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MAIZE-BASED SYSTEMS AS AFFECTED BY FORAGE GRASS AND NITROGEN FERTILIZATION: ELUCIDATING ^{15}N-FERTILIZER RECOVERY, GREENHOUSE GAS EMISSIONS, N-CYCLE FUNCTIONAL GENES IN SOIL AND CROP YIELDS

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ABSTRACT

Due to the interest in N use efficiency (NUE) and sustainable agricultural systems, the adoption of integrated systems, such as the intercropping of maize with forage grasses can be of great relevance, allowing the use of the land throughout the year, besides avoiding losses of N through nitrate (NO_3^-) leaching, nitrous oxide (N_2O) emissions, ammonia volatilization (NH_3), and immobilization. Tropical forage grasses of the genus *Megathyrsus* and *Urochloa* can suppress soil–nitrification by releasing inhibitory substances, reducing N losses and increasing fertilizer N recovery of the cash crop in rotation. In this way, understanding the N transformations in the soil by microorganisms and the fertilizer recovery in the system are very important. Firstly, the first two chapters are about a 3-year (2014-2017) field experiment conducted in southeastern Brazil, were forage grasses Guinea grass (Megathyrsus maximus cv. Tanzânia), palisade grass (Urochloa brizantha cv. Marandu), and ruzigrass (Urochloa ruziziensis cv. Comum) were cultivated in rotation with maize for grain in summer, to analyze the influence of forage grass and N fertilization in each study. In first chapter, maize was fertilized with 140 kg ha^{-1} N as $(^{15}NH_4)_2SO_4$ or not fertilized, and recovery of residual ^{15}N was quantified in the second season. In second chapter, the change was that the N source used was ammonium sulphate not labeled, and were analyzed nitrous oxide (N_2O), methane (CH_4), and NH_3 emissions from the system. In the third and fourth chapter, maize was intercropped with the same grasses previously mentioned. The N rates were 90, 180 and 270 kg ha^{-1} N and treatments without N fertilization. The objective was also to ascertain the effect of grasses and N fertilization from the analyzes carried out. The third chapter characterized the changes in N-cycle genes in the soil and measured the N_2O emissions. The fourth chapter assessed maize grain yield and forage production, bromatological quality, and estimated meat production. In the first season after ^{15}N application, 21%, 65%, and 33% of the N in maize grain, stover, and shoots, respectively, was derived from fertilizer. In the next season, of the total N found in maize grain, stover, and shoots, 2.2%, 1.9%, and 2.0%, respectively, was derived from the residual fertilizer applied in the previous year. There were no differences between forage grass species in the amount of ^{15}N recovered by maize, soil, and total N. In the first season of maize in rotation with forage grasses, Guinea grass, palisade grass, ruzigrass did not affect N_2O and NH_3 emission due to their apparent inability to suppress soil nitrification. However, N fertilization slightly increases cumulative N_2O emission. In maize intercropping with grasses, N fertilization increases the abundance of AOB (*amo*A of bacteria) more than AOA (*amo*A of archaea). N_2O emission was influenced by AOB, water-filled pore space (WFPS) and N fertilization. Nitrogen fertilization positively affects forage growth and nutritional quality, resulting in a higher maize grain yield, higher forage production and quality, and eventually higher estimated meat production. Moreover, Guinea grass resulted in the highest estimated meat production when fertilized with 270 kg ha^{-1} N. However, no evidence of biological inhibition by the grasses were confirmed.

Keywords: *Zea mays* L. *Urochloa*. *Megathyrsus*. N_2O. AOB. AOA. Estimated meat production.

RESUMO

Devido ao interesse na eficiência no uso do N (NUE) e em sistemas agrícolas sustentáveis, a adoção de sistemas integrados, como o consórcio de milho com gramíneas forrageiras, pode ser de grande relevância, permitindo o uso da terra ao longo do ano, além de evitar perdas de N por lixiviação de nitrato (NO_3^-), emissões de óxido nitroso (N_2O), volatilização de amônia (NH_3) e imobilização. Gramíneas forrageiras tropicais do gênero *Megathyrsus* e *Urochloa* podem suprimir a nitrificação do solo ao liberar substâncias inibidoras, reduzindo as perdas de N e aumentando a recuperação de N fertilizante da cultura comercial em sucessão. Desta forma, o entendimento das transformações do N no solo por microrganismos e a recuperação do fertilizante no sistema são muito importantes. Em primeiro lugar, os dois primeiros capítulos são a respeito de um experimento de campo de 3 anos (2014-2017) conduzido no sudeste do Brasil, onde gramíneas forrageiras capim colonião (*Megathyrsus maximus* cv. Tanzânia), capim braquiária (*Urochloa brizantha* cv. Marandu) e capim braquiária *(Urochloa ruziziensis* cv. Comum) foram cultivadas em rotação com milho para grão no verão, para analisar a influência da gramínea forrageira e da fertilização com N em cada estudo. No primeiro capítulo, o milho foi fertilizado com 140 kg ha^{-1} de N na forma de $(^{15}NH_4)_2SO_4$ ou não fertilizado, e a recuperação do ^{15}N residual foi quantificada na segunda safra. No segundo capítulo, a mudança foi que a fonte de N utilizada foi o sulfato de amônio não rotulado, e foram analisadas as emissões de óxido nitroso (N_2O), metano (CH_4) e NH_3 do sistema. No terceiro e quarto capítulos, o milho foi consorciado com as mesmas gramíneas mencionadas anteriormente. As doses de N foram 90, 180 e 270 kg ha^{-1} e os tratamentos sem adubação nitrogenada. O objetivo também foi verificar o efeito das gramíneas e da fertilização com N a partir das análises realizadas. O terceiro capítulo caracterizou as mudanças nos genes do ciclo N no solo e mediu as emissões de N_2O. O quarto capítulo avaliou o rendimento de grãos de milho e a produção de forragem, a qualidade bromatológica e a estimativa da produção de carne. Na primeira safra após a aplicação de ^{15}N, 21%, 65% e 33% do N no grão de milho, palha e brotos, respectivamente, foi derivado de fertilizante. Na safra seguinte, do total de N encontrado nos grãos, caules e ramos de milho, 2,2%, 1,9% e 2,0%, respectivamente, foram derivados do fertilizante residual aplicado no ano anterior. Não houve diferenças entre as espécies de gramíneas forrageiras na quantidade de ^{15}N recuperado pelo milho, solo e N. total. Na primeira temporada de milho em rotação com gramíneas forrageiras, capim-Guiné, capim-paliçada, ruzigrass não afetou a emissão de N_2O e NH_3 devido à sua aparente incapacidade de suprimir a nitrificação do solo. No entanto, a fertilização com N aumenta ligeiramente a emissão cumulativa de N_2O. No consórcio de milho com gramíneas, a fertilização com N aumenta a abundância de AOB (*amo*A de bactérias) mais do que AOA (*amo*A de arquéias). A emissão de N_2O foi influenciada por AOB, espaço poroso cheio de água (WFPS) e fertilização de N. A fertilização nitrogenada afeta positivamente o crescimento das forragens e a qualidade nutricional, resultando em maior rendimento de grãos de milho, maior produção e qualidade das forragens, e eventualmente maior produção de carne estimada. Além disso, o capim colonião resultou na maior produção de carne estimada quando fertilizado com 270 kg ha^{-1} N. No entanto, não foram confirmadas provas de inibição biológica por parte das gramíneas.

Palavras-chave: *Zea mays* L. *Urochloa*. *Megathyrsus*. N_2O. AOB. AOA. Produção estimada de carne.

FIGURE LIST

TABLE LIST

SUMMARY

GENERAL INTRODUCTION

Maize (*Zea Mays* L.) is among the most important food sources in the world, playing a key role (Ileri et al., 2018) in ensuring food security. It has been used for animal feed, human nutrition and, more recently, for bioethanol production (Ranum et al., 2014). As a C_4 plant, maize is capable of achieving high dry matter yields accumulating large amounts of nutrients (Uzun et al., 2020). Usually, nitrogen (N) is the nutrient most required by maize (Teixeira et al., 2014; Wang et al., 2017). Nitrogen defficiency can limit crop yields, since this element is a constituent of important molecules such as amino acids, proteins, nucleic acids, nitrogenous bases, and chlorophyll (Moreira and Siqueira, 2002). One of the most studied topics regarding N in agricultural systems refers to strategies to improve N use efficiency (NUE) by crops, which can be achieved through conservation practices, like systems with different species intercropped (Adewopo et al., 2014; Rosolem et al., 2017).

The adoption of integrated systems, such as the intercropping of maize with forage grasses has been of great relevance for tropical agriculture, allowing the use of land throughout the year (Crusciol et al., 2009; Kichel et al., 2009). However, increasing the NUE through conservation systems is paramount, as it is necessary to ensure adequate soil N availability for plants and to reduce losses in the agricultural systems (Rosolem et al., 2017). The volatilization of NH_3 from fertilizer sources is an important issue when urea is applied on the soil surface in no-till systems (Mariano et al., 2012), due to the higher activity of the urease enzyme when compared to conventional tillage (Silva et al., 2017). However, in Brazil, with the N rates currently used and split applications, the risk of NO_3^- reaching the groundwater is relatively low (Villalba et al., 2014). In addition, since N fertilizer is critical to sustain or increase the crop yield, the application of high N rates can lead to subsequent high N_2O emissions in N-fertilized soils compared with those unfertilized (McSwiney and Robertson, 2005; Martins et al., 2015). No-till systems also largely affect the organic matter in the soil (Sá et al., 2015., Souza et al., 2016), and during the anaerobic decomposition of organic matter, CH_4 production can occur (Dutaur and Verchot, 2007). Therefore, crop management strategies to decrease leaching losses and N_2O emissions, as well as to increase NUE are fundamental for achieving adequate sustainability levels in agricultural systems (Rosolem, et al., 2017).

Despite the several benefits from the no-till systems in relation to conventional cropping systems, such as improvements in the chemical, physical and biological properties of the soil, and reduction of CO_2 emissions (Lal et al., 2007), maize-grass intercropped systems can result in competition between species for N, which can compromise crop yields (Borghi et al. 2014). The rotation and intercropping of maize with tropical grasses of the genus *Urochloa* (syn. *Brachiaria*) and *Megathyrsus* (syn. *Panicum*) is very common for integrated crop-livestock systems in tropical Brazil (Salton et al., 2014). In addition, it has been suggested that forage species of the genus *Urochloa* and *Megathyrsus* can affect microbiological processes within the N cycle, as well as N availability and losses (Subbarao et al., 2012). Subbarao et al. (2012) reported that biological nitrification inhibition (BNI) capacity was much higher in *U. humidicola* than in *U. decumbens*, *M. maximus*, *Lolium perenne*, *U. brizantha,* cereal and vegetable crops studied in a sand-vermiculite culture for 60 days. Thus, using ^{15}N to study fertilizer N recovery by cash crops intercropped with forage species is fundamental (Rocha et al., 2019) to assess whether the fate of fertilizer N in the soil-plant system is affected by BNI, when this process is active.

The N transformations in the soil by microorganisms can occur in several ways (Zhang et al., 2006), and the understanding of these processes is essential in the search for efficient and sustainable agricultural systems. In the atmosphere, N is found as a diatomic molecule (N_2), which can be fixed by a specialized microbiota, by a process known as biological N fixation (BNF; Cardoso, 1992). In the soil, this element can be found in organic and mineral forms (Cantarella, 2007). The soil N dynamics is complex, mostly driven by soil microorganisms as follows: i) BNF: the enzyme nitrogenase, which is encoded by the *nif*H gene, breaks the N_2 triple bond to reduce N_2 to ammonia (NH_3; Zhang et al., 2006); ii) nitrification: NH_4^+ is converted to NO_3^- via the action of ammonia monooxygenase (*amo*A). This process comprises two phases: oxidation of NH_4^+ to nitrite (NO_2^-) and oxidation of NO_2^- to NO_3^-; iii) denitrification: copper nitrite reductase (*nir*K), iron nitrite reductase (*nir*S) and nitric oxide reductase (*nor*B; Levy-Booth et al., 2014) oxidize NO_3^- successively to NO_2^-, NO and finally N_2O; iv) the only route for converting N_2O to N_2 (Sun et al., 2019) is nitrous oxide reductase (*nos*Z; Henry et al., 2006).

Considering all these aspects, the following hypotheses can be formulated:

1. Forage species with high BNI capacity would increase maize grain yield and ^{15}N recovery in the soil-plant system by suppressing soil nitrification and therefore decreasing fertilizer N losses;
2. Tropical forage grasses would affect N cycle-associated genes and mitigate N_2O emissions in N-fertilized maize;
3. Nitrogen fertilization would increase maize yield, as well as dry matter yield and bromatological quality of forage grasses.

Based on these hypotheses, the objectives of this study were: i) to estimate N_2O, CH_4, and NH_3 emissions in maize-based rotation systems as affected by tropical forage grasses; ii) assess whether rotation with tropical forage grasses influences maize dry matter yield, N accumulation, ^{15}N recovery, and the fate of the N fertilizer in the plant-litter-soil system over two growing seasons; iii) to characterize changes in total bacterial and archaeal abundances and in microbial populations involved in N-fixation (*nif*H), ammonia oxidation (AOA and AOB), and denitrification (*nir*S and *nos*Z) and to measure N_2O emissions in the maize intercropped with forage grasses; and iv) to assess maize grain yield and forage production, bromatological quality and estimated meat production in maize-forage grass intercropped systems.

The first chapter of this thesis, entitled: “Fate of ^{15}N fertilizer applied to maize in rotation with tropical forage grasses” was published in *Field Crops Research*. Chapter 2, entitled: “Effect of tropical grass and nitrogen fertilization on nitrous oxide, methane, and ammonia emissions of maize-based rotation systems” was published in *Atmospheric Environment*; and the third chapter, entitled: “Functional N-cycle genes in soil and N_2O emissions in a maize/tropical forage grasses intercropping system” was recently submitted to *Science of the Total Environment*. Lastly, chapter 4, entitled “Bromatological quality and estimated meat production in maize intercropping with tropical forage grasses with N fertilization” will be submitted in due course to *Grass and Forage Science*.

FINAL CONSIDERATIONS

This study brings the importance of understanding some mechanisms that govern the integrated production systems such as intercropping and crop rotation in conservationist systems, through the choice of N fertilizer rates and forage grass species. These systems prove to be sustainable, since they allow the use of land throughout the year in addition to the recovery of degraded pastures. In addition, the discussions of the 21st century that address climate change across the planet (Oertel et al., 2016) show us the essentiality of studying systems that can result in more sustainable environments, aiming the reducing hunger in the world, by increasing production without increasing the area cultivated. In this way, the NUE, as well as the reduction of losses of this nutrient in the soil and in the atmosphere, become fundamental in the current agricultural systems, since N is one of the most limiting nutrients for plants (Teixeira et al., 2014; Wang et al., 2017). According to the results obtained, it was possible to understand the interactions between agricultural management and plant-soil-microorganism relationships can affect the N cycle (Bowles et al., 2013) and plant yield. Despite reports suggest that BNI by *Urochloa* spp. and *Megathyrsus* spp. decrease N loss in the system, it was not evident in N-rich environments. Regarding to maize rotation with forage grasses, there was no difference among the forage grass species in the distribution and fate of ^{15}N in the plant–litter–soil system and, consequently, in unrecovered–N (i.e., potential losses). The amount of residual labeled N taken up by maize in the second growing season was very low. Guinea grass, palisade grass and ruzigrass did not affect N_2O and NH_3 emission due to their apparent inability to suppress soil nitrification. However, N fertilization slightly increased cumulative N_2O emission in the second maize season and decreased soil CH_4 uptake in the fertilized palisade grass and ruzigrass relative to unfertilized palisade grass in the second forage season.

Regarding the results of maize intercropped with forage grasses, cumulative N_2O emissions were higher in the N-fertilized treatments than those without N addition. Moreover, a close relationship was observed among soil characteristics (NH_4^+-N, NO_3^--N, and WFPS), gene abundance (AOB) and N_2O emissions. No relationship was found between denitrifying genes and N_2O emissions. This suggests that although N modifies the soil microbiome, in addition to the abundance of AOB being highly linked to the increase in N_2O emissions in the intercropped system, the increase in N-N_2O

emissions to the system is small in relation to productivity gains of the system, suggesting a decreasing the specific emission, that is, the emission per unit of product.

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