Figure S1 – Nitrogen extraction by the corn grains, shoot dry matter (straw), and total dry matter of corn that received N fertilization in the 2017/2018 crop season. *Treatments followed by the same letter do not differ at 5% significance level by the Scott-Knott test. The vertical bars indicate the standard error of the mean (n=3).

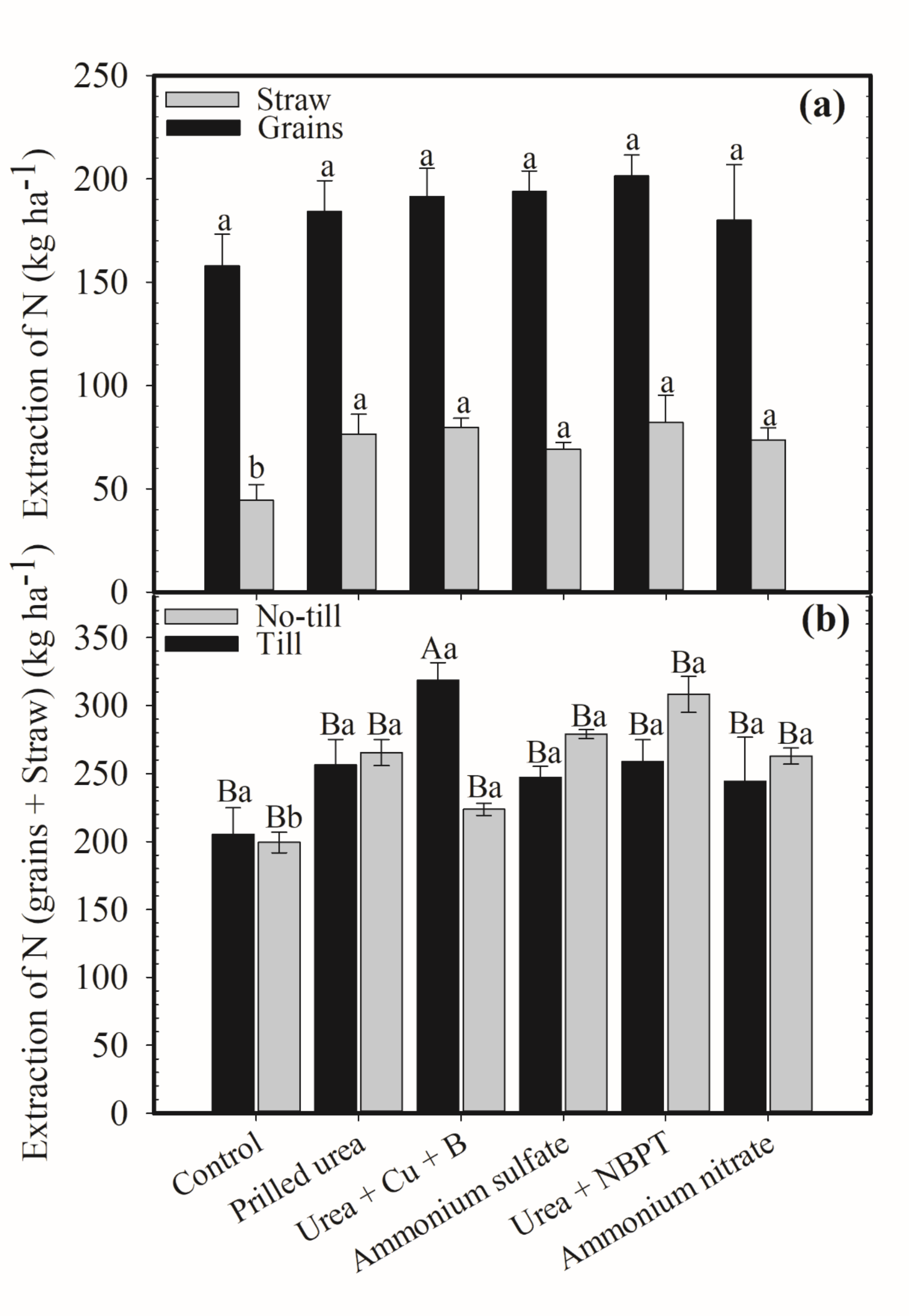

Figure S2 – Nitrogen extraction by the corn grains, shoot dry matter (straw) (a), and total dry matter of corn that received N fertilization (b) in the 2018/2019 crop season. *Treatments followed by the same letter do not differ at 5% significance level by the Scott-Knott test. The vertical bars indicate the standard error of the mean (n=3).

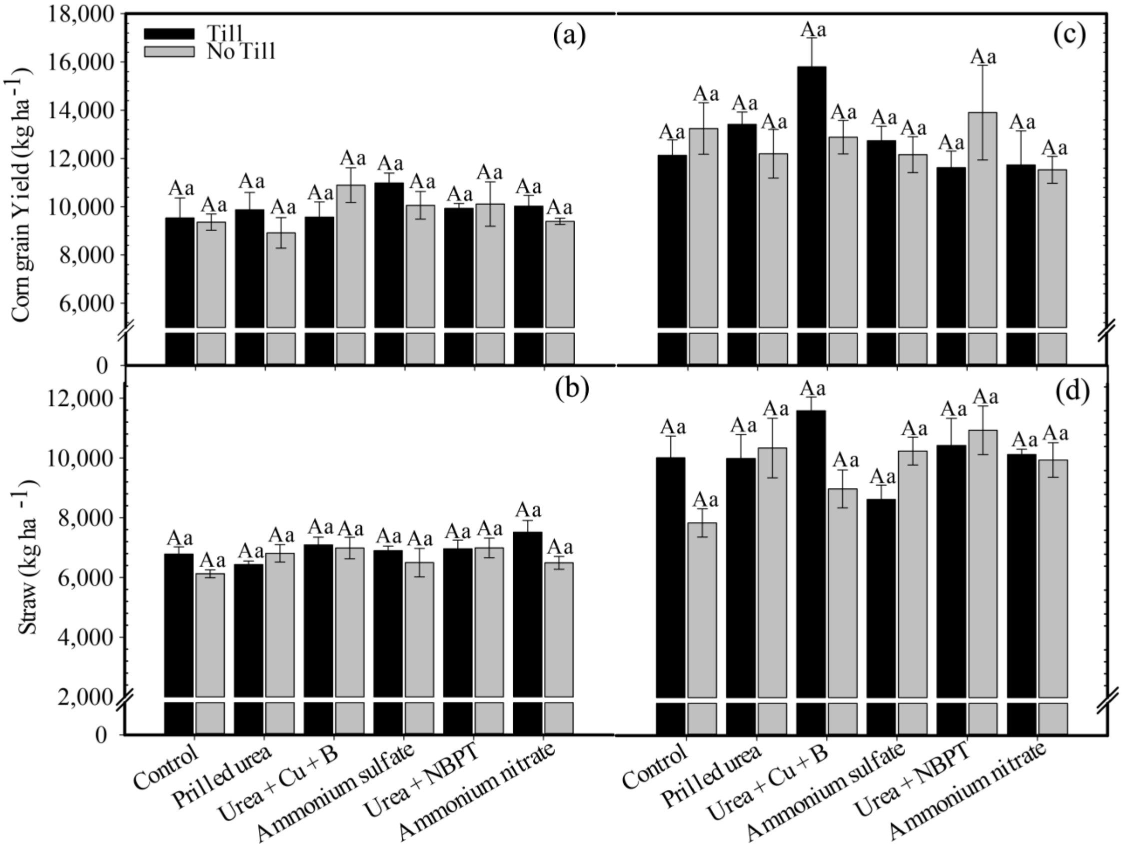

Figure S3 – Corn grain yield and straw production in the 2017/2018 (a and b) and 2018/2019 (c and d) crop seasons that received N top dressing fertilization. Treatments followed by the same upper letter in the bars do not differ within tillage systems (NT and T), and followed by the same lower letter do not differ within N sources at 5% significance level by the Scott-Knott test. The vertical bars indicate the standard error of the mean (n=3).

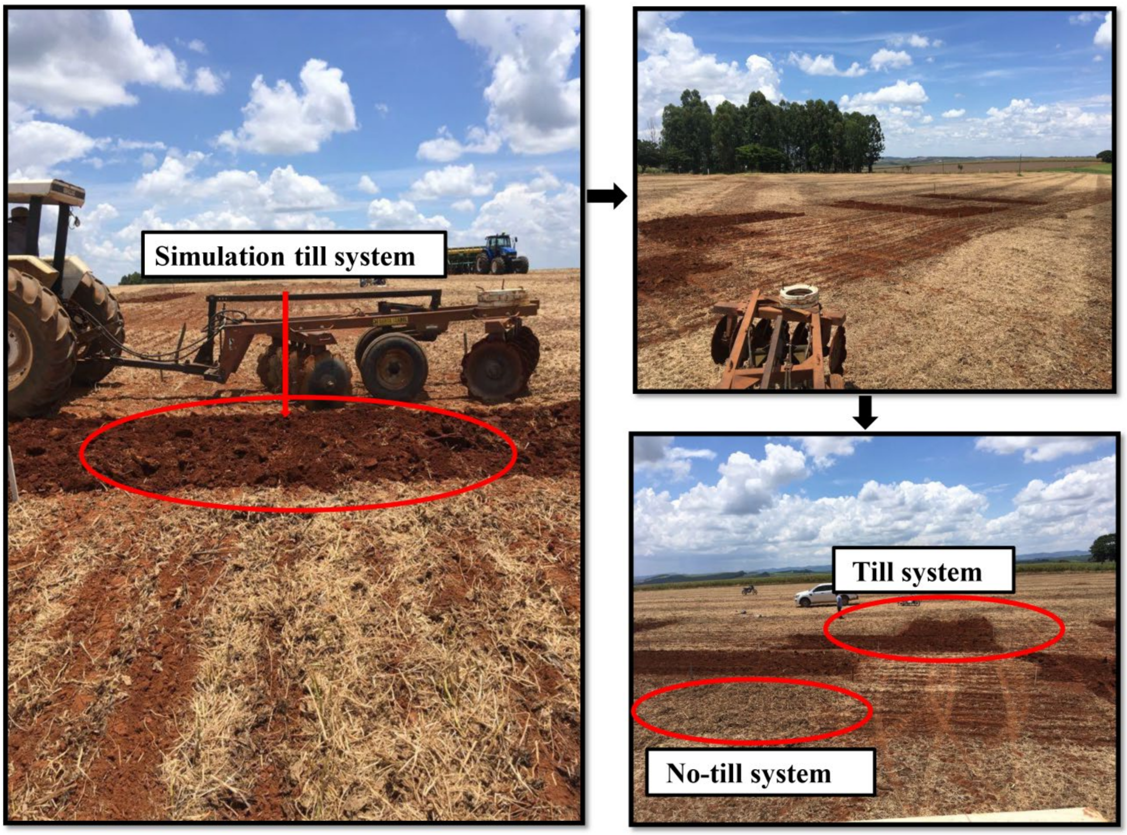

Figure S4 – Scheme of the simulation of conventional tillage within a no-till area before the sowing of soybean.

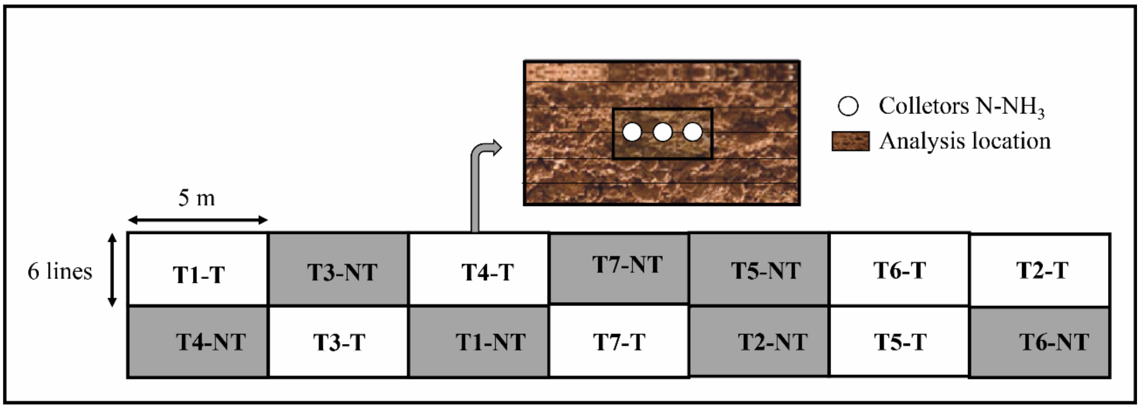

Figure S5 – Design of the distribution of treatments in field, referring to only one block of the experiment. NT: no-till system, T: conventional tillage system, T1: control, T2: PU, T3: UNBPT, T4: UCuB, T5: AN, and T6: AS.

Figure S6 – Rainfall, maximum and minimum temperatures, and relative air humidity during the evaluation period of the losses of N-NH3 by volatilization in the 2017/2018 (a) and 2018/2019 (b) crop seasons.