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NITROGÊNIO HIDROLISÁVEL EM MEIO ALCALINO NO PERFIL DE SOLOS CULTIVADOS COM ARROZ IRRIGADO E SUA CONTRIBUIÇÃO PARA A NUTRIÇÃO DA CULTURA

Santa Maria, RS
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Tese apresentada ao Programa de Pós-Graduação em Ciência do Solo, Área de Concentração em Processos Químicos e Ciclagem de Elementos da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de **Doutor em Ciência do Solo**

Orientador: Prof. Dr. Leandro Souza da Silva

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2019

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“Você não pode mudar o vento, mas pode ajustar as velas do barco para chegar onde quer.”

(Confúncio)

“Sorte, é o nome que o vagabundo dá ao esforço que ele não faz.”

(Leandro Karmal)

RESUMO

NITROGÊNIO HIDROLISÁVEL EM MEIO ALCALINO NO PERFIL DE SOLOS CULTIVADOS COM ARROZ IRRIGADO E SUA CONTRIBUIÇÃO PARA A NUTRIÇÃO DA CULTURA

Autor: Gerson Laerson Drescher
Orientador: Leandro Souza da Silva

O arroz é uma importante fonte de alimento para a população mundial e uma cultura substancial para o desenvolvimento econômico do Sul do Brasil. O nitrogênio (N) é um dos nutrientes mais limitantes para a produção da cultura do arroz, sendo a atual recomendação da adubação nitrogenada para o arroz no Sul do Brasil baseada no teor de matéria orgânica do solo (MOS) da camada de 0-20 cm. Contudo, as doses de N recomendadas são muito próximas para as diferentes classes de MOS e, nem sempre, ocorre incremento em produtividade pela adição do fertilizante. Em solos sujeitos ao alagamento, a entrada da água afeta a dinâmica de decomposição da MOS e o ciclo do N, dificultando a predição da dose de N apenas pelo teor de MOS. Índices de disponibilidade de N mais precisos, a avaliação do N no perfil do solo e sua recuperação pelo arroz, bem como o efeito do N de maiores profundidades do solo sobre a morfologia radicular das plantas podem auxiliar para uma melhor compreensão da dinâmica do nutriente neste sistema de produção, contribuindo para aprimorar a recomendação do fertilizante nitrogenado para a cultura. Este trabalho objetiva ampliar a compreensão sobre a dinâmica do N em áreas cultivadas com arroz irrigado e sua relação com as propriedades do solo, bem como identificar a capacidade das plantas utilizarem o N disponível em maiores profundidades no solo. Foi conduzido um estudo avaliando o teor total e a quantidade de amônio fixo pela argila (CF-NH_4) extraída pelo Nitrogen Soil Test for Rice (N-STaR) em solos do Arkansas, Estados Unidos. Em solos do Sul do Brasil, foi avaliada a distribuição do N hidrolisável em meio alcalino (AH-N) no perfil do solo e sua relação com as propriedades do solo; foi estudada a recuperação e a dinâmica de absorção do N de diferentes profundidades pelo arroz; e, foi avaliado o efeito do N de diferentes profundidades do solo sobre o crescimento radicular e a nutrição das plantas. O CF-NH_4 representa 24.8 e 28.1% do teor de N total nas camadas de 0-15 e 15-30 cm, respectivamente, sendo que o N-STaR estima que uma pequena proporção (<15%) deste compartimento de N seja disponível as plantas. Estes resultados indicam que CF-NH_4 é um importante compartimento de N do solo, podendo ser uma fonte primária de N para o arroz. A distribuição do AH-N no perfil do solo é distinta entre solos e pode apresentar incrementos em profundidade. Embora as frações granulométricas estejam fortemente correlacionas com o teor de AH-N para alguns solos, o N total é a principal variável que determina a distribuição do AH-N no perfil do solo. O arroz utiliza efetivamente o N disponível em profundidades de até 45 cm e a recuperação do N é afetada pelas propriedades físicas do solo, especialmente a condutividade hidráulica. Maior recuperação de ^{15}N e N derivado do fertilizante ocorreu quando o N foi aplicado aos 10, 20 e 30 cm de profundidade, o que indica que a aplicação do N em subsuperfície pode aumentar a eficiência de utilização do nutriente pela cultura do arroz. A disponibilidade de N em profundidade aumentou o comprimento, área superficial e volume radicular e reduziu o diâmetro médio das raízes. O N localizado nas camadas superficiais do solo é absorvido pelas plantas em estágios mais responsivos à adubação, resultando em maior produção de massa seca. O N localizado aos 30 e 45 cm contribuiu para a nutrição das plantas no estágio reprodutivo, aumentando o teor de ^{15}N na folha índice, o teor de N no tecido, o teor clorofila, a taxa fotossintética e eficiência de carboxilação pela Rubisco no florescimento do arroz. O N disponível em profundidades superiores à 20 cm contribui para a nutrição das plantas e deve ser considerado em estudos futuros envolvendo a predição e calibração de índices de disponibilidade de N para o arroz irrigado por alagamento.

Palavras chave: Análise do solo. Balanço nutricional. Fertilizante nitrogenado. Matéria orgânica. *Oryza sativa* L. Recuperação de ^{15}N.

ABSTRACT

ALKALINE HYDROLIZABLE NITROGEN IN FLOODED RICE FIELDS SOIL PROFILE AND ITS CONTRIBUTION FOR RICE CROP NUTRITION

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Rice is an important food source for the world's population and is a substantial crop for Southern Brazil economic development. Nitrogen (N) is one of the most limiting nutrients for rice crop production, and the current N fertilizer recommendation for rice in Southern Brazil is based on soil organic matter (SOM) content at the 0-20 cm layer. However, the recommended N rates are very close for the different SOM classes and there is not always a yield increase by adding fertilizer. In flooded soils, the water input affects the dynamics of SOM decomposition and the N cycle, making it more difficult to predict the N rate based only on SOM content. More accurate N availability indexes, the N evaluation in soil profile and its recovery by rice plants, as well as the effect of N from greater soil depths on rice root morphology can help to improve our understanding on the nutrient dynamics in this production system, while contributing to improve the crop N fertilizer recommendation. This work aims to broaden our understanding of N dynamics in areas cultivated with rice and its relationship with soil properties, as well as identifying the capacity of plants to use N available at greater soil depths. A study was conducted to evaluate the total content and the amount of clay fixed ammonium (CF-NH_4) extracted by the Nitrogen Soil Test for Rice (N-STaR) in soils from Arkansas, United States. In soils from southern Brazil, we evaluate the alkaline hydrolyzable N (AH-N) distribution in soil profile and its relationship with soil properties; we investigated the rice N recovery and absorption dynamics from different soil depths; and the effect of N from different soil depths on rice root growth and plant nutrition was also evaluated. The CF-NH_4 represents 24.8 and 28.1% of the total N content at the 0-15 and 15-30 cm layers respectively, and the N-STaR estimates that a small proportion (<15%) of this N pool is plant available. These results indicate that CF-NH_4 is an important soil N pool and might be a primary N source for rice crop. The AH-N distribution in soil profile is distinct between soils and can have increments in depth. Although granulometric fractions are strongly correlated with AH-N content for some soils, the total N is the major variable dictating AH-N distribution in soil profile. Rice effectively uses available N from depths of up to 45 cm and N recovery is affected by soil physical properties, especially hydraulic conductivity. Higher ^{15}N recovery and N derived from fertilizer occurred when N was applied at the 10, 20 and 30 cm depths, which indicates that the N application in subsurface can increase the nutrient use efficiency by rice crop. The N availability in depth increased the root length, root surface area, and root volume while reducing the root average diameter. The N located at the soil superficial layers is absorbed by the plants in more responsive stages to fertilization, resulting in greater dry mass production. The N located at 30 and 45 cm contributed to plant nutrition at the reproductive stage, increasing the ^{15}N content in the index leaves, the N content in tissue, the chlorophyll content, the photosynthesis rate and the carboxylation efficiency by Rubisco at rice heading. The N available in greater depths than 20 cm contributes to plant nutrition and should be considered in future studies involving the prediction and calibration of N availability indexes for flooded rice.

Keywords: Soil analysis. Nutritional balance. Nitrogen fertilizer. Organic matter. *Oryza sativa* L. ^{15}N recovery.

LISTA DE ABREVIATURAS E SIGLAS

AH-N	Alkaline Hydrolyzable Nitrogen
ANOVA	Analysis of Variance
ASM	Alfisol soil from Santa Maria
BD	Soil Bulk Density
C	Carbono
CF-NH_4	Clay Fixed Ammonium
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CNPq	Conselho Nacional de Desenvolvimento Científico e Tecnológico
DCB	Ditionito Citrato-Bicarbonato de Sódio
DOM	Dissolved Organic Matter
DOM	Dissolved Organic Nitrogen
DVD	Destilação a Vapor Direta
ESM	Entisol soil from Santa Maria
EA	Entisol soil from Agudo
HC	Hydraulic Conductivity
IC	Intercellular CO_2 Concentration
IRGA	Infrared Gas Analyzer
LSD	Least Significant Difference
MacP	Macro Porosity
MicP	Micro Porosity
MOS	Matéria Orgânica do Solo
N	Nitrogênio
NC	Condition Number
NDFF	Nitrogen Derived From Fertilizer
NDFS	Nitrogen Derived From Soil
N-STaR	Nitrogen Soil Test for Rice
NECF-NH_4	N-STaR Extractable Clay-Fixed Ammonium
NR	Nitrogen Recovery
NRCF-NH_4	N-STaR Recovery of Total Clay-Fixed Ammonium
PhotoR	Photosynthetic Rate

Photo/IC	Instantaneous Carboxylation Efficiency
PR	Penetration Resistance
PVC	Polyvinyl Chloride
SC	Stomatal Conductance
SOM	Soil Organic Matter
TCF-NH_4	Total Clay Fixed Ammonium
TC	Total Carbon
TN	Total Nitrogen
TP	Total Porosity
TR	Transpiration Rate
WUE	Water Use Efficiency

SUMÁRIO

1. INTRODUÇÃO

Entre os elementos essenciais ao desenvolvimento das plantas, o nitrogênio (N) ocupa uma posição de destaque. Embora esteja presente em grandes quantidades em alguns solos, sua baixa disponibilidade aliada à grande necessidade pelas plantas, faz com que este seja um dos nutrientes mais limitantes à produtividade da maioria das culturas. Para a cultura do arroz irrigado, o N é um dos nutrientes requeridos em maior quantidade (FAGERIA et al., 2003), sendo um dos mais importantes fatores que afetam a produção. O N interfere nos componentes do rendimento da cultura, principalmente no número de panículas por área, número de espiguetas por panículas, fertilidade das espiguetas e massa de grãos. Dentre os componentes, o número de panículas apresenta maior relação com a produtividade, podendo ser influenciado conforme a aplicação e eficiência de aproveitamento do nutriente (FAGERIA et al., 2003). Neste sentido, para maximizar a produtividade da cultura e minimizar as perdas do nutriente, é necessário realizar um adequado manejo da adubação nitrogenada.

O manejo da adubação visa o fornecimento dos nutrientes de forma adequada e no momento correto para à melhor nutrição mineral das plantas, considerando o fornecimento de nutrientes pelo solo. Boa parte dos nutrientes que as plantas absorvem provém das reservas do solo e, deste modo, o desafio é conhecer a real capacidade do solo em disponibilizar os nutrientes necessários às plantas, para que se possa melhorar a eficiência no aproveitamento dos nutrientes aplicados via fertilizantes, minimizando os custos de adubação, bem como os potenciais riscos ao meio ambiente. O N disponível do solo é praticamente todo proveniente da decomposição e mineralização da matéria orgânica do solo (MOS). Assim, uma avaliação simplista do grau de disponibilidade de N no solo pode ser baseada na análise do teor de MOS. Contudo, nem sempre existe uma boa associação entre o teor de MOS e o N acumulado pelas plantas ou rendimento relativo para diversas culturas.

A cultura do arroz irrigado por inundação é um caso típico deste processo, pois em decorrência das condições de má drenagem e ao alagamento ocasionado pela irrigação, a dinâmica da MOS é diferenciada, tanto com relação aos produtos formados quanto à velocidade da decomposição. Este fato é decorrente da condição singular deste sistema de produção, que envolve períodos de atividade biológica em condições de aerobiose e anaerobiose, os quais afetam a mineralização da MOS e, consequentemente, a disponibilidade de N às plantas, dificultando a estimativa da dose de fertilizante nitrogenado a ser indicada para a cultura do arroz irrigado a partir do seu teor total. Além disso, o nível de adubação nitrogenada que possibilita a obtenção do máximo rendimento econômico de grãos de arroz depende da interação de vários fatores, destacando-se a disponibilidade de N no solo, a adição de N via fixação biológica e através da água de irrigação, o tipo de planta e cultivar de arroz, bem como as condições climáticas, particularmente a temperatura e a radiação solar. Em função destes processos, a produtividade do arroz irrigado pode ser muito variável com o teor de MOS, sendo obtidos tanto baixos quanto elevados rendimentos relativos em solos com baixo ou alto teor de MOS, independente da adubação nitrogenada.

Neste sentido, a avaliação da disponibilidade de N por métodos que determinam frações orgânicas hidrolisáveis, que estão mais disponíveis ao ataque microbiano e que apresentam maior potencial de mineralização e disponibilização às plantas, pode proporcionar melhor associação com a quantidade de N absorvida e acumulada pelas plantas. Deste modo, o N hidrolisável em meio alcalino (AH-N) determinado por meio do procedimento de destilação a vapor direta (DVD), avalia frações lábeis da MOS, predominantemente o NH_4^+ N orgânico, como amino açúcares (por exemplo glucosamina e asparagina) e aminoácidos (por exemplo glutamina), trazendo melhorias na estimativa do N disponibilizado durante o ciclo da cultura do arroz irrigado. Com testes nas principais regiões produtoras de arroz do Arkansas nos Estados Unidos, foram obtidas boas correlações entre o teor AH-N e o teor de N mineralizado, N acumulado e o rendimento relativo da cultura do arroz. Estes resultados permitiram a calibração do índice AH-N e, o desenvolvimento de um protocolo de recomendação de adubação nitrogenada para arroz produzido em solos argilo-siltosos, chamado Nitrogen Soil Test for Rice (N-STaR) (ROBERTS et al., 2011; 2012). Contudo, para que a calibração do índice AH-N tivesse êxito, foi nessessário o estabelecimento de protocolos de amostragem do solo específicos para solos arenosos e argilosos em função da disponibilidade e distribuição das formas de N lábil no perfil do solo.

O trabalho realizado por Drescher et al. (2016), utilizando 25 solos das principais regiões produtoras de arroz irrigado do estado do Rio Grande do Sul (RS), também verificou elevada correlação entre o teor de AH-N e o N mineralizado em incubação anaeróbia e o N acumulado pelas plantas de arroz cultivadas em casa de vegetação. Estes resultados demonstram o potencial de utilização do índice AH-N para indicar a disponibilidade de N para a cultura do arroz irrigado no RS, podendo trazer avanços expressivos para a melhoria do sistema de recomendação de N para a cultura. Contudo, a distribuição e estabilização das formas de N-lábil no perfil do solo está muito relacionada às propriedades físicas e químicas do solo, o que faz com que solos que possuem granulometria mais fina (fração silte e argila <50 µm), maior teor de óxidos e algilominerais 2:1 como a montmorilonita, exerçam uma maior estabilização da MOS no solo, interferindo na migração e distribuição de formas de N no perfil do solo. Todavia, a influência dos processos de redução do solo sobre a solubilização da MOS e do N estabilizados pela fração mineral, a capacidade das plantas e dos métodos de extração do N de acessarem o N fixado pela argila não é bem entendida, e muito menos a migração e distribuição de formas lábeis, como o AH-N, no perfil do solo.

Estudos avaliando o crescimento radicular da cultura do arroz irrigado apontam que o sistema radicular dificilmente ultrapassaria os 40 cm de profundidade em virtude do ambiente fortemente reduzido em maiores (YOSHIDA, 1981; ABICHEQUER; BOHNEN, 2008). Contudo, compartimentos com maior concentração de nutrientes, como N e P, podem promover o crescimento radicular das plantas, aumentando o volume de solo explorado. Neste sentido, a presença de N lábil em maiores profundidades do solo pode promover o crescimento radicular em maiores profundidades, contribuindo para a absorção de nutrientes e nutrição das plantas. Ademais, a mineralização do N em profundidades superiores às amostradas para fins de recomendação da adubação pode contribuir para a disponibilização do nutriente às plantas e estar associada a falta de resposta à adubação nitrogenada em alguns solos. No entanto, muito pouco se conhece sobre a capacidade das plantas utilizarem do N distribuído ao longo do perfil do solo, bem como do seu efeito sobre o desenvolvimento e o crescimento radicular da cultura do arroz no Sul do Brasil.

Em decorrência dos processos dinâmicos associados ao sistema de produção do arroz por alagamento e da heterogeneidade das propriedades químicas, físicas e mineralógicas dos solos cultivados com arroz irrigado no Sul do Brasil, a profundidade de amostragem do solo, que está em consonância com o enraizamento efetivo da cultura para aquisição do N, pode apresentar variações conforme as características do solo. Neste sentido, há necessidade de investigar a interação entre estas propriedades visando propiciar uma adequada capacidade preditiva do AH-N, o que é necessário para uma correta calibração do método e a melhoria do atual sistema de recomendação de adubação nitrogenado para a cultura do arroz irrigado por alagamento no Sul do Brasil.

2. REFERENCIAL TEÓRICO

O teor de MOS é utilizado como índice de disponibilidade de N para o manejo da adubação nitrogenada em muitas regiões, incluindo o Sul do Brasil (SBCS, 2016; SOSBAI, 2018), pelo fato de grande parte do N estar no solo na forma orgânica, podendo estar presente em compostos húmicos livres ou associados com colóides minerais ou ainda como componentes de biomassa microbiana (MULVANEY; KHAN, 2001). É por meio da decomposição da MOS que o N é liberado na forma mineral em diferentes velocidades, dependendo da sua recalcitrância e proteção física ao ataque microbiano (CAMARGO et al., 1997). Deste modo, a disponibilidade do N está relacionada com a intensidade da decomposição e mineralização exercida pela atividade microbiana, disponibilizando o N na forma mineral às plantas. Assim, a quantidade de N mineralizado é função do conteúdo e da taxa de mineralização do N orgânico (CAMARGO et al., 1997), existindo relação direta entre o N mineralizado e o N absorvido pelas plantas.

As tabelas de recomendação das doses de N para a cultura do arroz irrigado são baseadas em classes de teor de MOS (SBCS, 2016; SOSBAI, 2018), a partir das quais se obtêm as faixas de interpretação de disponibilidade de N. No entanto, para uma mesma expectativa de produtividade de arroz, as doses de N a serem adicionadas ao solo são muito próximas para as diferentes classes de MOS. Isto acontece porque, para o arroz irrigado, não existe uma boa correlação entre rendimento relativo e teores de MOS (SCIVITTARO; MACHADO, 2004). Nos solos alagados, a entrada da água faz com que ocorram alterações de ordem física, química e biológica, as quais afetam a dinâmica da decomposição da MOS, bem como o ciclo do N, o que dificulta a predição da dose de fertilizante nitrogenado a ser indicado para o arroz irrigado apenas a partir do teor de MOS (DRESCHER et al, 2016). Dessa forma, a associação da MOS com o N absorvido pelas plantas ou com o rendimento relativo do arroz irrigado é baixa, diferentemente do que ocorre em solos bem drenados (RHODEN et al., 2006b).

Os processos que ocorrem no solo após o alagamento são dinâmicos e interligados, sendo a avaliação do N do solo disponível às plantas muito complexa e difícil para a cultura do arroz (FAGERIA; STONE, 2003). Contudo, a predição adequada da quantidade de N inorgânico liberado a partir da mineralização da MOS é essencial para o desenvolvimento de práticas que maximizem a eficiência no uso do N, minimizando impactos adversos ao meio ambiente (LI et al., 2003), principalmente quando se considera a grande mobilidade e dinâmica do N, tanto em ambientes de sequeiro, como em ambientes alagados (RHODEN et al., 2006a).

Em função disso, diversos estudos têm sido conduzidos para o desenvolvimento e avaliação de métodos de análise de solo com potencial de estimar a disponibilidade de N para a cultura do arroz irrigado. Entre os métodos, podem-se destacar as análises químicas, como as extrações ácidas, alcalinas e salinas, com diferentes intensidades de extração (WILSON et al., 1994; ANH; OLK 2001, 2002); métodos biológicos, como as incubações aeróbicas e anaeróbicas (LI et al., 2003; RHODEN et al., 2006a; VILLASEÑOR et al., 2015); e os métodos com avaliação de varáveis da planta, para estimar a disponibilidade de N ao longo do ciclo de desenvolvimento da cultura (SILVA et al., 2007; SILVA et al., 2008; POCOJESKI et al., 2012). No entanto, dos métodos e procedimentos desenvolvidos até o momento, nenhum conseguiu se adequar a rotina dos laboratórios ou possui uma adequada calibração de campo de acordo com o rendimento da cultura.

Visando melhorar o manejo da adubação nitrogenada para o arroz irrigado no estado do Arkansas nos Estados Unidos, Roberts et al. (2011, 2012) desenvolveram um protocolo de recomendação de adubação nitrogenada para arroz denominado N-STaR. O princípio deste protocolo é estimar a quantidade de N que o solo pode fornecer durante o período de crescimento da cultura, permitindo ajustes na dose de fertilizante à ser aplicado para o arroz e a otimização do uso do fertilizante nitrogenado. O N-STaR consiste de um procedimento de destilação alcalina que quantifica formas de N orgânico potencialmente mineralizáveis, como amino açúcares (por exemplo glucosamina e asparagina) e aminoácidos (por exemplo glutamina) mais N-inorgânico como o N-NH_4 (ROBERTS et al., 2009a), denominados de AH-N.

No sistema de recomendação de adubação N-StaR, 10 amostras de solo são coletadas por área representativa e encaminhadas para o N-STaR Soil Test Laboratory, onde serão secas em estufa a 65 °C, moídas, peneiradas (<2 mm) e analisadas em processo de destilação alcalina (NaOH 10 mol L^{-1}) para quantificar o teor de AH-N. Posteriormente, os clientes recebem as recomendações de dose de N correspondentes a três espectativas de rendimento: econômico, ótimo e máximo (correspondentes a 90, 95 e 100 % do rendimento relativo da cultura). Deste modo, o produtor dispõe de recomedações específicas para sua área de produção, podendo aumentar sua lucratividade por meio de um manejo mais eficiente da adubação nitrogenada. Contudo, para que a calibração do índice AH-N tivesse sucesso, foi necessário ajustes na profundidade de amostragem em relação ao demais nutrientes com amostragem do solo na camada de 0-45 cm para solos siltosos (ROBERTS et al., 2012) e na camada de 0-30 cm em solos argilosos (FULFORD et al., 2013). Estes ajustes, possivelmente, são decorrentes da distribuição do AH-N no perfil do solo e da distinta capacidade das plantas recuperarem o N de maiores profundidades em solos com granulometria e características mineralógicas distintas.

Para que um método analítico tenha sucesso e possa ser utilizado pelos laboratórios de análise de solo, alguns fatores precisam ser considerados, como a amostragem do solo adequada e o método apropriado, cujos resultados se correlacionem com a absorção de nutrientes e a produtividade de grãos da cultura (ROBERTS et al., 2013). Deste modo, com o AH-N são avaliadas predominantemente as frações: N-NH_4^+ + N-orgânico (principalmente na forma de amino açúcares e aminoácidos) (ROBERTS et al., 2009a). Estas frações orgânicas hidrolisáveis estão mais disponíveis ao ataque microbiano, sendo mineralizadas e disponibilizadas às plantas com maior facilidade. Esta característica proporciona ao AH-N melhor associação com a quantidade de N absorvida e acumulada pelas plantas, que aliada à rapidez e ao custo da análise, torna o método com bom potencial de ser utilizado para a recomendação do N à cultura.

Neste sentido, em trabalho realizado por Roberts et al. (2011), foi possível verificar elevada correlação entre o AH-N estimado por DVD e as variáveis da planta medidas em solos do estado do Arkansas. Quando o N total absorvido pelas plantas e o rendimento da cultura foram relacionados com o AH-N, foram observados coeficientes de determinação de 0,46 e 0,73, respectivamente. Ao estabelecer modelos de regressão para a relação entre a taxa de N necessária para alcançar 95% de rendimento de grãos relativa com o AH-N, Roberts et al. (2011) atingiram elevado coeficiente de determinação (0,89) quando se considerou a camada de amostragem de 0 a 45 cm. Esses resultados evidenciam que o AH-N pode ser correlacionado e calibrado para a cultura do arroz irrigado, podendo aumentar a sustentabilidade da produção agrícola em longo prazo, enquanto reduz potenciais impactos ambientais provenientes do excesso de fertilização nitrogenada.

Da mesma forma, no Brasil, em trabalho realizado por Drescher et al. (2016) utilizando 25 solos das principais regiões produtoras de arroz irrigado do Rio Grande do Sul (RS), foi possível verificar elevada correlação do teor AH-N extraído por destilação a vapor direta (DVD) com o N disponível após 28 dias de incubação anaeróbia (r=0,78) e com o N acumulado pelas plantas de arroz após 45 dias de cultivo em casa de vegetação (r=0,72). Neste trabalho, os autores verificaram melhor correlação entre o AH-N em relação aos índices de extração intensa N total e MOS. Estes resultados demonstram o potencial de utilização do AH-N como índice de disponibilidade de N para a cultura do arroz irrigado no Sul do Brasil, podendo trazer avanços expressivos para a melhoria do sistema de recomendação de N para a cultura.

No entanto, embora os resultados encontrados por Drescher et al. (2016) nos solos do RS utilizando o índice AH-N sejam promissores, existem poucas informações sobre a distribuição do AH-N no perfil do solo e as propriedades do solo que influenciam o seu teor no solo. Formas de N em disponíveis em maiores profundidades do solo podem afetar o crescimento radicular das plantas, bem como contribuir para a nutrição das plantas, afetando a quantidade de N acumulado, teor de clorofila e taxa fotossintética, promovendo maior produtividade da cultura. Ademais, muito pouco se conhece sobre a capacidade de recuperação pela planta e a contribuição do N de camadas mais profundas do perfil do solo na nutrição das plantas de arroz. Estes fatores podem interferir no teor de AH-N, bem como na quantidade de N assimilado pela planta, precisando ser identificados e levados em consideração para que o método mantenha uma boa capacidade preditiva e possa ser calibrado eficientemente para o arroz irrigado no Sul do Brasil.

2.1 PROFUNDIDADE DE AMOSTRAGEM DO SOLO

Em trabalho realizado nos Estados Unidos, Roberts et al. (2009b) observaram um comportamento distinto na distribuição dos teores de N total e AH-N no perfil de 16 solos do estado do Arkansas, sendo que os maiores teores de N total sempre ocorreram nos primeiros 15 cm de profundidade, enquanto que os teores de AH-N variavam em profundidade. Neste trabalho, foi verificado que, para alguns solos, camadas com mais de 30 cm de profundidade apresentaram ampla variação no teor de AH-N, o qual aumentou, diminuiu, ou se manteve constante em comparação com as concentrações quantificadas nas camadas mais superficiais. Em função deste contraste entre N total e AH-N, os autores salientam a importância do N localizado em camadas mais profundas que 30 cm para a nutrição das plantas. Este comportamento pode ajudar a entender os casos de falta de resposta do arroz irrigado à adubação nitrogenada em alguns solos com baixo teor de MOS, ao mesmo tempo em que realça a necessidade da amostragem de solo em consonância com a profundidade de enraizamento da cultura para manter a capacidade preditiva do método (MULVANEY et al., 2006). Este fato se evidenciou claramente no trabalho de Roberts et al. (2011), que estabeleceram modelos de regressão para a relação entre a dose de N necessária para alcançar 95% de rendimento relativo de grãos com o teor de AH-N quantificado por DVD em diferentes profundidades. Neste trabalho, os autores atingiram o melhor coeficiente de determinação (0,89) quando se considerou a camada de amostragem de 0 a 45 cm.

Diante da elevada influência da profundidade de amostragem sobre a capacidade preditiva do AH-N para a recomendação da adubação nitrogenada para o arroz, Roberts et al. (2012) avaliaram a distribuição dos teores de N e carbono (C) totais no perfil do solo e a sua calibração para a cultura do arroz. Os resultados da calibração para N e C totais proporcionaram um menor coeficiente de determinação do que a calibração do AH-N determinado por DVD. Contudo, Roberts et al. (2012) observaram que a calibração dos teores de N e C totais também foi altamente influenciada pela profundidade de amostragem do solo, sendo que a profundidade de amostragem de solo tradicional, realizada na camada de 0 a 15 cm para análise de rotina do solo no Arkansas, muitas vezes apresentou os menores coeficientes de determinação.

A influência da profundidade sobre a calibração dos teores de AH-N e os teores de N e C totais verificados nos solos do Arkansas pode ser ocasionada pela migração de matéria orgânica dissolvida no perfil do solo, processo importante no transporte vertical de nutrientes no solo (KAISER; ZECH, 2000). Esta migração no perfil é decorrente do aumento da solubilidade de matéria orgânica em condições anaeróbicas, o que favorece uma maior mobilização em profundidade (GRYBOS et al., 2007, 2009). Outro fator também relacionado com a mobilidade de compostos orgânicos (metabólitos intermediários provenientes da decomposição da MOS, como ácidos orgânicos de baixo peso molecular e C proveniente dos resíduos vegetais recém adicionados ao solo) é a dissolução e solubilização de oxihidróxidos de Fe em função da redução do solo ocasionada pelo alagamento (KÖGEL-KNABNER et al., 2010). Oxihidróxidos de Fe atuam como principais absorventes para MOS dissolvida em condições de solo oxidado (KAISER et al., 1996, KAISER; GUGGENBERGER, 2000), os quais podem ser dissolvidos e solubilizados sob condição de redução. Este processo reduz a retenção e sorção, sendo considerada a principal razão para a concentração e fluxo de carbono orgânico dissolvido em horizontes mais profundos do solo sob condições anaeróbicas (NELSON et al., 1993; HAGEDORN et al., 2000; KALBITZ et al., 2000).

Deste modo, é possível que solos cultivados com arroz irrigado no estado do RS possam apresentar elevados teores de N-lábil em maiores profundidades e que não estão sendo determinadas pelo atual sistema de amostragem. Neste caso, se não houver impedimento físico para o crescimento radicular (como uma camada de solo mais adensada, comumente utilizada para reduzir as perdas da água de irrigação por percolação no perfil do solo), é provável que as raízes das plantas de arroz se desenvolvam e consigam assimilar estas formas de N em maiores profundidades. Além disso, em solos com maior condutividade hidráulica, a assimilação de nutrientes absorvidos predominantemente por fluxo de massa, como o N, pode ser facilitada, o que viria a contribuir para a utilização do N disponível em maiores profundidades pela cultura do arroz. Assim, estes processos podem ajudar a explicar a falta de resposta à adubação nitrogenada em solos que apresentam baixo teor de MOS

Diante disso, é necessário investigar a presença e a distribuição do AH-N no perfil do solo, uma vez que seu comportamento deve ser distinto em relação ao que foi observado nos solos do estado do Arkansas. Os solos cultivados com arroz irrigado no Sul do Brasil apresentam elevada heterogeneidade em termos de textura, condutividade hidráulica e teores de MOS e AH-N, onde o fluxo e migração de compostos orgânicos em profundidade pode se expressar de maneira distinta, o que pode influenciar a nutrição das plantas e a resposta à adubação.

2.2 CARACTERÍSTICAS E PROPRIEDES DO SOLO

Outro aspecto que afeta o teor de AH-N extraído e sua relação com o N acumulado pelas plantas, além da profundidade de amostragem de solo, é a estabilização e proteção da MOS pela fração mineral do solo. As condições anaeróbicas e, mais significativamente, as interações com as partículas do solo são essenciais na proteção do húmus contra o ataque microbiano e sua conversão para CO_2. Diversos estudos sobre a biodegradabilidade do húmus em solos de clima temperado suportam esta ideia, com relatos de aumento do conteúdo de C orgânico e da idade de húmus com a diminuição do tamanho das partículas (SPOSITO, 2008). Neste sentido, a granulometria e a mineralogia do solo são fatores que atuam nos processos de estabilização, podendo interferir na capacidade de extração do DVD, conforme verificado por Roberts et al. (2009a).

No solo, tanto em condições aeróbicas quanto anaeróbicas, existem mecanismos (químicos e físicos) que conferem proteção e estabilidade à MOS, dificultando a sua decomposição, como a recalcitrância molecular, inacessibilidade ou proteção física e interação química ou coloidal (CHRISTENSEN, 1996). Diversos tipos de substâncias orgânicas podem reagir com substâncias minerais no solo, formando complexos organo-minerais de vários graus de estabilidade contra o ataque biológico e químico (ALLISON, 1973). Essa interação ocorre através da associação dos grupos funcionais da MOS com os minerais do solo (Tabela 1), influenciando suas propriedades químicas e físicas (CANELLAS, et al., 2008).

Tabela 1 - Mecanismos de associação entre grupamentos funcionais da matéria orgânica e minerais do solo (adaptado de Sposito, 1989).

Mecanismo	**Grupos funcionais envolvidos**
Troca de cátions	Amino, NH ligado a anel aromático, N heterocíclico
Protonação	Amino, N heterocíclico, carbonila, carboxilato
Troca de ânions	Carboxilato
Ligação com a água	Amino, carbonila, carboxilato, OH alcoólico
Ligação com cátions	Carbonila, aminas, carboxilato, OH alcoólico
Troca de ligantes	Carboxilato
Pontes de hidrogênio	Amino, carbonila, carboxila, OH fenólico
Interações de van der Waals	Unidades orgânicas não carregadas

Deste modo, a proteção física pode ser decorrente do encapsulamento da MOS em agregados do solo ou do seu revestimento por partículas de silte e argila (SKJEMSTAD et al., 1993). A MOS pode também ser físico-quimicamente estabilizada por meio da adsorção na superfície de minerais da fração argila (TISDALL; OADES, 1982). Deste modo, a proteção física consiste na formação de uma barreira que impede ou dificulta o acesso dos microrganismos decompositores à MOS, cuja intensidade depende da quantidade de entradas de compostos orgânicos e do grau de saturação com matéria orgânica nos locais de proteção nas partículas de silte e argila (HASSINK et al., 1997).

Alguns estudos apontam que a estabilidade da MOS é mais dependente dos mecanismos físicos de proteção do que da recalcitrância (SKJEMSTAD, 1993; LADD et al., 1993). A argila interfere diretamente neste processo de estabilização, pois forma complexos organo-minerais, contribuindo para o aumento da estabilidade e, consequentemente da proteção física das MOS (CAMARGO et al., 1997). Deste modo, o teor de argila está relacionado com a dinâmica da mineralização de N (DRESCHER et al., 2015) e, consequentemente, pode afetar a resposta da cultura do arroz à adubação nitrogenada, mesmo em solos com alto teor de MOS.

Deste modo, é evidente que as propriedades físicas e mineralógicas do solo estão diretamente relacionadas com a dinâmica e estabilidade da MOS e do N no solo. A redução do tamanho de partícula da fração mineral do solo (fração argila e silte < 50 µm) está positivamente correlacionada com aumento do teor de MOS (QUIROGA et al., 1996, SPOSITO, 2008), pois atua na proteção contra a hidrólise enzimática dos microrganismos do solo. Este processo é evidenciado no trabalho de Blanco-Moure et al. (2016), que verificaram que duas frações organominerais isoladas, C no exterior e dentro de microagregados estáveis, constituíram a maior parte do C orgânico do solo (54 e 26%, respectivamente) e não foram consideravelmente afetados pelo manejo do solo. Neste trabalho, os autores ressaltam o papel da argila na estabilização química através de complexos de argila e material orgânico e, a proteção física como os principais mecanismos de preservação do C nos solos estudados.

O acúmulo de C orgânico e N no subsolo de solos de várzea é descrita por Li et al. (2005) e Zhang e He (2004). Os autores sugerem que o C orgânico no subsolo é originado do movimento descendente de C orgânico dissolvido ou da associação coloidal de organo-minerais, embora, não forneçam nenhuma evidência para tais processos. Contudo, os resultados de Maie et al, (2004) demonstram que a MOS no subsolo de solos cultivados com arroz irrigado é originada, pelo menos parcialmente, a partir de MOS dissolvida lixiviada, proveniente da camada arável. Parte deste material deve ser proveniente de compostos de baixo peso molecular, que são estabilizados por meio de interação com os óxidos de ferro.

O processo de estabilização de compostos nitrogenados pela fração mineral do solo, mais especificamente pelos óxidos, é realçado no trabalho de Leinweber e Schulten (2000) que avaliaram a capacidade de extração e a composição de compostos nitrogenados não hidrolisáveis em meio ácido. A presença das formas Fe^{2+}/Mn^{3+} e Fe^{3+}/Mn^{4+}, entre outras, no solo é dependente do potencial redox (PANNAMPERUMA, 1972). A redução do potencial redox do solo pode resultar na solubilização parcial de óxidos pedogênicos extraídos com ditionito citrato-bicarbonato de sódio (DCB), liberando os íons na forma Fe^{2+}/Mn^{3+} (BRÜMMER, 1974). Aspectos relacionados à energia e geometria tornam pouco provável que ligações covalentes sejam formadas diretamente entre a matéria orgânica e silicatos do solo. Este processo pode ser demostrado por meio de modelos moleculares, que apontam a ocorrência de pontes de hidrogênio e ligações organo-minerais com cátions metálicos (SCHULTEN; LEINWEBER, 2000). Leinweber e Schulten (2000) relatam que a dinâmica de redução do solo, mineralogia de óxidos pedogênicos e a ciclagem de compostos nitrogenados no solo podem estar interligadas. Diante disso, se percebe que existe elevada interação entre a fração mineral e orgânica do solo, e que algumas formas de N podem ser solubilizadas pelos processos de redução do solo, sendo disponibilizadas para as plantas.

Em solos cultivados com arroz irrigado, o NH_4^+ é considerado a principal forma de N inorgânico, o qual pode estar presente na solução do solo, adsorvido aos coloides do solo, e nas entrecamadas de argilominerais, sendo denominado de NH_4^+ fixo pela argila ou NH_4^+ não trocável (CF-NH_4) (KEERTHISINGHE et al., 1985). O CF-NH_4 pode constituir um compartimento de N importante para muitos solos, estando o seu conteúdo relacionado ao material de origem do solo, textura, teor de argila, mineralogia, saturação de K nas entrecamadas de argilominerais 2:1 e condições de umidade (NIEDER et al., 2011). Zhang et al. (2007) avaliaram o teor de CF-NH_4 e a capacidade máxima de fixação de NH_4 em solos da China, e verificaram que o teor de CF-NH_4 pode representar até 17% do teor de N total em solos de arroz irrigado e até e 40% do teor de N total em solos bem drenados. O CF-NH_4 pode ser separado em duas categorias principais: o NH_4^+ fixo nativo e o NH_4^+ recentemente fixado (PARAMASIVAM; BREITENBECK, 2000; NIEDER et al., 2011). O NH_4^+ recentemente fixado é uma fração de N mais dinâmica, sendo afetada pela adição de fertilizantes nitrogenados e pela mineralização da MOS. Deste modo, esta fração está envolvida no ciclo geral do N (CRUSH; EVANS, 1988) e está mais disponível para as plantas do que o NH_4^+ fixo nativo do solo (NOMMIK; VAHTRAS, 1982; NIEDER et al., 2011).

Estudos investigando o CF-NH_4 demonstraram que uma parte deste compartimento nitrogenado pode ser acessível às plantas (MENGEL et al., 1990; SCHERER; AHRENS, 1996; LU et al., 2010) e microflora (NOMMIK; VAHTRAS, 1982; DRURY; BEAUCHAMP, 1991; BREITENBECK; PARAMASIVAM, 1995), indicando que o CF-NH_4 é um componente dinâmico do ciclo do N do solo e pode desempenhar um papel importante na nutrição das plantas. Keerthisinghe et al. (1985) relataram que o NH_4^+ trocável, e em alguns solos o CF-NH_4, são as frações de N do solo mais importantes para o arroz irrigado. Deste modo, a natureza e a dinâmica do CF-NH_4 pode influenciar a disponibilidade de N do solo e, portanto, o NH_4^+ recentemente fixado deveria ser considerado no sistema de recomendação do fertilizante nitrogenado visando aumentar a eficiência de recuperação do N pelas plantas, bem como reduzir as perdas de N do solo. Contudo, poucos estudos foram desenvolvidos visando avaliar este compartimento do N do solo e sua relação com índices de disponibilidade de N e o seu efeito sobre a recomendação da adubação nitrogenada para as culturas.

Solos que apresentam variação quanto à mineralogia e granulometria exercem diferentes graus de proteção físico-química do N orgânico e inorgânico do solo, o que interferir na extração do AH-N por DVD. Em decorrência disso, para implementar o sistema de recomendação da adubação nitrogenada N-STaR para a cultura do arroz irrigado no estado do Arkansas, utilizando o índice AH-N, houve necessidade de ajustes no sistema de amostragem. Foram estabelecidos protocolos específicos para solos arenosos e argilosos, variando a profundidade de amostragem conforme o tipo de solo para atingir uma adequada calibração entre o teor de N extraído pelo DVD e o rendimento relativo da cultura (ROBERTS et al., 2012; FULFORD et al., 2013).

Nos solos cultivados com arroz irrigado no Sul do Brasil existe uma variação muito ampla das propriedades físicas, no teor de MOS e no teor de AH-N (DRESCHER et al., 2016).

Estas características podem influenciar o movimento dos compostos nitrogenados no perfil do solo, bem como afetar a capacidade preditiva do índice AH-N. Embora a análise por DVD seja uma extração branda, avaliando frações mais lábeis da MOS, prontamente disponíveis à degradação microbiana, o teor de AH-N extraído pode sofrer a influência do teor da argila e do conteúdo de CF-NH_4 do solo. Solos com frações granulométricas menores (isto é, silte e argila < 50 µm) podem proporcionar maior proteção à MOS e aos compostos nitrogenados, reduzindo a quantidade de N extraída. Por sua vez, o processo de alagamento e de redução do solo, podem favorecer a solubilização e a migração de formas de N lábil no perfil do solo, as quais podem ser utilizadas pelas plantas, mas não são determinadas pela análise do solo na camada superficial (0-20 cm).

Outro fator que pode influenciar a capacidade de extração do AH-N pelo DVD são os processos de preparo da amostra de solo. Os procedimentos de secagem, moagem e peneiramento (peneira com malha de abertura de 2 mm) ocasionam a desestruturação do solo e a ruptura de agregados, expondo superfícies minerais e frações de N orgânico e N inorgânico à solução extratora. Ademais, a base utilizada no procedimento de destilação alcalina é o hidróxido de sódio (NaOH) e os íons Na^+ da solução extratora, em virtude do seu raio iônico, podem expandir as lâminas dos argilominerais, favorecendo a troca e a extração do NH_4^+ recentemente fixo nas arestas dos argilominerais. Este procedimento, do ponto de vista de um índice de disponibilidade de N, é vantajoso, pois além de contabilizar frações de N orgânico lábeis, NH_4^+ trocável e em solução, também estaria acessando uma parcela do NH_4^+ recentemente fixo pela argila. Contudo, a intensidade e a importância destes processos ainda são pouco conhecidas.

Diante disso, há necessidade de se investigar a interação das propriedades físico-químicas do solo com o AH-N, a distribuição do AH-N no perfil do solo e a capacidade das plantas utilizarem o N localizado em maiores profundidades no solo e o seu efeito sobre a morfologia radicular das plantas. Uma melhor compreensão destes processos é necessária para identificar variáveis que precisam ser levadas em consideradas no processo de correlação e calibração do índice AH-N para a cultura do arroz no Sul do Brasil, almejando um aprimoramento do atual sistema de recomendação de adubação nitrogenada da cultura e um sistema de produção mais sustentável.

2.3 DISPONIBILIDADE DE NUTRIENTENTES EM MAIOR PROFUNDIDADE DO SOLO: CRESCIMENTO RADICULAR, RECUPERAÇÃO PELAS PLANTAS E NUTRIÇÃO DA CULTURA

A absorção eficiente dos nutrientes é um dos principais fatores que pode levar ao aumento do rendimento do arroz irrigado, podendo reduzir a necessidade de adubação em virtude da redução das perdas e melhor aproveitamento dos nutrientes. A eficiência de absorção de nutrientes pode estar relacionada com a morfologia e a distribuição do sistema radicular no solo (ABICHEQUER; BOHNEN, 2008). Espécies e variedades de plantas diferenciam-se quanto à capacidade de absorver nutrientes devido à variação na dimensão e morfologia do sistema radicular e/ou na cinética de absorção de nutrientes (ANGHINONI et al., 1989). Deste modo, a arquitetura radicular e a distribuição das raízes ao longo do perfil do solo também pode influenciar a absorção de nutrientes pelo arroz (ABICHEQUER; BOHNEN, 2008). Para a cultura do arroz, existe relação entre altos rendimentos de grãos e a dimensão e o padrão de distribuição do sistema radicular no solo, sendo vantajosa a localização das raízes em maiores profundidades (MORITA; YAMAZAKI, 1993), pois um maior volume de solo é explorado, facilitando a absorção de nutrientes de maiores profundidades do solo.

Estudos anteriores apontam que a cultura do arroz irrigado por alagamento raramente desenvolve raízes abaixo dos 40 cm de profundidade, em virtude do ambiente fortemente reduzido e da maior concentração de substâncias tóxicas (como Fe^{+2} e H_2S) que inibem a expansão das raízes (YOSHIDA, 1981). Comportamento similar foi observado por Abichequer e Bohnen (2008), que verificaram que mais de 84% das raízes do arroz estavam localizadas na camada de 0-20 cm, sendo que poucas raízes se desenvolveram em maiores profundidades. Por outro lado, Beyrouty et al. (1987) verificaram que o crescimento radicular máximo do arroz se estende até os 72 cm de profundidade no florescimento da cultura, o que indica que as plantas poderiam efetivamente absorver nutrientes destas camadas, contribuindo para a nutrição da cultura.

O padrão de enraizamento está relacionado às características genéticas da planta (FAGERIA; STONE, 2006; FAGERIA; MOREIRA, 2011), porém o crescimento das raízes pode ser modificado por características químicas e físicas do solo. Embora o aprofundamento das raízes do arroz possa ser prejudicado no ambiente anaeróbico em que o arroz irrigado é cultivado, tanto pelo ambiente reduzido quanto pela presença de camadas de solo compactadas em subsuperfície (utilizadas para evitar a percolação e perda de água no perfil), o esgotamento de nutrientes da camada superficial e a concentração de nutrientes em maior profundidade, como formas lábeis de N ou P, podem estimular o crescimento das raízes e da própria planta. O trabalho de Lima et al. (2010) demonstra que compartimentos mais ricos em NH_4^+ promovem o crescimento radicular, principalmente por meio de um estímulo para a emissão de raízes secundárias. Neste mesmo sentido, Mounier et al. (2014) apontam que uma maior concentração de NO_3^- estimula a o crescimento/elongação das raízes, forma de N presente na rizosfera do arroz em virtude do processo de nitrificação nesta zona aeróbica. Ademais, a maior solubilização de P em solos submetidos ao alagamento também pode favorecer o crescimento das raízes em profundidade, uma vez que maiores concentrações de P também favorecem o desenvolvimento radicular das plantas (CRUSCIOL et al., 2013).

O trabalho de Mulvaney et al. (2006) ressalta que a mineralização de N em profundidades superiores à 30 cm poderia contribuir para a nutrição das plantas e estar associada a falta de resposta a adubação nitrogenada. Neste sentido, Roberts et al. (2013) verificaram que o arroz cultivado no Arkansas (Estados Unidos) consegue utilizar efetivamente o N localizado em profundidades de até 60 cm, contribuindo para a nutrição da cultura. Diante disso, desponta a necessidade de avaliar o efeito da disponibilidade de N em maiores profundidades do solo sobre o crescimento e morfologia radicular, informações estas carentes para o Sul do Brasil, mas que podem auxiliar para o aumento da eficiência da utilização do N pela cultura. Adicionalmente, não existe informações referentes a capacidade de recuperação e da dinâmica de absorção do N de maiores profundidades pela cultura do arroz irrigado por alagamento. Uma alternativa para estudar este fenômeno é a utilização da técnica isotópica com ^{15}N.

As técnicas baseadas no uso de ^{15}N possibilitam uma melhor compreensão da dinâmica desse nutriente no sistema solo-planta-atmosfera (ALVES et al., 2005). Os autores apontam que a técnica parte do princípio de que qualquer material existente na natureza que possua N em sua constituição apresentará uma proporção de 0,3663% de átomos de ^{15}N em relação ao total de N, que em sua maioria é composto do isótopo ^{14}N. Assim, se uma planta cresce utilizando N de duas fontes (ex. solo e fertilizante) e uma das fontes for artificialmente enriquecida com ^{15}N, é possível determinar a contribuição de cada uma para a nutrição da planta (ALVES et al., 2005). Estudos mais detalhados de cada fonte também podem ser desenvolvidos, bem como avaliação da eficiência de fertilização, recuperação do N aplicado via fertilizante ou resíduos, perdas de N do sistema, contribuição da FBN, entre outros. Deste modo, a utilização de isótopos permite avaliações quantitativas (estoques e fluxos) de forma acurada e precisa, importantes para dimensionar o resultado dos fatores envolvidos em determinado sistema de produção e permitindo a adoção de estratégias que visem maximizar a eficiência de utilização de nutrientes, como o N.

3. HIPÓTESES

i) O N fixado pela argila é uma fração representativa do teor de N total do solo cultivado com arroz irrigado no Arkansas, e as frações mais fracamente adsorvidas são extraídas pelo método N-STaR;

ii) O teor de N hidrolisável em meio alcalino é influenciado pela umidade da amostra em detrimento da expansão da entrecamada das lâminas dos argilominerais, aumentando a exposição de superfícies à solução extratora, bem como pelo uso de bases com maior potencial de extração do amônio adsorvido pela argila;

iii) O N lábil extraído como N hidrolisável em meio alcalino varia com a profundidade do solo, não é um compartimento constante do N total do solo e é influenciado pelo aumento do teor de argila;

iv) O N disponível em maiores profundidades no perfil do solo contribui para a nutrição das plantas de arroz;

v) O N disponível em maiores profundidades do solo estimula o crescimento radicular, aumenta o volume de solo explorado pelas raízes e a absorção de N pela planta ao final do ciclo, o que promove maior teor de clorofila e taxa fotossintética neste período.

4. OJETIVOS

4.1 OBJETIVO GERAL

Ampliar a compreensão sobre a dinâmica do N em áreas cultivadas com arroz irrigado e sua relação com as propriedades físico-químicas do solo, bem como identificar a capacidade das plantas de arroz utilizarem o N disponível em maiores profundidades no perfil do solo.

4.2 OBJETIVOS ESPECÍFICOS

i) Determinar o teor total de amônio fixado pela argila e o percentual recuperado pelo método N-STaR em solos cultivados com arroz irrigado no Arkansas;

ii) Avaliar se o teor de umidade da amostra de solo e a base utilizada no processo de extração do N influenciam o índice de disponibilidade de nitrogênio N-STaR;

iii) Verificar a distribuição dos teores de N hidrolisável em meio alcalino no perfil dos solos cultivados com arroz no Sul do Brasil e a sua relação com as propriedades físico-químicas do solo;

iv) Investigar a dinâmica de absorção do N e a sua recuperação de diferentes profundidades do solo pela cultura do arroz no Sul do Brasil;

v) Avaliar o efeito da localização do N no perfil do solo sobre a morfologia radicular e a nutrição do arroz irrigado.

5. RESULTADOS

5.1 ESTUDO I

Quantifying clay-fixed ammonium in paddy soils and determining its availability using alkaline distillation[1]

Abstract

The Nitrogen Soil Test for Rice (N-StaR, *Oryza sativa* L.) has been calibrated and is being used to predict rice nitrogen (N) fertilizer rates. Research has identified the organic forms of N quantified via N-STaR (NH_4 released by distillation with 10 M NaOH), but no research has looked at the ability of N-STaR to quantify clay-fixed NH_4 (CF-NH_4) which can contribute to plant N uptake. This laboratory trial was initiated to determine the amount of CF-NH_4 quantified via N-STaR as well as the influence of soil drying conditions (oven dry vs. field moist) and alkali distillation method (NaOH vs. KOH) on the CF-NH_4 recovered. Seven soils representing five clayey and two loamy soil textures were collected from various rice-producing regions across Arkansas from the 0- to 15-cm and 15- to 30-cm soil depths. Total CF-NH_4 and CF-NH_4 recovered by N-STaR varied greatly across soils and soil depths with a range of 35 to 299 mg N kg soil^{-1} and 6 to 21 mg N kg soil^{-1}, respectively. The N-STaR recovered as much as 15% of total CF-NH_4 and recovery was greatest for silt loam soils. Alkaline hydrolyzable-N (AH-N) concentrations were strongly influenced by soil drying conditions and the alkali used with a higher level (22%) of AH-N recovered from oven-dry than field-moist soils and a higher (7%) recovery of AH-N with NaOH than KOH**.** These results indicate that the N-STaR method recovers a small, but significant percentage of total CF-NH_4 and may capture the portion of CF-NH_4 that may be plant available during the growing season.

Keywords: nitrogen, alkaline hydrolyzable-nitrogen, soil fertility

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[1] Artigo elaborado de acordo com as normas da revista Soil Science Society of America Journal; https://dl.sciencesocieties.org/publications/style/.

Introduction

Fertilizer N represents a large input cost for most cereal-crop-production systems, an effort to these costs can improve profitability and potentially reduce the impact of agriculture on the environment. One tool that is being tested for rice is N-Star (Roberts et al., 2012a). Crops obtain N from a multitude of sources and processes including organic matter mineralization (Sahrawat, 2006), microbial biomass turnover (Sainz et al., 2004), ammonium (NH_4^+) adsorbed to soil colloids (Norman and Gilmour, 1987; Nieder et al., 2011), N fertilization (Wienhold, 2007), and a small fraction that originates from irrigation water and other environmental and biotic sources (Hirzel et al., 2011). In lowland rice soils, NH_4^+ is considered the most important inorganic-N form, and may be present in soil solution, adsorbed to soil colloids, and in the interlayer of selected minerals in what is referred to as nonexchangeable NH_4^+ (Keerthisinghe et al., 1985).

The nonexchangeable NH_4^+ pool is often referred to as CF-NH_4, the fraction of soil N that is bonded in clay mineral interlayers. Clay-fixed NH_4^+ is an important soil-N pool, and its content is related to parent material, texture, clay content, clay mineral composition, K saturation of the interlayers of 2:1 clay minerals, and moisture conditions (Nieder et al., 2011). Vermiculite, illite, and smectite are the clay minerals in soils that are chiefly responsible for NH_4^+ fixation, as a result of NH_4^+ replacement for interlayer cations (Ca^{2+}, Mg^{2+}, Na^+, and H^+) in the expanded lattice of these clay minerals when hydrated (Stevenson and Dhariwal, 1959). Zhang et al. (2007) evaluated CF-NH_4 content and maximum capacity of NH_4^+ fixation in soils from China, and CF-NH_4 content ranged from 135 to 412 mg kg^{-1} for paddy soils and 86 to 436 mg kg^{-1} for upland soils, representing up to 17% and 40% of soil total N, respectively.

The CF-NH_4 can be separated into two main categories, native-fixed NH_4^+ and recently-fixed NH_4^+ (Paramasivam and Breitenbeck, 2000; Nieder et al., 2011). The mechanisms leading to the formation of native and recently fixed NH_4^+ lead to differences in bioavailability. Native-fixed NH_4^+ is formed in soils containing appreciable amounts of 2:1 clay minerals during pedologic development, when NH_4^+ is deposited or entrapped (Paramasivam and Breitenbeck, 2000). Additional NH_4^+ can be fixed when mineral N fertilizers are applied (Chen et al. 1989; Smith et al. 1994), and also from soil organic matter mineralization (Nieder et al. 1995), a pool referred to as recently fixed NH_4^+. Recently-fixed NH_4 takes place in the interlayer sites of the clay lattices at a medium stage of collapse (in transitional zone, near the edge of the clay crystal), where native-fixed NH_4^+ (trapped in the center of the interlayers) and exchangeable ions meet (Nommik and Vahtras 1982). The native-fixed NH_4^+ ions seem fairly immobile, while recently-fixed NH_4^+ apparently have a more dynamic nature, been involved in the general soil N cycle (Crush and Evans, 1988), and available to plants to a greater degree than native-fixed NH_4^+ (Nommik and Vahtras, 1982; Nieder et al., 2011).

Schneiders and Scherer (1998) observed that NH_4^+ ions added with mineral fertilizer or mineralized from organic compounds are temporarily fixed in the interlayers of clay minerals, but afterwards are available to rice plants. The research by Schneiders and Scherer (1998) highlighted that the CF-NH_4 pool plays an important role in the N turnover of paddy soils and N nutrition of lowland rice. Moreover, clay mineralogy, K^+ saturation of the minerals, soil microflora (Nieder et al., 2011), plant root vicinity (Mengel et al. 1990; Scherer and Ahrens, 1996), soil moisture content (Mengel, 1981), and redox potential (Schneiders and Scherer, 1998) reportedly play important roles in CF-NH_4 release. Despite the aforementioned research, the CF-NH_4 availability to plants and soil microorganisms is perhaps the least understood N form in soil (Drury and Beauchamp, 1991). Our lack of understanding is due, in part, to the limitations of various methods employed for determination of CF-NH_4 (Paramasivam and Breitenbeck, 2000).

Temporal changes in recently fixed NH_4^+ content suggest that this N pool is actively involved in N dynamics during the crop growing season (Kudeyarov, 1981; Nieder et al., 1996), depending on N fertilizer application timing and microbial demand relative to crop uptake (Kudeyarov, 1981). Research has shown that a part of the CF-NH_4 pool can be accessible to plants (Mengel et al., 1990; Scherer and Ahrens, 1996; Lu et al., 2010) and microflora (Nommik and Vahtras 1982; Drury and Beauchamp 1991; Breitenbeck and Paramasivam, 1995), indicating that CF-NH_4 is a dynamic component of the soil-N cycle and may have an important role in plant N nutrition. Keerthisinghe et al. (1985) reported that exchangeable NH_4^+, and in some soils, CF-NH_4, are the most important soil N fractions for lowland rice. The dynamic nature of soil CF-NH_4 can influence on soil N availability to the growing crop and therefore the recently fixed NH_4^+ pool should be considered in fertilizer management programs to increase fertilizer N recovery efficiency and reduce soil N losses.

Recently, University of Arkansas researchers have developed a soil-based N test for rice called N-STaR (Roberts et al., 2011, 2012b). The N-STaR is an alkaline distillation method that quantifies easily mineralized organic-N forms, such as amino acids (e.g., glutamine) and amino sugars (e.g., glucosamine) plus inorganic NH_4^+, estimating the amount of N that a soil can supply during the growing season allowing adjustment of the N fertilizer rate needed to maximize rice yield. Research has provided a better understanding of the organic-N forms and the amount quantified by N-STaR (Roberts et al., 2009a; Roberts et al., 2012b). However, the amounts of CF-NH_4 recovered by N-STaR, the effect of sample moisture condition, and the use of a different alkali for the sample distillation have not yet been investigated. The objectives of this study were to determine: 1) the amount of CF-NH_4 in rice soil from Arkansas, 2) the availability of CF-NH_4 to plants using N-STaR; 3) the effects of soil moisture, soil drying and two different alkali solutions on the amounts of N-STaR-N for a given soil.

Materials and Methods

Soil Sampling and Analysis

Seven rice fields representing five clayey and two loamy soil textures were sampled using a Dutch Auger Probe (AMS Inc., American Falls, Idaho) during 2017 (Table 1). The soil samples were collected prior rice crop seeding and soil moisture condition was close to field capacity. The seven soils will be referred to by the site-no listed in Table 1. Soil samples were obtained from the 0- to 30-cm depth and split into two 15-cm depth increments (i.e., 0–15 and 15–30 cm). The soil layers were chosen based on the soil depth (0-15 cm) used for soil fertility diagnostic and the soil depth (0-30 cm) used for N-STaR N rates recommendations for rice cropped in clay soils in Arkansas state, USA. Each soil sample included six soil cores which were composited, thoroughly mixed and split into two subsamples. One subsample was oven dried at 65°C for 48 h, ground to pass a 2-mm sieve, and stored at room temperature in cardboard containers. The second subsample was maintained at field-moisture content, passed through a sieve with 4-mm openings to get a homogeneous sample, stored in a sealed plastic bag and kept refrigerated (~2.5°C). Prior to chemical analysis, the soil moisture content was determined (Topp and Ferré, 2002). Selected soil physical and chemical properties as well as the location and taxonomic classification of each soil are presented in Table 1.

Table 1. Selected chemical and physical characteristics of the seven soils.

Soil	Soil Series	Taxonomic Class	Location (GPS)	Soil Depth	pH†	NO_3^-‡	NH_4^+‡	TN§	TC§	OM¶	Silt#	Clay#	GMC††
				cm	-	mg kg^{-1}		%					
1	Sharkey	very-fine, smectitic, thermic Chromic Epiaquerts	35°41'39" N	0-15	6.96	32.01	14.86	0.12	1.39	4.11	29.9	67.3	28.0
			90°03'39" W	15-30	6.53	5.14	12.13	0.08	1.01	3.60	24.1	61.4	27.5
2	Sharkey	very-fine, smectitic, thermic Chromic Epiaquerts	35°39'59" N	0-15	6.95	1.69	12.17	0.11	1.40	3.49	34.7	51.1	22.6
			90°04'55" W	15-30	6.98	1.39	11.11	0.08	1.05	2.95	35.9	49.8	21.6
3	Alligator	very-fine, smectitic, thermic Chromic Dystraquerts	35°20'52" N	0-15	6.38	6.61	15.43	0.09	0.95	3.69	28.4	68.4	27.3
			90°35'29" W	15-30	6.23	2.79	7.60	0.06	0.79	3.47	27.1	71.0	26.7
4	Calloway	fine-silty, mixed, active, thermic Aquic Fraglossudalfs	35°07'28" N	0-15	7.45	26.46	4.37	0.06	0.66	1.80	79.6	16.3	16.6
			90°55'36" W	15-30	7.09	19.06	2.72	0.03	0.28	1.30	79.7	16.3	17.3
5	Tichnor	fine-silty, mixed, active, thermic Typic Endoaqualfs	34°28'20" N	0-15	5.83	7.94	6.21	0.06	0.57	1.87	75.9	20.1	16.3
			91°25'26" W	15-30	7.00	5.06	4.54	0.03	0.34	1.55	76.0	21.4	16.5
6	Desha	very-fine, smectitic, thermic Vertic Hapludolls	33°48'05" N	0-15	7.78	1.20	6.29	0.08	0.93	2.96	46.5	52.8	28.2
			91°17'12" W	15-30	7.47	5.57	6.71	0.04	0.37	2.36	35.0	64.6	28.3
7	Roellen	fine, smectitic, thermic Vertic Epiaquolls	35°13'44" N	0-15	6.31	1.31	16.16	0.13	1.79	4.23	42.2	57.4	26.8
			92°53'19" W	15-30	6.53	0.42	11.45	0.07	1.17	3.57	36.0	62.7	25.7

† Determined on a 1:2 (m:v) soil-to-water suspension (Sikora and Kissel 2014);
‡ Determined using salicylate colorimetric techniques (Mulvaney, 1996);
§ TN, total N, TC, total carbon, and clay-fixed NH_4^+–N determined using dry combustion (Bremner, 1996; Nelson and Sommers, 1996);
¶ Determined using loss-on-ignition method (Zhang and Wang 2014);
# Determined using hydrometer method (Gee and Or, 2002).
††Gravimetric moisture content- determined using thermo gravimetric method at 65°C (Topp and Ferré, 2002).

Total clay fixed ammonium (TCF-NH_4) was measured using dry combustion (Liang et al., 1999) following removal of organic-N and exchangeable inorganic-N , with modifications at drying procedure. Briefly, 20 mL of 2 mol L^{-1} KOH–KOBr were added to 1 g of dry and finely ground (crushed with a mortar and pestle and passed through a sieve with 0.15-mm openings) soil in a 200 mL glass beaker. The beaker was swirled to mix the soil and KOBr solution, covered and kept at room temperature (21°C) for 2 h before 60 mL of deionized water was added, heated to approximately 99°C for 5 min and then allowed to slowly cool and settle overnight (16 h) at room temperature.

Following soil pretreatment with 2 mol L^{-1} KOH–KOBr, the clear supernatant was decanted and three sequential extractions with 0.5 mol L^{-1} KCl were used to remove exchangeable NH_4^+-N. The soil residue was quantitatively transferred to a 50 mL glass centrifuge tube with 30 mL of 0.5 mol L^{-1} KCl, shaken for 5 min, centrifuged at 959 times g for 10 minutes, and the entire process repeated. Upon completion of the third extraction, the clear supernatant was discarded, and the contents of the centrifuge tube were oven-dried overnight at 105°C. Pretreated, oven-dried soil was removed from the centrifuge tube, crushed with a mortar and pestle, weighed, and stored at room temperature prior to analysis of CF-NH_4. TCF-NH_4 was quantified by dry combustion of pretreated soil residue using an Elementar CN Variomax (Elementar Americas, Inc., Mt. Laurel, NJ). N-STaR extractable clay-fixed ammonium (NECF-NH_4) was also quantified on soil residue by N-STaR procedure (Bushong et al., 2008; Roberts et al., 2009a). One g of soil residue was distilled with 10 mL of 10 mol L^{-1} NaOH. A total of 35 mL of distillate was collected in 40 g L^{-1} boric acid indicator (4% w/v aqueous solution with bromocresol green-methyl red indicator) and the N concentration of the distillate determined by acidimetric titration (719 S Titrino, Metrohm Ltd., Herisau, Switzerland).

The effect of soil moisture and alkali used during N-STaR distillation was evaluated by distilling 1 g of soil subsamples with 10 mL of 10 mol L^{-1} NaOH and a duplicate sample with KOH, following the same titration procedure used to determine NECF-NH_4. Distillation of soil samples with NaOH and KOH was proposed by Barshad (1951) as a methodology for CF-NH_4 evaluation. In Barshad's alkaline-distillation method, CF-NH_4 is measured by the difference of NH_4^+ released between the two distillations, assuming that NaOH distillation accesses clay-fixed NH_4^+-N + exchangeable NH_4^+-N + organic-N, while KOH distillation accesses only exchangeable NH_4^+ plus organic-N, due to the blocking effect of K^+ ions for fixed NH_4^+ release. Thus, the relationship between alkaline-distillation CF-NH_4 and NECF-NH_4^+ was also evaluated. All analyses were performed with four replicates.

Statistical analysis

The experiment was a completely randomized design with a factorial arrangement. Each treatment was replicated four times with replicate being a random effect in the ANOVA model. ANOVA was conducted to determine the influence of soil, soil depth and their interaction (fixed effects) on TCF-NH_4 and NECF-NH_4, and N-STaR recovery of total clay-fixed ammonium (NRCF-NH_4). For the quantification of AH-N, the influence of drying condition and alkali distillation method and their interaction (fixed effects), ANOVA was conducted separately for each soil and soil depth with replicate as a random effect. When the interaction was non-significant, one way ANOVA conducted. Quantification of AH-N was evaluated for each soil and soil depth separately, considering that previous research has already shown and discussed the differences on AH-N among soils and depths (Roberts et al. 2009b, 2011). Means were compared using Fisher's least significant difference (LSD) at the 0.05 probability level. Linear regression model was used to describe the relationship between TCF-NH_4 and soil TN for each soil depth, and exponential model was adjusted to describe

NECF-NH_4 and alkaline distillation method relationship. Regression analysis was completed on data means and statistical significance was evaluated at the 0.05 probability level. The influence of soil clay content on total and NECF-NH_4 and NRCF-NH_4 was evaluated with Pearson correlation coefficient on data means, and significance was evaluated with Student's t test. Statistical analyses and figures were performed with Sigma Plot 12.0 software (Systat Software Inc., San Jose, CA), and linear regressions slopes and intercepts were compared at 0.05 probability level with BioStat 5.0 software (AnalystSoft Inc., Walnut, CA).

Results and Discussion

Quantification of total and available clay fixed ammonium

There was a significant soil x soil depth interaction ($P < 0.01$) for the quantification of TCF-NH_4 via dry combustion, NECF-NH_4, and NRCF-NH_4. TCF-NH_4 ranged from 35 to 286 mg kg^{-1} in the 0 to 15 cm depth and 71 to 299 mg kg^{-1} in the 15 to 30 cm depth (Table 2).

Table 2. Total clay-fixed NH_4^+ (TCF-NH_4) concentration, CF-NH_4 extracted by N-STaR (NECF-NH_4), and N-STaR recovery of TCF-NH_4 (NRCF-NH_4) as influenced by soil and sampling depth.

Soil	TCF-NH_4		NECF-NH_4		NRCF-NH_4	
	0-15	15-30	0-15	15-30	0-15	15-30
	……………… mg kg^{-1} ………………				……… % ………	
1	258	295	17	21	6.4	6.8
2	222	214	16	12	7.3	5.8
3	286	299	17	19	6.1	6.3
4	35	71	7	6	15.3	8.3
5	67	82	11	10	12.6	12.4
6	225	282	14	14	6.4	5.1
7	232	215	15	16	5.8	7.5
LSD$_{(p<0.05)}$†	22.77		2.50		2.2	
$r_{\text{clay content }(p<0.001)}$‡	0.97		0.89		-0.83	

†Fisher's least significant difference (LSD) at the 0.05 probability level.
‡Pearson's correlation coefficient with clay content, significant at 0.001 probability level.

Significant differences occurred among soils with the lowest TCF-NH_4 measured in the two silt loams, soils 4 and 5, indicating silt loam soils have less CF-NH_4 than clay soils. Differences in CF-NH_4 among soils has been reported due to differences in parent material and mineralogy (Nieder et al., 2011), clay content (Juang, 1990), degree of K^+ saturation at the exchange complex of soil colloids and K^+ saturation of the interlayers of 2:1 clay minerals (Doram and Evans, 1983), and soil moisture conditions (Black and Waring, 1972). The TCF-NH_4 content we found in the seven soils from Arkansas is similar to results with upland soils from Germany (Steffens and Sparks, 1997) and Canada (Liang et al., 1999) and lowland soils from China (Zhang et al., 2003; 2007).

A significant and positive linear relationship was observed between TCF-NH_4 and soil TN concentration at each sampling depth (Fig. 1). The coefficient of determination (R^2) was numerically greater for soil from the 0 to 15 cm depth (R^2 = 0.58) than for the 15 to 30 cm depth. The slope suggests that CF-NH_4 was 24.8, and 28.1% of soil TN for the 0-15, and 15-30 cm sampling depths, respectively. Despite the absence of statistical difference between the linear slopes (p= 0.5593), the CF-NH_4 represents a numerically greater percentage of soil TN in subsurface (15–30 cm) soil compared to surface (0–15 cm) soil. In a soil profile, the CF-NH_4 content as percentage of soil TN (inorganic plus organic) generally increases with soil depth (Black and Waring, 1972; Doram and Evans, 1983; Zhang et al., 2003) because soil organic matter content usually decreases as depth increases with soil depth. Moreover, at the soil 15-30 cm depth might be higher K depletion due to plant absorption, and therefore a lower K saturation of 2:1 clay interlayer, which could lead to greater NH_4 fixation potential by clay minerals. Nieder et al. (2011) reviewed the published literature and reported similar amounts of soil TN present as CF-NH_4, which indicates CF-NH_4 is an important pool of soil-N, representing up to 28.1 % of soil TN for Arkansas's paddy soils.

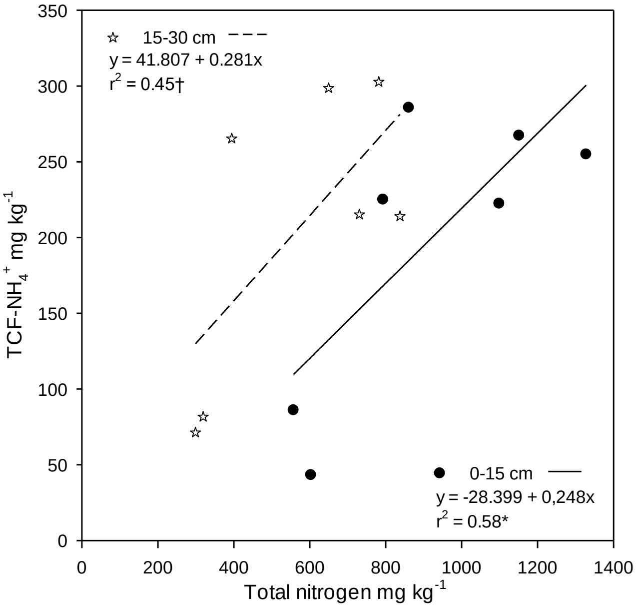

Figure 1– Total clay-fixed ammonium (TCF-NH_4) determined using dry combustion, regressed on soil total N at a sampling depth of 0- to 15-cm and 15- to 30-cm. † and * denotes statistical significance of the linear regression at the 0.1 and 0.05 level of probability. Regression equations were compared and showed statically difference for intercept (p= 0.0049), while the difference between slopes is non-significant (p= 0.5593).

NECF-NH_4 content followed the same trend of TCF-NH_4, with lower values for silty soils and higher values for clay soils (Table 2). Nonetheless, NECF-NH_4 content was much lower than TCF-NH_4, ranging from 7 to 17 mg kg^{-1} in the 0 to 15 cm depth and 6 to 21 mg kg^{-1} in the 15 to 30 cm depth. The highest NECF-NH_4 and TCF-NH_4 contents were observed at sites 1 and 3, which also had the highest clay and exchangeable NH_4^+ contents (Table 1). The higher clay and exchangeable NH_4^+ contents (Table 1) suggest a chemical equilibrium between fixed NH_4^+, exchangeable NH_4^+ and NH_4^+ in soil solution (Nommik and Vahtras, 1982). Juang (1990) showed that fixed NH_4^+ is highly correlated with clay content, which is supported by the high Pearson correlation coefficients of 0.97 and 0.89 for TCF-NH_4 and NECF-NH_4, respectively (Table 2).

Different from the TCF-NH_4 content, the NRCF-NH_4, was greater for the silt loam soils, sites 4 and 5, which might be related to different soil mineralogy and a probably lower CEC, exchangeable K^+, and K^+ saturation at 2:1 clay minerals. Silt loam soils with a lower solution and exchangeable K concentration would allow greater opening of 2:1 clay interlayer wedge site, where NH_4^+ could be easily replaced by the Na ions from N-STaR's alkaline solution. On the other hand, the clay soils would experience an edge blocking effect from more solution and exchangeable K^+, which would also explain the negative correlation ($r=-0.83$) between NRCF-NH_4 and soil clay content.

Overall, the NRCF-NH_4, the amount of NECF-NH_4 expressed as a percentage of TCF-NH_4, was 6 to 15% (Table 2). However, the amount of NRCF-NH_4 could be overestimated compared to the regular N-STaR soil analysis method. The drying procedure post-KOBr treatment (at 105 °C) may have broken some clay mineral lattices and exposed more surfaces where fixed NH_4^+ could be extracted by the NaOH solution used with N-STaR method. Thus, the amount of NECF-NH_4 and NRCF-NH_4 could be higher than at samples dried at lower temperatures.

The percentage of CF-NH_4 extracted by N-STaR and predicted to be plant available was relatively low (≤15%) regardless of soil textural class suggesting that only a small fraction of TCF-NH_4 may be available for plant uptake. The NRCF-NH_4 is similar to previous research conducted to determine CF-NH_4 availability for crops, indicating that N-STaR can provide an index of CF-NH_4 availability to crops. The percentage of TCF-NH_4 released is suggested to range from 4 to 25% in different soils (Steffens and Sparks, 1997). According to Smith et al. (1994), an average of 8 and 4% of CF-NH_4 was released from the surface and subsurface layers, respectively, from several soils across the United States.

According to Nieder et al. (2011), the inability to distinguish between recently CF-NH_4 (i.e., available NH_4^+) and native CF-NH_4 (i.e., unavailable NH_4^+) obscures the assessment of the biological importance of CF-NH_4. Recently fixed NH_4^+ entrapped near the edges of the clay lattices is not completely retained against alkaline extraction and could therefore be a likely contributor to plant available-N (Paramasivam and Breitenbeck, 2000). Plant species influence the CF-NH_4 pool through different mechanisms (Nieder et al., 2011). Plants can take up soluble or exchangeable NH_4^+ from the vicinity of NH_4^+-fixing clays and thus promote diffusion of ions out of the interlayers (Mengel et al. 1990; Scherer and Ahrens, 1996). Plant roots affect CF-NH_4 pool indirectly by releasing exudates that promote the activity of soil microflora and microbial-N uptake (Marschner, 2012). For rice plants, the rhizosphere may have even more importance for the CF-NH_4 release.

Rice roots not only deplete the soil solution NH_4^+, but also release O_2 in the rhizosphere where the conversion of NH_4^+ to NO_3^- by aerobic microflora may mobilize the CF-NH_4 out of mineral interlayers by diffusion (Zhang and Scherer, 2002). Additionally, O_2 secretion promotes Fe^{2+} oxidation, resulting in a decrease at the rhizosphere pH, and therefore increasing H^+ concentration (Zhang and Scherer, 2002). So, in lower pH soil, H^+ ions can penetrate into the wedge zones of clay minerals and displace specifically adsorbed cations

(Sparks and Liebhard, 1982), a process that might increase CF-NH_4 release at the rice rhizosphere (Zhang and Scherer, 2002). Thus, due to CF-NH_4 dynamics during plant growth and flooding processes on rice paddies, the amount of CF-NH_4 available to plants may be greater than the amount of CF-NH_4 released using N-STaR alkaline distillation.

Quantification of AH-N by NaOH and KOH distillations

A significant drying condition x alkali interaction ($P<0.05$) was observed for the quantification of AH-N for soils 1, 2 and 3 at both soil depths and soil 6 at the 0 to 15 cm depth (Table 3). The amount of AH-N extracted from soils 4, 5, and 7 was affected only by one or both of the main effects. Alkaline hydrolyzable-N determined using NaOH ranged from 46 to 182 mg N kg soil^{-1} across all soils and depths, while the AH-N range extracted with KOH was numerically (on average 7%) lower, ranging from 44 to 176 mg N kg soil^{-1} (Table 4).

Table 3. Analysis of variance (ANOVA) *p* values for the quantification of alkaline hydrolyzable-N in seven soils, as influenced by drying condition (DC), alkali distillation method (A) and main effects of interactions.

Soil depth	Source of Variation	df	Soil site						
			1	2	3	4	5	6	7
			p value						
0-15 cm	DC	1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	A	1	<0.001	<0.001	<0.001	0.877	0.014	0.427	0.808
	DC x A	1	<0.001	0.001	0.003	0.771	0.211	0.049	0.826
15-30 cm	DC	1	<0.001	<0.001	<0.001	<0.001	0.002	0.454	<0.001
	A	1	<0.001	<0.001	0.008	0.866	0.335	<0.001	<0.001
	DC x A	1	0.003	0.024	0.007	0.363	0.323	0.475	0.744

Table 4. Mean alkaline hydrolyzable-N (AH-N) concentration estimated by the NaOH and KOH distillations, as influenced by oven drying in seven soils from Arkansas.

Soil	Drying	Alkali distillation method					
		NaOH	KOH	Average‡	NaOH	KOH	Average
		0-15 cm			15-30 cm		
		mg AH-N kg^{-1}					
1	Oven dry	182	176	-	157	132	-
	Field moist	152	129	-	121	110	-
	Average§	-	-		-	-	
$LSD_{(p<0.05)}$†		3		-	6		-
2	Oven dry	165	163	-	133	127	-
	Field moist	134	112	-	103	88	-
	Average	-	-		-	-	
$LSD_{(p<0.05)}$		7		-	6		-
3	Oven dry	136	128	-	127	113	-
	Field moist	109	88	-	71	71	-
	Average	-	-		-	-	
$LSD_{(p<0.05)}$		5		-	7		-
4	Oven dry	-	-	100	-	-	59
	Field moist	-	-	89	-	-	45
	Average	-	-		-	-	
$LSD_{(p<0.05)}$		-		3	-		5
5	Oven dry	-	-	108	-	-	78
	Field moist	-	-	92	-	-	71
	Average	102	98		-		
$LSD_{(p<0.05)}$		3		3	-		4
6	Oven dry	113	115	-	-	-	-
	Field moist	103	99	-	-	-	-
	Average	-	-	-	78	67	-
$LSD_{(p<0.05)}$		5		-	5		-
7	Oven dry	-	-	189	-	-	144
	Field moist	-	-	134	-	-	109
	Average	-	-		133	120	
$LSD_{(p<0.05)}$		-		8	6		6

†Fisher's least significant difference (LSD) at the 0.05 probability level.

‡Compares over alkaline hydrolyzable N values between soil drying conditions, averaged across alkaline distillation methods.

§Compares alkaline hydrolyzable N values between alkaline distillation methods, averaged across drying condition.

The AH-N concentrations measured in this study were similar to the results of Roberts et al. (2012b) who reported that AH-N, quantified to a 60-cm depth, had a wide range even within a particular soil textural class. The difference between the NaOH and KOH methods can be attributed to the differences between cations that expand (Ca^{2+}, Mg^{2+}, Na^+, and H^+) or contract the clay lattice (K^+, Rb^+, Cs^+) (Stevenson and Dhariwal, 1959), with the NaOH extracting more AH-N than KOH.

The alkali used for the extraction of labile organic-N and mineral NH_4^+-N by N-STaR had a significant influence on the amount of AH-N quantified during distillation. This effect might be even greater for soils with a predominance of vermiculite, illite, and montmorillonite clay minerals, due to a higher capacity CF-NH_4. Therefore, for soils with these clay mineral types, the evaluation of both alkalis (i.e., NaOH and KOH) is necessary in the process of N-STaR correlation and calibration, because the amount of CF-NH_4 extracted with NaOH could lead to an overestimation of N-STaR index, and therefore influence N fertilizer recommendations.

Oven drying had significant effect on AH-N recovery for both methods in 13 of the 14 soil and soil depth comparisons (Table 3). Oven dry soil had equal or greater (on average 22%) amounts of AH-N compared to field moist soil (Table 4). The tendency for more AH-N to be extracted from oven dried soil might be due to grinding procedure or clay layer separation (Scott and Smith, 1968), which breaks soil aggregates and clay mineral lattices, exposing more surfaces where fixed NH_4^+ can be replaced by other cations, such as Na^+ and K^+ present in the extractant, and therefore be hydrolyzed during the distillation procedures. On the other hand, for field moist samples, NH_4^+ replacement should occur just at the edges of expanded lattices. Moreover, similar to K^+, the types of clay mineralogy and drying temperature affect NH_4^+ fixation and release. Previous studies have reported increases of NH_4^+ release after drying field moist soil samples (Nelson and Bremner, 1972; Frye and

Hutcheson, 1981; Nina and Sigunga, 2012). This behavior can be ascribed to decomposition of organic and inorganic-N sources, such as ammonium phosphate, which increases solubility by heating, releasing NH_4^+ ions that can be retained by cation exchange sites of soil colloids (Frye and Hutcheson, 1981; Nina and Sigunga, 2012). Hence, the higher AH-N values observed it the oven dry samples can be a result from an increase in NH_4^+ release during both heating/drying and grinding of the soil samples.

CF-NH_4 determined by N-STaR and the difference in alkaline-distillation method

Clay-fixed NH_4^+ extracted using N-STaR had an exponential relationship (r^2=0.73) with the difference between the two alkaline-distillation methods (Fig. 2).

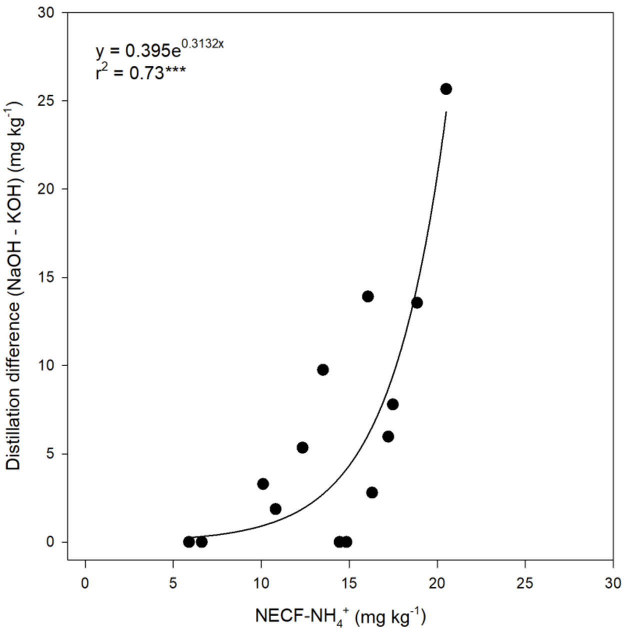

Figure 2– Clay-fixed ammonium extracted with Nitrogen Soil Test for Rice - N-STaR (NECF-NH_4) regressed on alkaline-distillation (NaOH – KOH) CF-NH_4. *** denotes statistical significance of the regression equation at the 0.001 level of probability.

Despite the similarities in the amount of CF-NH_4 recovered for most soils, some amount of recently fixed NH_4^+ seems to be accessible to N-STaR and the alkaline-distillation (NaOH – KOH) alike. This behavior might be related to the extraction of part of recently fixed NH_4^+ (i.e. NH_4^+ fixed at the edges of the clay mineral) during KOH distillation, which is not removed during KOH-KOBr pretreatment and KCl extractions. Thus, this amount of recently fixed NH_4 is accounted for as CF-NH_4 by the N-STaR method, but not by the alkaline-distillation. Another effect, as aforementioned, could be the greater oven-drying temperature (105 °C) used after the KOBr treatment, releasing more NH_4^+ and increasing the amount of NECF-NH_4 compared to the NaOH extraction following 65 °C oven-drying temperature (possibly 10 mg kg^{-1} of NH_4^+ visible the low end at figure 2). This effect can explain the predominantly lower content of CF-NH_4 across soils and depths using the alkaline-distillation, with the greatest differences for the silt loam sites 4 and 5.

Summary

Research with two silt loam and five clay soils used for rice and soybean production in Arkansas showed that CF-NH_4 represents 24.8, and 28.1% of soil TN for the 0-15 and 15-30 cm sampling depths, respectively, and clay-textured soils have substantially greater amounts of CF-NH_4 than silt loam soils. A greater proportion of the CF-NH_4 for the clay textured soils was determined to be extractable and potentially available for plant uptake than for the silt loam soil. These results indicate that CF-NH_4 is an important soil-N pool in Arkansas's clay soils which could be a potential fate of added fertilizer-N and also a primary source of N for the rice crop.

The N-STaR method estimated that only a small portion (≤15%) of TCF-NH_4 was available for plant uptake in the soils investigated. Release of CF-NH_4 using alkaline steam distillation suggests that soil test methods such as N-STaR may quantify an important plant available portion of soil TCF-NH_4 as AH-N. Also, N-STaR use of NaOH appears to be more sensitive in accounting for recent/weakly fixed NH_4^+ compared to when KOH is utilized with the alkaline-distillation method. Nevertheless, the fact that N-STaR is able to access CF-NH_4 pool reinforces the results of previous research (Roberts et al., 2011, 2012) showing the ability of N-STaR to provide an index of plant available-N for rice. The N-STaR method is able to extract the primary soil-N pools that plants have access to during the growing season: easily mineralizable organic-N forms, mineral-N as solution and exchangeable NH_4^+ (Roberts et al., 2009a), and as shown in the present study nonexchangeable NH_4^+ that is fixed at the edges of clay minerals.

Oven drying and alkali type influenced the amount of AH-N extracted from the soils studied. The heating/drying and grinding procedures conducted on the soil samples increased NH_4 release and resulted in higher AH-N content than in field-moist, unground soil samples. Distillation with NaOH provided an average 7% higher extraction of AH-N than KOH. Thus, in order to accurately predict potentially mineralizable soil-N, soil drying conditions and alkali should be considered in future studies and calibration processes, since they can influence N-STaR index and therefore fertilizer N rate recommendations.

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Figure captions

Figure 1– Total clay-fixed ammonium (TCF-NH_4) determined using dry combustion, regressed on soil total nitrogen at a sampling depth of 0- to 15-cm and 15- to 30-cm. † and * denotes statistical significance of the linear regression at the 0.1 and 0.05 level of probability. Regression equations were compared and showed statically difference for intercept (p= 0.0049), while difference between slopes is non-significant (p= 0.5593).

Figure 2. Clay fixed ammonium extracted with Nitrogen Soil Test for Rice - N-STaR (NECF-NH_4) regressed on alkaline-distillation (NaOH – KOH) CF-NH_4. *** denotes statistical significance of the regression equation at the 0.001 level of probability

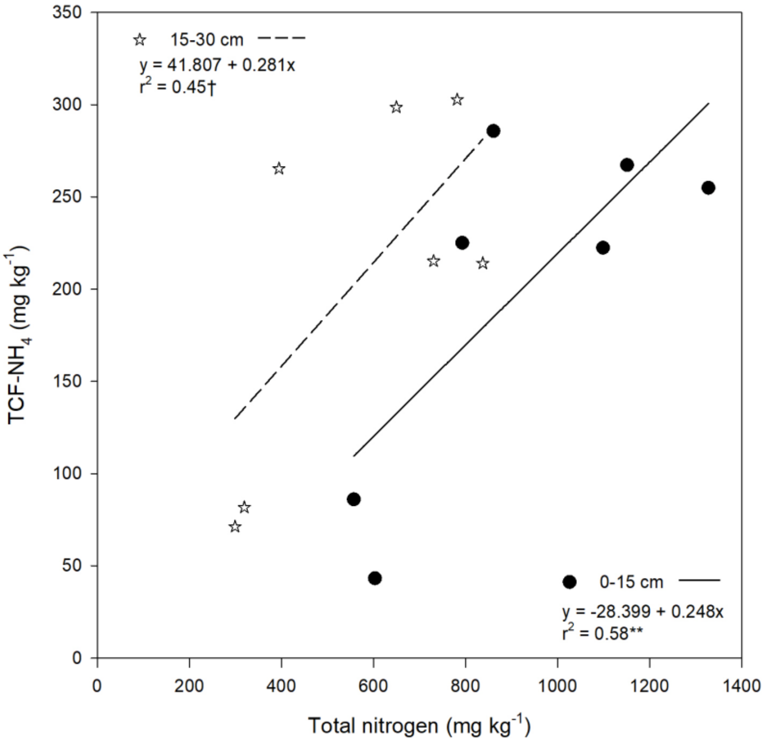

Figure 1. Total clay-fixed ammonium (TCF-NH_4) determined using dry combustion, regressed on soil total N at sampling depths of 0- to 15-cm and 15- to 30-cm. † and * denotes statistical significance of the linear regression at the 0.10 and 0.05 levels of probability. Regression equations were compared and showed statically difference for intercept (p= 0.0049), while difference between slopes is non-significant (p=0.5593).

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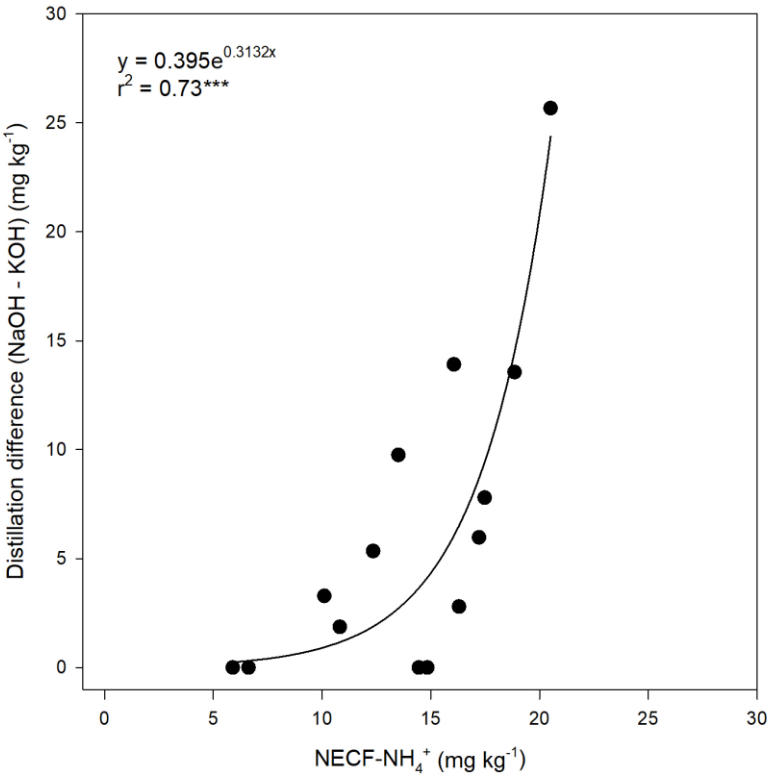

Figure 2. Clay-fixed ammonium extracted with Nitrogen Soil Test for Rice - N-STaR (NECF-NH_4) regressed on alkaline-distillation (NaOH – KOH) CF-NH_4. *** denotes statistical significance of the regression equation at the 0.001 level of probability.

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5.2 ESTUDO II

Alkaline hydrolysable nitrogen in paddy soils and properties that dictate its distribution in soil profile[1]

Abstract

Nitrogen (N) is a key nutrient for rice production and its bioavailability in paddy soils is strongly coupled to soil organic matter (SOM) cycling. A better understanding of soil potentially available N forms, such as alkaline hydrolyzable nitrogen (AH-N), distribution in depth will support the development of best management practices to improve N use efficiency for rice while minimizing adverse environmental effects. Fifteen rice fields from Rio Grande do Sul state were selected and stratified soil samples were taken to a depth of 60 cm before crop establishment. Selected soil physical and chemical properties were analyzed to evaluate their relationship with AH-N content in soil profile. The AH-N bellow 20 cm varied and increased, decreased, or remained constant compared with the concentrations quantified in the 0 to 20 cm depth. Although clay and clay + silt contents were highly correlated to AH-N for some soils, the major property dictating AH-N distribution in depth is total N (TN) as its correlation is mainly by direct effect. The AH-N is not a constant N pool in depth as the proportion of TN recovered as AH-N across soil sites and depths presented a high amplitude, indicating that AH-N can be affected by soil management practices even when TN has no major changes. The distinct distribution of AH-N among soil sites and depths indicates that greater depths than 0 to 20 cm should be considered for a correct prediction of N availability for flooded rice in Southern Brazil.

Keywords: Fertilizer recommendation, Nitrogen availability, Organic matter, Organomineral interaction, *Oryza sativa* L., Soil analysis

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Introduction

Global crop yields have been continuously increasing to meet population growth and food demand (Guo et al., 2017). However, to maximize grain yield, farmers often use more nitrogen (N) fertilizer than the minimum required for maximum crop growth (West et al., 2014), which represents high environmental risk, such as eutrophication of waters, loss of biodiversity, global warming and stratospheric ozone depletion, often found in high-income areas (Rütting et al., 2018). On the other hand, the limited access to mineral fertilizers can lead to low yields and insufficient food supply in low-income areas (Sutton et al., 2011). This characteristic raises the worldwide challenge to improve soil fertility and N management for different type of climates, soils and crop conditions around the world (Rütting et al., 2018).

Brazil is among the top ten rice producers in the world with about 11 million tons, standing out as the largest producer of Latin America and from outside Asia (GRiSP, 2013). Rice is a major food supply for Brazilian population and is a substantial crop for Southern Brazil economic development, which accounts for more than 70% of Brazilian rice production (SOSBAI, 2016). Nitrogen is one of the most limiting nutrients for rice production due to its importance to reach high yields (Fageria et al., 2014), the large proportion in production costs (Deng et al., 2012) as well as the nutrient complex dynamics in the environment and high loss potential from the soil system (Chowdary et al., 2004; Canfled et al., 2010). Therefore, in order to increase efficiency and minimize potential environmental consequences, a complete understanding of soil N will support the development of best management practices to improve the use of soil N supply in agricultural production systems while minimizing adverse environmental effects (Li et al., 2014).

The bioavailability of N in paddy soils is strongly coupled to soil organic matter (SOM) cycling and is a crucial factor in determining crop yield (Kader et al., 2013). Therefore, the N fertilizer recommendation for flooded rice in many regions, such as in Southern Brazil, is based on soil SOM analysis at the 0 to 20 cm soil layer (SBCS, 2016). So, the N rate is interpreted form SOM classes; however, for a similar rice yield estimation, the N rates are very close for different classes of SOM, and frequently there is not an adequate response to N fertilizers application. This behavior can be related to poor relationships of total organic carbon with mineralizable N, rice yield or N uptake by wetland rice (Cassman et al., 1996; Adhikari et al., 1999), resulting in a lack of correlation between SOM levels and rice relative yield (Scivittaro and Machado, 2004).

Mineralization of organic N in paddy soils is affected by soil properties and redox conditions. Soil clay content can negatively affect N mineralization due to SOM binding to mineral particles (Hassink, 1992), while pH, moisture and temperature are often non-linearly related to N dynamics (Paul et al., 2003). In flooded rice production system, the unique water management involving aerobic and anaerobic conditions due to dry-wet cycles results in remarkable changes in soil biological, chemical, and physical properties, influencing the transformation and translocation of N fertilizer in soil (Kadiyala et al., 2015) as well as affecting the dynamics of SOM decomposition, N cycle and plant N uptake patterns. Furthermore, rice plant response to N fertilizer application is also affected by environmental conditions (Scivittaro and Machado, 2004), timing and rate of N application (Dunn et al., 2016) as well as the N addition in soil system via biological N fixation by non-symbiotic bacteria communities (Bei et al., 2013; Ma et al., 2018). These aspects in soil system and rice production difficult soil N availability prediction by SOM content.

Reliable prediction of N mineralization in wetland soils will improve the sustainability of agriculture as it allows farmers to determine the rate of N fertilizer application required to optimize crop yield (Kader et al., 2013). Thus, the evaluation of N availability by more sensitive methods that determine organic-N fractions, more readily available to microbial consumption and mineralization, can provide a better estimation of the N availability during the crop season and result in better association with plant N uptake and yield. Roberts et al. (2011, 2012) have developed a soil-based N test for rice production systems in Arkansas, United States of America, called Nitrogen Soil Test for Rice (N-STaR). The N-STaR soil-based N test is an alkaline distillation method that quantifies easily mineralizable organic-N forms, such as amino acids (e.g., glutamine) and amino sugars (e.g., glucosamine) plus inorganic-N as NH_4^+-N (Roberts et al., 2009a) considered alkaline hydrolizable N (AH-N). However, Roberts et al. (2009b) reported that the AH-N is not evenly distributed in soil profile and that some sites can have significant increases in AH-N concentration in greater depths than those commonly sampled for soil analysis. Thus, for the successful calibration of the N-STaR method, soil samples should be taken deeper than conventional sampled, such as at 0 to 45cm depth for silt loam soils in Arkansas (Roberts et al., 2011, 2012).

Based on a good correlation between AH-N quantified by direct steam distillation with N mineralization in incubation experiment and N uptake by rice plants, Drescher et al. (2016) reported a potential use of AH-N as an index to predict N availability in Southern Brazil paddy soils. Although, there is yet no information about AH-N distribution in depth for Southern Brazil rice paddies and soil properties that are associated with these N forms. The successful calibration of N-STaR for rice in Southern Brazil depends on further investigation of AH-N distribution in depth for Southern Brazil paddy soils, including a better understanding of soil properties associated with these N forms. We hypothesized that the labile organic-N forms extracted as AH-N vary in soil depth, are not a constant proportion of total N and are affected by soil particle size fractions.

The objective of this research was to (a) examine the variation of AH-N in depth at Southern Brazil paddies and (b) verify soil properties that dictate AH-N distribution in soil profile.

Material and methods

Fifteen rice fields from Central Depression region of Rio Grande do Sul state, Brazil, were selected representing a variety of physical and chemical properties. The fifteen soils will be referred to by the site-no listed in Table I. Stratified soil samples were taken before crop establishment to a depth of 60 cm (i.e., 0-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30-35, 35-40, 40-50, and 50-60 cm deep) by opening a trench in soil profile and using a spatula and a ruler to take samples at each depth. Soil samples were oven dried at 55 °C for 48 h, ground to pass a 2 mm sieve, and stored at room temperature in plastic containers.

The soil AH-N content was determined using direct steam distillation (Roberts et al., 2009a) with three replicates. Selected soil physical and chemical properties were also analyzed to evaluate their relationship with AH-N content in soil profile: total C (TC) and total N (TN) were determined using dry combustion at elemental analyzer (Thermo Scientific, Flash EA 1112, Milan, Italy); NO_3^--N and NH_4^+-N were extracted with 2 mol L^{-1} KCl and determined using salicylate colorimetric techniques (Mulvaney, 1996); pH was determined on a 1:2 (m:v) soil water suspension, P and K extracted with Melich-1 and Ca, Mg, Mn and Al extracted with 1 mol L^{-1} KCl (Tedesco et al, 1995); total clay fixed ammonium (TCF-NH4) was measured using dry combustion (Liang et al., 1999) at elemental analyzer (Thermo Scientific, Flash EA 1112, Milan, Italy) following removal of organic-N and exchangeable inorganic-N with 2 mol L^{-1} KOH–KOBr pretreatment for 10 min (Zhang and Scherer, 1998); granulometric fractions were determined using pipette method (Donagema et al., 2011). Soil physical and chemical properties at the 0 to 20 cm depth and are presented in Table I. Previous study developed by Roberts et al. (2009b) reported that AH-N is not a constant fraction of TN. Thus, the amount of TN recovered as AH-N (i.e., the amount of AH-N expressed as TN) in soil profile was also evaluated.

Statistical analysis

Analysis of variance (ANOVA) was conducted to determine the influence of soil depth on AH-N content for each soil. Means were compared using Fisher's least significant difference (LSD) at the 0.05 probability level. The relationship between selected physical and chemical properties and AH-N content was evaluated by the Pearson's linear correlation coefficient (r) in depth for each soil site and in a global analysis for all soil sites and depths. The significance of r values was determined by Student's t test. Subsequently, path analysis was performed to study the causal path (i.e., direct effects) of soil properties on AH-N (dependent variable). Path analysis permits the partitioning of simple correlation coefficients between dependent variables (AH-N content) and independent variables (soil properties) into direct and indirect effects. Therefore, only the soil properties with highest correlation ($r>0.40$) with AH-N content in the global analysis were used (i.e., TN, TC, Mn, sand, clay, and clay + silt). Prior, proceeding with the path coefficients estimation, the multicollinearity diagnostic was set in the explanatory variables (i.e., TN, TC, Mn, sand, clay, and clay + silt) correlation matrix according to the condition number (NC) criteria proposed by Montgomery and Peck (1981). When the multicollinearity degree is considered weak ($NC<100$), there is no serious issue to be analyzed (Cruz et al., 2004) and path analysis can be performed.

Results

The AH-N content in soil profile had a different behavior among soil sites and significative ($p<0.05$) differences between soil layers (Fig. 1). Alkaline hydrolyzable N bellow 20 cm (Fig. 1) varied and increased, decreased, or remained constant compared with the concentrations quantified in the 0 to 20 cm depth at each site (Table I). For soil sites 1, 2, 8, 9 and 14 a gradual and constant reduction in AH-N content was observed as depth increased, while at soil sites 10 and 15 the AH-N content decreased only after 30cm deep and sites 5 and 7 had little changes in AH-N content as depth increased. On the other hand, soil sites 4, 6, 12, and 15 had an increase in AH-N content at certain depths while soil site 13 presented an expressive increase in AH-N content in depths below 30 cm, reaching higher concentration at 60 cm deep than at soil surface.

By comparing specific soil depths, it is possible to verify a wide variation in AH-N content among soil sites. Furthermore, soil sites intrinsic physical properties such as clay and clay + silt contents seem to effect AH-N content and its distribution in depth, which can be verified by the high positive correlation coefficients at soil sites 6, 10 and 13 for clay content (0.76, 0.61, and 0.92, respectively) and 6, 9, 10 and 13 for clay + silt content (0.65, 0.95, 0.82, and 0.93, respectively) (Table II). However, for certain soil sites these properties can also influence the AH-N distribution negatively, as observed at sites 11 and 15 for clay content (-0.83 and -0.71, respectively) and at sites 1, 3, 4, 5, and 15 for clay + silt content (-0.72, -0.75, -0.76, -0.60, and -0.61, respectively) (Table II).

Pearson's linear correlation coefficient matrix (Table III) shows the relationship of the AH-N content and selected soil chemical and physical properties on a global analysis for all soil sites and layers. The AH-N content had highest and positive correlation with TN content, followed by TC, clay, clay + silt and Mn, while sand content presented negative correlation (0.86, 0.80, 0.48, 0.43, 0.42, and -0.43, respectively). Some other soil properties such as TCF-NH_4, despite low r value (0.18), also presented significant correlation ($p<0.05$). This fact is probably due to a numeric effect of the high number of samples used in the correlation process (n=143). Therefore, only the above-mentioned properties were used for path analysis (Fig. 2).

Soil TN is the most important property dictating AH-N pool, as it can be observed through the high correlation coefficient ($r=0.86$, $p<0.001$), which is mainly by direct effect (1.617) (Fig. 2). The high direct estimation (above 1) of TN can be related also to the indirect effect of other variables, especially TC as there is a high correlation between these variables (0.95, $p<0.001$), which influences the explanatory variables multicollinearity. On the other hand, TC had also high correlation with AH-N ($r=0.80$, $p<0.001$) but it was caused mainly through the indirect effect of TN as TC direct effect on AH-N was negative (-0.734). The Mn content had low and negative direct effect on AH-N, while for clay + silt the effect was also negative, but higher (-0.068 and -0.429, respectively). Furthermore, the direct effect of clay content on AH-N was very low (0.038). Similar to TC behavior, the high correlation observed for these variables with AH-N is related to the indirect effect of TN content. Opposite to this, sand content had negative correlation ($r=-0.43$, $p<0.001$) and a negative direct effect on AH-N (-0.452), which is related to the constant decrease in TN and AH-N as sand content increases.

The high direct association of TN content with AH-N, or the indirect effect via other soil properties shows a true cause and effect relationship between these two variables. Thus, this behavior raises the question: is AH-N pool is a constant proportion of TN across soil depth? To answer this, we evaluate the recovery of TN as AH-N in depth, which is illustrated in Fig. 3. It is possible to observe that AH-N pool is not a constant proportion of TN in depth neither across soil sites. There is a high amplitude in TN recovery as AH-N across depth for certain soil sites (e.g., 1, 2, 7, 10, 14, and 15) or at a same layer across soil sites (e.g., 0-5, 5-10, and 30-35 cm). Moreover, stands out the expressive increase of TN recovery as AH-N in depth for sites 1, 2, 7, and 10. This result shows that despite of presenting a decrease in AH-N content in depth (Fig. 1), the AH-N represents a larger proportion of TN in greater depths for these soil sites.

Discussion

The AH-N content changes across soil depth (Fig. 1) are in accordance with previous research of Roberts et al. (2009b), who outlined that AH-N behavior at greater depths than 30 cm cannot be predicted with the superficial soil layer analysis, which implies in specific soil sampling and calibrations for different soils. Paddy fields are flooded during the period of rice growth, and the flooded water continuously percolates through the plow layer to the sublayer, leaching organic and inorganic materials into the subsoil layer (Maie et al., 2004; Kögel-Knabner et al. 2010). Therefore, dissolved organic matter (DOM) leached by the percolation water from the plow layer is expected to contribute to SOM (Maie et al., 2004), inorganic N, and organic N forms, such as AH-N in the subsoil. The leaching of dissolved organic N (DON) as well as NH_4^+ or NO_3^- in agroecosystem varies widely upon management practice (i.e., tillage, rotation, fertilization, and irrigation) (Chantigny, 2003). For example, the input of mineral N can promote not only leaching of inorganic N but also the release and leaching of DON (Lu et al., 2011), while the application of manure trend to increase DON leaching compared to fertilization with mineral N (Embacher et al., 2008). Moreover, research developed by Song et al. (2015) showed that DON input by precipitation and irrigation had an extremely significant effect on the DON leaching amount. Thus, the authors pointed out that DON inputs from precipitation and irrigation are the most important factors for DON leaching in the rice seasons, which could largely conceal the influence of N fertilization.

The DON and the DOM in soil are SOM mobile fractions, and their movement in the pedosphere is affected by chemical factors such as solubility in water and the presence of binding cations and sesquioxides, and physical factors such as soil pore size (Zsolnay, 1996). Furthermore, the changes in physical state and valence of Mn and Fe ions (i.e., insoluble Mn^{4+} and Fe^{3+} changes into water-soluble Mn^{2+} and Fe^{2+}) due to the reduced conditions in rice fields are reported to affect the binding force to SOM and increase the solubility of DON in water (Hagedorn et al., 2000; Qualls, 2000), and consequently, its mobility downwards in soil profile. Therefore, this movement of SOM fractions in soil profile can be related to the wide variation in AH-N content observed among soils and for specific soil depths, as the AH-N content can be influenced by certain soil management practices over time, such as crop rotation, soil manipulation (i.e., land leveling), tillage and naturally inherent variations in N cycling (Roberts et al., 2009b). Moreover, plant derived C seems to play a larger role for DOM in paddy soils than in upland soils (Lu et al., 2002, 2004). Thus, an increase in DON concentrations and fluxes in paddy soils as result of long periods with anaerobic conditions combined with soil physical factors, such as the pore size (Zsolnay, 1996) and pores connectivity, may help to explain the distinct AH-N concentrations among soil sites and layers.

The high and positive correlation observed for AH-N and clay and clay + silt at soils 6, 9, 10 and 13, indicates that this N fraction could be stabilized by soil minerals and protected against soil microorganism's enzymatic hydrolysis. Therefore, soil particle size fractions would have large influence over AH-N content at these soils, as a higher proportion of clay and silt would have a higher probability to stabilize the SOM and organic N. However, Roberts et al. (2009b) pointed out that AH-N can be influenced by certain soil management practices over time, therefore the AH-N pool would be more related to plant residues addition and its quality than soil particle size, i.e., soils with low clay content would also be able to present higher content of AH-N. This effect could be the reason why, for certain soils, there is a low correlation between soil particle size and AH-N content. So, if we look at the global analysis of soil properties (Table III), the correlation between AH-N and particle size fractions (clay and clay + silt < 50 µm) decreased (0.48 and 0.43, respectively), since the granulometric fraction alone has no effect on these N forms of soil. This behavior could also be related to the diversity of soil characteristics and management practices in the areas, as lower correlation coefficients were also observed for TN and TC and particle size content (clay and clay + silt < 50 µm) (0.43 and 0.38; 0.28 and 0.25, respectively).

We highlight that AH-N pool is not a constant proportion of TN in depth neither across soil sites, as there is a high amplitude in TN recovery as AH-N across depth and soil sites (Fig. 3). The expressive increase of TN recovery as AH-N soil sites 1, 2, 7, and 10 shows that AH-N represents a larger proportion of TN in greater depths for these soil sites. This behavior is probably related to the DOM and NH_4^+ leaching from the plow layer, contributing to an increase of organic N forms and inorganic N and, therefore, resulting in an increase of AH-N in subsoil layers. The leaching of DON as well as NH_4^+ or NO_3^- in agroecosystem varied widely upon soil management practice (i.e., tillage, rotation, fertilization, and irrigation) (Chantigny, 2003). Additionally, the increase in soluble Mn^{+2} and Fe^{+2} ions concentration in soil solution due to soil reduction (Ponnamperuma, 1972), can increase NH_4^+ release form cation exchange sites and its mobility in soil, especially for variable charged soils (Xiong et al., 2010), contributing to AH-N concentration increase in depth as well as increase the proportion of AH-N at soil TN for greater depths.

The accumulation of organic C and N in the subsoil of paddy soils is described by Li et al. (2005) and Zhang and He (2004). The authors suggest that the organic C in the subsoil originates from the downward movement of dissolved organic carbon (DOC) or the colloidal association of organo-minerals, although, they provide no evidence for such processes. However, the results of Maie et al. (2004) demonstrate that SOM in the subsoil of floodplain soils originates at least partially from DOM leached from the arable layer. Part of this material must come from low molecular weight compounds, which could then be stabilized by interaction with the iron oxides in paddy soils dry cycles. This behavior would help explain the low direct relationship of AH-N with clay and clay + silt content, as the presence of AH-N in deeper soil layers is probable more related to the solubility and migration of the organic compounds and NH_4^+-N in soil than with the increase of clay and clay + silt contents. The soil particle size may be important for the stabilization of TN, TC, and SOM, but not for more active fractions of SOM such as amino acids and amino sugars analyzed by alkaline distillation. Furthermore, results of Kader et al. (2013) also suggest that neither SOM quantity nor soil texture, but rather SOM quality or other factors, have a more strongly effect on the anaerobic N mineralization in short and medium soil incubations. Since AH-N is not a constant fraction of soil TN, it undergoes variations independently of soil clay and clay + silt contents.

This behavior, from the point of view of a N availability index is an advantage, as AH-N would be more sensitive to changes in soil management practices, plat residues deposition, and N cycle than the TN and SOM contents. Considering that rice plants are able to develop roots and explore soil depths greater than 20 cm (Yoshida, 1981), and therefore use efficiently available N below 20 cm deep, the AH-N distribution in soil sites 13 and 15 may help to explain the lack of rice response to N fertilization in some soils, even with low SOM content. Thus, it is necessary more information on the best soil sampling depth for AH-N calibration for rice crop in Southern Brazil. The results of this study indicate that greater soil depths than 0 to 20 cm should be considered for a correct prediction of soil N availability with AH-N for flooded rice in Southern Brazil and some soil properties, such as TN and clay + silt content, can indicate its distribution in soil profile.

Conclusions

The AH-N distribution in soil profile is distinct among soil sites and can have increment in depth, which indicates that greater soil depths than 0 to 20 cm should be considered for a correct prediction of N availability for flooded rice in Southern Brazil. Despite of the high correlation of certain soil properties with AH-N, the major property that dictates its distribution is soil is TN. The AH-N is not a constant N pool in depth. The TN recovery as AH-N across soil sites and depths presented a high amplitude, indicating that AH-N can be affected by soil management practices even when TN has no major changes. These characteristics reinforces the potential use of AH-N as soil index to predict N availability for flooded rice in Southern Brazil, as it is a more sensitive soil index than TN and SOM.

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TABLE I
Soil site-no, location, class (U.S Soil Taxonomy), and selected physical and chemical properties at the 0 to 20 cm depth.

Soil	Location	Soil Class	AH-N	NO_3^-	NH_4^+	TCF-NH4	TN	TC	Sand	Silt	Clay
			.... mg kg^{-1}				.... g kg^{-1}				
1	Santa Vitória do Palmar	Alfisol	101	14.3	21.0	0.0	0.7	9.1	613	241	147
2	Santa Maria	Alfisol	123	0.6	37.9	0.0	0.8	9.8	263	571	166
3	Pelotas	Alfisol	120	3.4	16.6	0.0	0.9	11.4	423	431	145
4	Cachoeirinha	Alfisol	147	23.9	170.7	0.0	0.7	7.7	299	528	174
5	Camaquã	Alfisol	84	0.6	28.2	0.0	0.6	6.9	467	373	160
6	Santa Maria	Alfisol	120	2.3	89.3	0.0	1.9	22.6	419	379	203
7	Cachoeira do Sul	Alfisol	93	2.5	20.5	0.0	0.6	6.8	479	337	184
8	Itajaí	Alfisol	133	1.5	61.2	307.1	1.2	9.7	266	476	257
9	Santa Maria	Entisol	279	2.9	47.7	40.3	2.2	26.6	161	503	336
10	Paraíso do Sul	Alfisol	124	1.6	21.7	0.0	1.1	11.8	474	176	350
11	Cachoeira do Sul	Alfisol	84	3.7	15.9	0.0	0.7	8.0	233	478	289
12	Uruguaiana	Entisol	229	6.0	44.1	35.2	1.3	15.7	469	224	306
13	Agudo	Alfisol	97	3.4	15.4	0.0	0.8	8.8	492	156	351
14	Cachoeira do Sul	Entisol	180	1.1	23.2	18.3	1.4	14.7	286	297	417
15	Agudo	Entisol	217	1.8	34.3	139.3	2.0	21.0	84	474	441

TABLE II

Pearson's correlation coefficient for AH-N and soil sites properties in depth.

Soil	n	Pearson's correlation	TN	TC	Sand	Silt	Clay	Clay + Silt
1	10	r	0.98 ***	0.47 ns	0.72 *	-0.54 ns	0.02 ns	-0.72 *
2	10	r	0.99 ***	0.99 ***	0.31 ns	-0.53 ns	0.49 ns	-0.31 ns
3	10	r	0.97 ***	0.97 ***	0.75 **	-0.87 ***	-0.43 ns	-0.75 **
4	10	r	0.94 ***	0.88 ***	0.76 **	-0.75 **	-0.31 ns	-0.76 **
5	10	r	0.83 **	0.79 **	0.60 §	-0.22 ns	-0.45 ns	-0.60 §
6	10	r	0.61 §	0.51 ns	-0.65 *	0.29 ns	0.76 **	0.65 *
7	10	r	0.83 **	0.78 **	0.41 ns	0.00 ns	-0.49 ns	-0.41 ns
8	8	r	0.99 ***	0.98 ***	0.14 ns	-0.30 ns	0.01 ns	-0.14 ns
9	10	r	0.98 ***	0.95 ***	-0.95 ***	0.90 ***	0.23 ns	0.95 ***
10	10	r	0.99 ***	0.99 ***	-0.82 ***	0.61 §	0.61 §	0.82 ***
11	10	r	0.92 ***	0.92 ***	-0.49 ns	0.91 ***	-0.83 ***	0.49 ns
12	5	r	0.97 **	0.97 **	-0.12 ns	0.89 *	-0.09 ns	0.12 ns
13	10	r	0.94 ***	0.92 ***	-0.93 ***	0.93 ***	0.92 ***	0.93 ***
14	10	r	0.98 ***	0.97 ***	0.45 ns	-0.31 ns	-0.42 ns	-0.45 ns
15	10	r	0.72 *	0.75 **	0.61 §	0.43 ns	-0.71 **	-0.61 §

^{ns} Non-significant; § p<0.10; * p<0.05; ** p<0.01; *** p<0.001.

TABLE III
Pearson’s linear correlation coefficient (n=143) matrix for alkaline hydrolysable nitrogen (AH-N) content and selected chemical and physical properties.

	AH-N	NO_3^-	NH_4^+	TCF-NH_4	TN	TC	Ca	Mg	Mn	P	K	Al	pH	Sand	Silt	Clay	Clay+Silt
AH-N	1.00	0.20 **	0.14 ns	0.18 *	0.86 ***	0.80 ***	0.34 ***	0.28 ***	0.42 ***	0.20 *	0.33 ***	0.15 ns	-0.19 *	-0.43 ***	0.05 ns	0.48 ***	0.43 ***
NO_3^-		1.00	0.53 ***	-0.12 ns	0.03 ns	0.05 ns	0.01 ns	-0.05 ns	-0.16 *	0.19 *	0.12 *	-0.26 ***	0.15 ns	0.15 ns	0.03 ns	-0.23 **	-0.15 ns
NH_4^+			1.00	0.01 ns	0.10 ns	0.09 ns	-0.16 *	-0.22 **	-0.18 *	-0.01 ns	-0.14 ns	-0.10 ns	-0.13 ns	-0.20 *	0.39 ***	-0.18 *	0.20 *
TCF-NH_4				1.00	0.21 **	0.04 ns	0.04 ns	0.06 ns	0.35 ***	0.07 ns	0.03 ns	-0.23 **	0.02 ns	-0.38 ***	0.20 *	0.25 ***	0.38 ***
TN					1.00	0.95 ***	0.24 ***	0.17 *	0.40 ***	0.21 **	0.42 ***	0.29 ***	-0.25 ***	-0.38 ***	0.03 ns	0.43 ***	0.38 ***
TC						1.00	0.27 ***	0.16 ns	0.24 ***	0.17 *	0.41 ***	0.29 ***	-0.14 ns	-0.25 ***	0.03 ns	0.28 ***	0.25 ***
Ca							1.00	0.80 ***	0.39 ***	0.07 ns	0.42 ***	-0.27 ***	0.49 ***	0.05 ns	-0.44 ***	0.42 ***	-0.05 ns
Mg								1.00	0.18 *	0.13 ns	0.30 ***	-0.38 ***	0.53 ***	-0.03 ns	-0.36 *	0.45 *	0.03 ns
Mn									1.00	0.14 ns	0.23 **	0.12 ns	-0.27 ***	-0.37 ***	-0.22 **	0.70 ***	0.37 ***
P										1.00	0.38 ***	-0.19 *	0.20 *	0.03 ns	-0.06 ns	0.04 ns	-0.03 ns
K											1.00	-0.07 ns	0.24 ***	0.20 *	-0.40 ***	0.20 *	-0.20 *
Al												1.00	-0.66 ***	-0.21 **	-0.06 ns	0.33 ***	0.21 **
pH													1.00	0.38 ***	-0.16 *	-0.29 ***	-0.38 ***
Sand														1.00	-0.64 ***	-0.52 ***	-1.00 ***
Silt															1.00	-0.32 ***	0.64 ***
Clay																1.00	0.52 ***
Clay+Silt																	1.00

^{ns} Non-significant; * p<0.05; ** p<0.01; *** p<0.001.

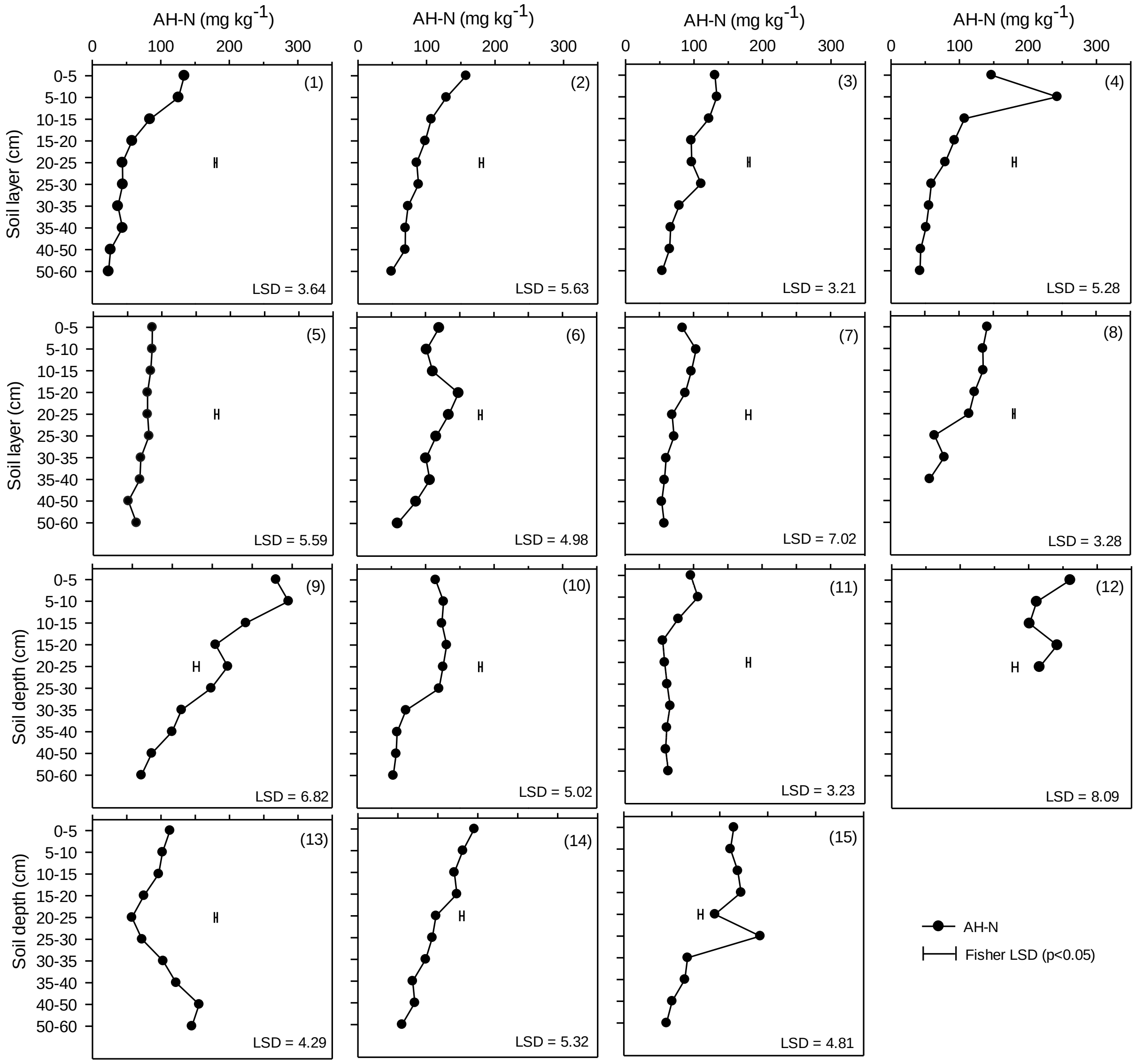

Fig. 1 Alkaline hydrolysable nitrogen (AH-N) distribution in depth in paddy soils from Southern Brazil.

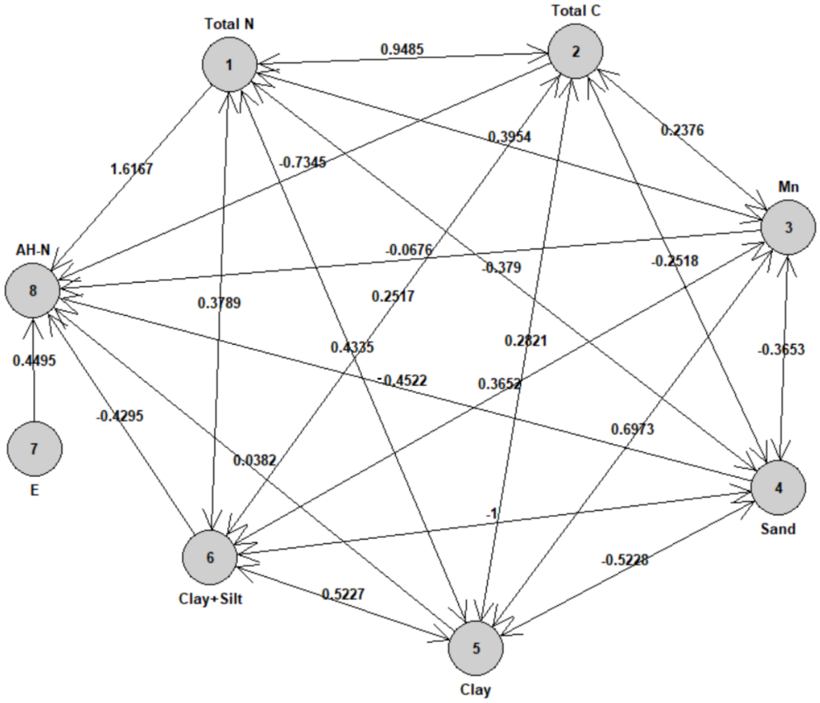

Fig. 2 Path analysis diagram illustrating the direct effect of soil properties on AH-N in paddy soils from Southern Brazil.

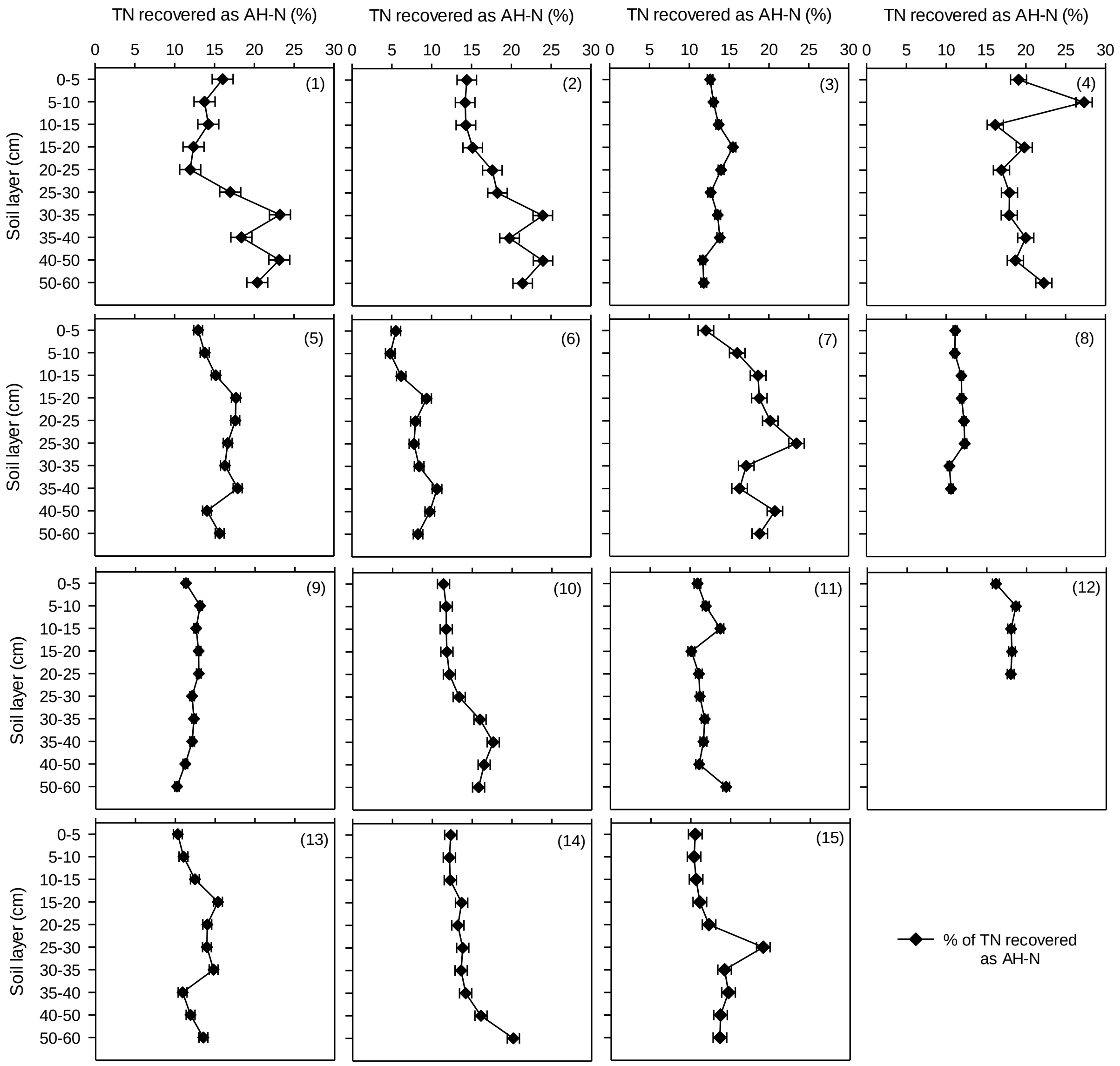

Fig. 3 Total nitrogen recovered as alkaline hydrolysable nitrogen (AH-N) in depth in paddy soils from Southern Brazil. Horizontal bars represent the standard error.

5.3 ESTUDO III

Contribution of nitrogen from different soil depths to flooded rice nutrition[1]

Abstract

Background and aims Soil organic nitrogen (N) availability is a limiting factor for rice (*Oryza sativa* L.) production. Nitrogen forms from deeper soil layers can contribute to plant nutrition while influencing rice crop response to N fertilizer applications. This study aims to investigate the N absorption dynamics and the N recovery by depth for flooded rice in Southern Brazil.
Methods A greenhouse experiment was conducted with undisturbed soils collected from three locations - Alfisol soil from Santa Maria (ASM), Entisol soil from Santa Maria (ESM), and Entisol soil from Agudo (EA) - varying in their physical and chemical properties. Enriched ^{15}N was applied at the soil surface and injected at 0.10, 0.20, 0.30, and 0.45-m depths. Rice plants were grown and ^{15}N absorption and recovery by the rice was evaluated.
Results The N placed in near surface layers was rapidly assimilated by rice plants whereas N located at greater depths was absorbed over time, resulting in a similar ^{15}N concentration in leaves among treatments at rice heading. Highest ^{15}N recovery by shoots was observed when N fertilizer was applied at 0.10, 0.20, and 0.30-m depths for all soils. The highest ^{15}N recovery with N application at 0.45-m was observed at ESM soil, which is related to higher soil hydraulic conductivity (HC) at this site.
Conclusions The ^{15}N recovery from 0.45-m indicates that, rice plants use N from deeper soil layers which are not commonly sampled for routine soil analysis and absorption at these deeper soil layers is related to soil HC. To use potentially available organic-N indexes deeper soil layers should be considered in the correlation and calibration process for rice in Southern Brazil.

Keywords ^{15}N uptake. N derived from fertilizer. Fertilizer use efficiency. Paddy soil. *Oryza sativa* L.

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Introduction

Nitrogen (N) is one of the most critical nutrients in crop production as it is an important constituent of proteins, nucleic acids, porphyrins, and alkaloids (Schulten and Schnitzer 1998). Plants can acquire N through their roots from the soil as inorganic-N (nitrate (NO_3^-) and ammonium (NH_4^+)) and organic (e.g. urea, amino acids, peptides) forms (Kiba and Krapp 2016). Although organic-N forms can contribute to plant N nutrition in specific habitats such as in boreal ecosystems (Jones and Kielland 2012; Werdin-Pfisterer et al. 2012), NO_3^- and NH_4^+ are considered the primary N sources for crops, due to their prevalence in agricultural soils (Paungfoo-Lonhienne et al. 2012). However, the two inorganic-N forms in soil make up only a very small proportion of the total N stock in any ecosystems, while the organic nitrogen mainly existing in soil organic matter (SOM) constitutes the dominant fraction of soil N (Li et al. 2014). Over 90% of the N in the surface layer of most soils is organically combined (Stevenson 1982) if the fixed N by clay minerals is included (Li et al. 2009).

There are multitude sources and processes that contribute to the N supply of crops such as SOM mineralization (Sahrawat 2006), microbial biomass turnover (Sainz et al. 2004), NH_4^+ adsorbed to soil colloids (Nieder et al. 2011; Norman and Gilmour 1987), N fertilization (Wienhold 2007), irrigation water and other environmental and biotic sources (Hirzel et al. 2012). Soil N is a significant component of plant N uptake (Li et al. 2014; Kadiyala et al. 2015), however, in most cases fertilizer N is required to maximize yield. Thus, the organic-N not only constitutes the dominant part of soil N, but also plays a key role in soil N cycling and crop production (Li et al. 2014). Since crop production depends to a large extent on soil N supplying capacity, to determine the amount of soil N potentially available for plant uptake is a key process for improving N use efficiency and must be considered for N fertilizer management.

Recently, Roberts et al. (2011, 2012) developed a soil-based N test for rice production systems in Arkansas called N-STaR. The N-STaR soil-based N test is an alkaline distillation method that quantifies easily mineralized organic-N forms, such as amino acids (e.g., glutamine) and amino sugars (e.g., glucosamine) plus inorganic-N as NH_4^+-N (Roberts et al. 2009a), estimating the amount of N that a soil can supply during the growing season and allows site-specific adjustment of the N fertilizer rate needed to maximize rice yield. Furthermore, Drescher et al. (2016) reported potential use of the alkaline hydrolyzable N (AH-N) quantified by direct steam distillation as a soil index to predict N availability in Southern Brazil paddy soils as the AH-N provided the best correlation with N mineralization in incubation experiment and N uptake by rice plants amongst all soil indexes tested. Nonetheless, Roberts et al. (2009b) reported that AH-N is not evenly distributed in soil profile and that some sites can have significant increases in AH-N concentration in greater depths than those commonly sampled for soil analysis. Thus, for the successful calibration of the N-STaR method soil samples should be taken at 0-0.45-m depth for silt loam soils (Roberts et al. 2011, 2012).

In order to increase efficiency and minimize potential environmental consequences, a complete understanding of soil N will support the development of best management practices to improve the use of soil N supply in agricultural production systems while minimizing adverse environmental effects (Li et al. 2014). Subsoil N availability is dependent on the particular crop’s rooting depth, and N located below the crop’s rooting depth should be considered unavailable (Roberts et al. 2009b). Previous research concerning the AH-N distribution in the soil profile (Roberts et al. 2009b), soil sampling depth (Roberts et al. 2011, 2012), and N recovery with depth (Roberts et al. 2013) for Arkansas rice production system suggests that the potential use of AH-N as soil index for rice production in Southern Brazil (Drescher et al. 2016) warrants further investigation. Currently, there is no information about N recovery with depth by flooded rice in Southern Brazil and how N from greater depths contributes to plant nutrition.

In this study, we hypothesized that available N from greater depths in the soil profile contributes to rice plant nutrition and N absorption dynamics is distinct between near surface and deeper soil layers, which can influence rice response to fertilizer application, and N use efficiency. To investigate the N recovery and absorption over time, a greenhouse study was conducted with undisturbed soil samples and ^{15}N injection at different depths within the soil profile. The objectives of this study were (a) to investigate the N absorption dynamics and (b) the N recovery by rice at different soil depths in Southern Brazil.

Materials and Methods

Soil sampling and analysis

Three sites commonly cropped with rice in Southern Brazil were selected - Alfisol soil from Santa Maria – ASM (29°43'3.15"S; 53°42'21.67"W), Entisol soil from Santa Maria - ESM (29°43'8.38"S; 53°42'21.84"W) and Entisol soil from Agudo – EA (29°40'6.16"S; 53°18'50.22"W) - representing a variety of physical and chemical properties for soils that are commonly cropped to rice. Stratified soil samples were taken before crop establishment to a depth of 0.60-m (i.e., 0.0-0.05, 0.05-0.10, 0.10-0.15, 0.15-0.20, 0.20-0.25, 0.25-0.30, 0.30-0.35, 0.35-0.40, 0.40-0.50, and 0.50-0.60m deep) and selected chemical and physical properties were analyzed: total nitrogen (TN) and total carbon (TC) were determined using dry combustion at elemental analyzer (Thermo Scientific, Flash EA 1112, Milan, Italy); AH-N was determined using direct steam distillation (Roberts et al. 2009a); NO_3^- and NH_4^+ were extracted with 2*M* KCl and determined using salicylate colorimetric techniques (Mulvaney 1996); pH was determined on a 1:2 (m:v) soil-to-water suspension; P and K were extracted with Mehlich-1 (Tedesco et al. 1995); particle size fractions were determined using pipette method (Donagema et al. 2011); soil bulk density (BD), total porosity (TP), macro porosity (MacP), and micro porosity (MicP) were determined using sand column (Reinert and Reichert 2006); penetration resistance (PR) was determined using a MARCONI-MA 933 electronic bench penetrometer at saturated samples (Tormena et al. 2007); hydraulic conductivity (HC) was determined using constant head permeameter method (Libardi 2005) (Table 1).

Undisturbed soil samples (columns) were collected at the same sites with a polyvinyl chloride (PVC) pipe (0.20 x 0.65m) coupled to a monolith collector and manually inserted into the soil up to 0.60-m deep (Figure 1a). After collection, the base (i.e., the side that reaches 0.60-m deep) of the PVC pipe was covered with a cap and sealed and the soil columns were placed in the greenhouse.

^{15}N injection and plant growth

Rice cultivation was done in a randomized block design, with two factors (three soil sites and five N placement depths) and three replications. Eight rice seedlings from the cultivar 'IRGA 424RI' were transplanted in each soil column. After transplanting, a water layer of approximately 0.01-m was applied, simulating the pre-germinated / water-seeded rice system, so that SOM mineralization would occur anaerobically. Three days after transplanting, only four plants per column were maintained, selecting the most developed ones, and the water layer was increased to 0.04-m and maintained for the duration of the trial.

An injection apparatus was constructed to facilitate the application of ^{15}N-labeled $(NH_4)_2SO_4$ solution (Figure 1b). The injection apparatus consisted of a crystal PVC hose with 6-mm diameter x 200-mm length. The PVC hose was sealed at one end and had 1-mm holes along the first 100-mm. At the other end of the hose, a three-way valve was coupled, which allowed the fertilizer injection with a syringe. To install the injection system on the columns, a 6-mm x 150-mm hole was drilled on one side of the soil column so that, N application was placed in the center of the soil column. After insertion of the injection apparatus, the crystal PVC hose was sealed at the PVC tube to avoid soil solution leakage.

To assess the N uptake by rice plant from various placement depths, 10-mL of a solution containing 196 mg N (rate equivalent to 62 kg N ha^{-1} – commonly used in the first N application in the standard two-way split N application) as 6.0 atom% ^{15}N-labeled ammonium sulfate ($(NH_4)_2SO_4$) was applied superficially (i.e., 0-m deep) and injected at 0.1, 0.2, 0.3 and 0.45-m depths when rice plants reached V3 stage (nine days after transplanting). After N injection, 10-mL of distillated water was injected to evacuate all ^{15}N solution from the injection apparatus. Fertilizer injection was performed only after the establishment of a permanent flood to ensure there was no downward movement of water in the soil profile or upward flow of the ^{15}N-labeled solution via capillary action (Figure 1b). A solution containing 346 and 137 mg L^{-1} of phosphorus and potassium as K_2HPO_4 (a rate equivalent to 40 and 70 kg P and K ha^{-1}, respectively) was also applied superficially to provide sufficient nutrients for optimum rice growth.

Plant tissue sampling and analysis

To evaluate N assimilation by rice plants across time, one plant per column was selected and the last two fully expanded leaves were sampled at 13, 28, 44 and 61 days after ^{15}N addition. Samples were oven dried at 65 °C, crushed with an agate mortar and pestle for further ^{15}N content determination. When rice plants were at the beginning of heading (70 days after rice transplanting and 61 days after ^{15}N addition) rice aboveground biomass was collected, oven dried at 65 ºC until constant mass, and dry matter production was determined for each soil column. After collecting plant aboveground biomass, the soil column was opened, and rice root samples were collected in one half of the longitudinal section (i.e., corresponding to two rice plants). The roots were separated from soil by washing under running water, using sieves with 2 and 1-mm openings. Once separated from the soil, rice root samples were washed with distillated water and oven dried at 65 °C until constant weight and root dry matter production was determined.

After drying, rice aboveground and root biomass was milled (particle size <1mm) to determine total N content in an elemental analyzer (Thermo Scientific, Flash EA 1112, Milan, Italy) and ^{15}N abundance by mass spectrometry (IRMS) (Thermo Scientific, Delta V Advantage, Bremen, Germany) coupled to an elemental analyzer (Thermo Scientific, Flash EA 1112, Milan, Italy). Total N uptake and ^{15}N recovery (shoot and root portions) were obtained from TN and ^{15}N content in plant tissue and dry matter production of each treatment.

Nitrogen uptake, ^{15}N atoms excess in plant tissue, N derived from fertilizer (NDFF), and rice plants nitrogen recovery (NR) were calculated by the following equations:

$$Total\ N\ uptake\ (mg) = \frac{\%\ N\ in\ sample\ x\ Dry\ mass\ production\ (g)}{100} x1000 \qquad \text{(Eq. 1)}$$

$$\%atom\ ^{15}N\ in\ excess\ in\ sample\ = \%atom\ ^{15}N\ in\ sample - 0.3663\% \qquad \text{(Eq. 2)}$$

$$NDFF\ (mg) = Total\ N\ uptake\ (mg)\ x \frac{\%atom\ ^{15}N\ in\ excess\ in\ sample}{\%atom\ ^{15}N\ in\ excess\ in\ fertilizer} \qquad \text{(Eq. 3)}$$

$$NR(\%) = \frac{NDFF(mg)}{Nfertilizer\ application\ rate\ (mg)} x100 \qquad \text{(Eq. 4)}$$

Statistical analysis

The experiment was a randomized block design with a 3x5 factorial arrangement. Each treatment was replicated three times with replicate being a random effect in the analysis of variance (ANOVA) model. Normality test (Kolmogorov-Smirnov and Lilliefors) and equal variance test were performed before running the ANOVA. The ANOVA was conducted to determine the influence of soil site, N application depth and their interaction (fixed effects) on ^{15}N recovery by rice plants. For ^{15}N content in rice leaves over time, ANOVA was conducted to determine the influence of N application depth, rice leaf sampling time and their interaction (fixed effects) for each soil site separately. Statistical means were compared using Fisher's least significant difference (LSD) at the 0.05 probability level.

Results

Soil sites used in the experiment had distinct chemical and physical properties (Table 1). Among these stands out the AH-N content, which was higher at ESM soil site, followed by

EA and ASM sites, respectively. The AH-N distribution in soil depth is also noticeably different among soil sites as ESM site had higher AH-N concentration at superficial layers while EA and ASM had increased AH-N content in deeper soil layers. There were also differences in soil sites particle size fractions. The ASM soil site presented the highest sand content, ESM the highest silt content and EA presented the highest clay and lowest sand contents. Another soil property that attracts attention is hydraulic conductivity (HC) due to its variance with depth and among soil sites. The ESM soil site presented highest HC, followed by EA and ASM sites, respectively. The three sites reduced expressively HC at the 0.10 to 0.30-m depth, at the same time as soil bulk density (BD) increased and macro porosity (MacP) reduced.

The ^{15}N concentration in rice leaves had a significant ($p<0.001$) N application depth by leaf collection time interaction (Table 2). At the first leaf sampling (13 days after ^{15}N labeled fertilizer injection in soil), the ^{15}N concentration in rice leaves was higher with superficial application of N fertilizer for all soil sites, followed by the application at 0.10-m deep (4.9%, 4.2% and 4.5%; and 1.3%, 1.7% and 2.9% for ASM, ESM and EA soil sites, respectively) (Figure 2). On the other hand, treatments with deeper N fertilizer application (0.30 and 0.45-m) presented very low or absent (less than 0.5%) ^{15}N concentration in leaves at this sampling. Over time, ^{15}N concentration in leaves decreased when N fertilizer was applied in superficial layers and increased in those treatments with the deepest application, i.e. at 0.20, 0.30 and 0.45-m depths. The N applied in deeper layers was absorbed and assimilated more expressively by rice plants only after 44 days of application for all soil sites. At the beginning of rice heading (70 days after rice transplanting and 61 days after ^{15}N addition), ^{15}N content in the rice leaves was very close for all treatments and values ranged between 1 and 2% for the ESM and EA soil sites, and between 1.4 to 3% for the ASM soil site.

The interaction of soil sites by N application depth was significant ($p<0.001$) for ^{15}N recovery by rice aboveground biomass, while for rice roots there was no interaction (Table 2).

Thus, for ^{15}N recovery by rice roots, only the main effect means were presented (Figure 3). Highest ^{15}N recovery by rice aboveground biomass was observed when N fertilizer was injected at 0.10, 0.20, and 0.30m depths for all soil sites (Figure 3). Among soil sites, there was no difference of ^{15}N recovery by rice aboveground biomass with superficial and 0.10-m N applications depths. The ESM soil site had significantly higher ^{15}N recovery at 0.45 and 0.20-m depths, while for 0.30-m depth there was no difference from ASM site. The EA soil site had lower ^{15}N recovery compared to ESM and ASM sites. The highest ^{15}N recovery (52%) at EA soil site was observed when N fertilizer was applied at 0.10-m, with constant decrease in depth. Opposite to this, the ASM and ESM soil sites kept similar recovery when ^{15}N was applied at 0.20 (54% and 61%, respectively) and 0.30-m (54% and 59%, respectively), and only decreased ^{15}N recovery at 0.45-m (22% and 36%, respectively). Nitrogen recovery by rice roots was low compared to aboveground biomass, with highest recoveries at ESM and ASM soil sites (4.6%) and superficial layers (5.9, 6.2 and 5.1% for superficial, 0.10 and 0.20-m depths, respectively).

Discussion

The N placed in superficial layers was rapidly assimilated by rice crop whereas N located at greater depths was absorbed and assimilated slower over the time (Figure 2). The expressive ^{15}N content increase in rice leaves after 44 days of application (Figure 2) in treatments with N application at 0.20, 0.30, and 0.45-m shows that the N located in deeper layers is an important N source to rice crop later in the season. In treatments with fertilizer application near the soil surface rice plants depleted the soil and fertilizer N sources, thus N remobilization would be the main N contribution for grain production. On the other hand, with N application in greater depths, plants used first available soil N pools accessible to root system, produced less dry mass, and increase N content in tissue by using fertilizer N from greater depths closer to the reproductive stage as rice roots tend to increase in depth throughout the growing season, with the maximum penetration depth occurring near harvest (Beyrouty et al. 1987). Apparently, rice plants accessed the N located in deeper layers when plants were in a phenological stage of higher N demand, which resulted in higher ^{15}N recovery than superficial N fertilizer application (Figure 3), especially for ESM and ASM soil sites.

The ^{15}N concentration in rice leaves and the higher efficiency of ^{15}N recovery by rice from 0.10, 0.20 and 0.30-m fertilizer placement depths is supported by previous studies that estimate 86% of rice total root length in the top 0.36-m of the soil profile when the rice crop is at the mid-tillering growth stage, growing rapidly, and actively taking up N and other nutrients (Beyrouty et al. 1987). In an anaerobic environment, such as flooded soils with impaired water percolation, rice roots seldom exceed a maximum depth of about 40 cm, and the actual root depth found in the field is controlled not only by genetic ability but also by environmental conditions (Yoshida 1981). As rice plants were growing, roots reached deeper soil layers and absorbed N from greater depths, increasing the N content in rice leaves over time, resulting in higher ^{15}N recovery in the rice aboveground biomass.

The highest ^{15}N recovery was observed when N was applied at 0.10, 0.20 and 0.30-m depths for ASM and ESM soil sites and at 0.10-m for EA soil site (Figure 3) and might be related to ammonium (NH_4^+) absorption to cation exchange sites and protection against lost. Thus, NH_4^+ was protected and available when rice roots reached these layers, resulting in a higher contribution of N form fertilizer. Sorption and desorption of NH_4^+ is an important mechanism in retention and release of N and affects the N bioavailability for plants and its leaching from the soil (Fen et al. 1982; Phillips 1999). Moreover, the addition of N fertilizer below the soil surface may have also led to an increase in ^{15}N recovery as there was no dilution in the flood water and potentially less N immobilization (Roberts et al. 2013). Although the experimental conditions were controlled and ^{15}N-labeled $(NH_4)_2SO_4$ solution injection was performed only after the establishment of a permanent flood, N concentration in soil solution might have been higher than young rice plants demanded when fertilizer was applied at soil surface, resulting in a higher flood water NH_4^+ concentration and in a probable N loss through ammonia (NH_3) volatilization (Yao et al. 2018; Zhang et al. 2017). This behavior can help to explain the lower ^{15}N recovery when N application was at soil surface compared to deeper layers. Furthermore, when N fertilizer was applied at 0.45-m there was also lower recovery than intermediately N placement depths probably due to the difficulty of plants to access the fertilizer in deeper layers at the beginning of vegetative stages. Beyond that, soil N is a significant component of plant N uptake and crop production (Kadiyala et al. 2015; Li et al. 2014) and must be considered for N fertilizers to be used efficiently (Roberts et al. 2009b). Norman et al. 1992, suggest that rice crop uses preferentially fertilizer N early in the season, while later in the season, when the plant has developed an extensive root system, N uptake would be primarily from soil N pools. Thus, the lower ^{15}N recovery when N fertilizer was applied superficially can also be related to the higher mineral N and AH-N content at soil superficial layers, as result of deposition and decomposition of rice crop residues and SOM mineralization. This effect can also explain the lower ^{15}N recovery by rice in EA soil, as this site presents higher potentially available N (i.e. AH-N) content in depth. Furthermore, a higher amount of available organic and inorganic-N might have resulted in a dilution effect, leading to higher overall N uptake, but with a lower percentage of fertilizer-N uptake.

Apparently, soil physical properties play an important role in rice ^{15}N recovery from greater depths in soil profile. Overall, ESM soil site had the highest ^{15}N recovery even when N fertilizer was injected at 0.45-m (Figure 3). This result can be a consequence of the higher HC verified at this soil site (Table 1). Soil physical properties, such as MacP, pores connectivity, and HC influence N absorption as plants uptake N from soil mainly through mass flow (Marschner 2012; Oyewole et al. 2014). Although there are similarities between certain soil site properties such as BD, total porosity, macro and micro porosity, small changes in pore radius and pore connectivity that have no substantial effect on BD and the fraction of pore classes (i.e., MacP/MicP) may have considerably influenced HC and the flow of saturated soil water (Drescher et al. 2016), allowing an increase in the N provided by mass flow. These findings indicate rice plants are actively using N from depths up to 0.45-m, and the intensity of N recovery is also related to soil physical properties. Despite of the effect of MacP and HC on ^{15}N recovery, it seems that BD alone did not influenced N uptake. Differences between BD values across soil sites might be more related to changes in site specific properties, such as sand and clay content. Moreover, due to saturated conditions, penetration resistance was predominantly low across soil sites profile and mechanical impedance probably had no effect on rice root growth and N uptake.

The significant interaction ($p<0.001$) between soil site and N application depth indicates that there is a potential for N uptake and assimilation to vary across depths and across locations, as reported in previous study by Roberts et al. (2013) in silt loam paddies from Arkansas. These same authors also reported higher N recovery by rice aboveground biomass with N fertilizer application at 0.30 and 0.45-m (93 and 40%, respectively) than we found in present study, which can be related to soil sites properties, rice variety as well as rice phenological stage when aboveground samples were collected. However, the N recovery with superficial N fertilizer application (around 40% at the three soil sites) is similar to previous research carried out by Liu et al. (2008) with basal $(NH_4)_2SO_4$ application (27%) and fertilizer combined with chicken manure (36.5%) and Kadiyala et al. (2015) who observed 41% and 32% of N recovery by flooded rice crop in a 2-year-field study. Nitrogen recovery efficiency for lowland rice is low, usually less than 50% (Fageria and Baligar 2005), which may be related to N loses from soil via nitrification-denitrification, NH_3 volatilization or leaching (Khatum et al. 2015).

The effective N uptake and recovery by rice plants in treatments with N at greater depths, shows that soil sites with higher potentially available N in the soil profile likely cause the rice plants to function more optimally in photosynthate supply and in converting most of the tillers into productive ones. The increased number of productive tillers and greater panicle length, as well as the decreased percentage of empty grains, contributes to an increase in grain yield (Hidayati et al. 2016). Therefore, N application in depth could be a strategy to increase N recovery and efficiency. Khatum et al. (2015) investigate the efficiency of N application at 0.04-0.05-m depth after rice transplanting in Bangladesh and reported highest agronomic use efficiency, apparent recovery efficiency and utilization efficiency among different N management options without sacrificing grain yield and could also save about 30% of urea rate effectively. Wu et al. (2017) also investigated the effects of N fertilizer placement on grain yield and N recovery efficiency in subtropical China and reported that, N application in depth (i.e., 0.12-m) could maintain a higher N supply in deep soil layers than N broadcasted and could significantly increase grain yield and apparent N recovery efficiency. Thus, N application in depth could be a strategy for increasing N use efficiency by flooded rice and reduce N loss (Yao et al. 2018; Zhang et al. 2017) and N fertilizer rates, representing an effective and promising practice to achieve food and environmental security.

The effective N uptake and recovery by flooded rice from depths up to 0.45-m indicates that rice crop is able to use N from greater depths than those commonly sampled (0 to 0.20-m) for soil analysis and N fertilizer recommendations (SBCS, 2016). The increments of AH-N in depth at some soil sites or the high concentrations in some layers, may help to explain way some soil sites, despite low SOM content, are not responsive to N fertilizer application (Roberts et al. 2009b). Thus, to use potentially available organic-N indexes such as AH-N for a rice crop in Southern Brazil, deeper soil layers should be accounted for in the correlation and calibration process.

Conclusions

The N from soil depths (>0.20-m) greater than those commonly sampled for fertilizer recommendations is effectively taken by the rice crop, especially in high hydraulic conductivity conditions. The N placed at superficial soil layers was rapidly taken up by the rice crop whereas N applied at greater depths was absorbed later in season, more likely contributing to plant nutrition in reproductive growth stages. The high ^{15}N recovery observed with N application at intermediate soil depths (i.e., 0.10, 0.20, and 0.30-m) does not only indicate effective N recovery with depth, but also a potential strategy to enhance N use efficiency by flooded rice. Rice plants effectively use N available in depths up to 0.45-m, therefore greater soil depths should be considered in future studies with correlation and calibration of soil-based N tests for rice crop N fertilizer recommendations.

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Table 1 Soil sites (Alfisol soil from Santa Maria - ASM, Entisol soil from Santa Maria - ESM and Entisol soil from Agudo - EA) selected chemical and physical properties

Soil site	Soil layer	Chemical properties								Physical properties								
		TC[a]	TN[a]	AH-	NO$_3^{-c}$	NH$_4^{+c}$	pH[d]	P[d]	K[d]	Sand[e]	Silt[e]	Clay[e]	BD[f]	TP[f]	MacP[f]	MicP[f]	PR[g]	HC[h]
	m	g kg^{-1}		mg kg^{-1}			-	mg kg^{-1}		g kg^{-1}			g cm^{-3}	-	-	-	Mpa	mmh^{-1}
ASM	0-0.5	25.9	2.2	120	5.5	92.4	4.6	22.5	258	491	355	153	1.38	0.46	0.11	0.34	0.15	34
	0.5-0.10	25.2	2.1	102	2.5	100	4.6	15.6	106	503	349	148	1.43	0.49	0.14	0.35	0.32	72
	0.10-0.15	21	1.8	111	0.8	67	4.8	11.3	78	381	383	237	1.72	0.38	0.01	0.36	0.83	4
	0.15-0.20	18.1	1.6	148	0.5	97.5	4.9	7.8	86	300	428	272	1.61	0.42	0.04	0.38	0.83	2
	0.20-0.25	19.3	1.7	134	0.3	72.8	4.9	5.3	94	377	397	227	1.63	0.4	0.04	0.37	1.03	15
	0.25-0.30	17.8	1.5	116	0.3	54.2	4.8	5.9	88	396	399	206	1.54	0.45	0.07	0.38	0.82	38
	0.30-0.35	15.6	1.2	101	0.4	62.3	5	4.5	76	408	387	205	1.53	0.44	0.04	0.4	0.98	18
	0.35-0.40	13.6	1	106	0.4	58.3	5	5	72	386	400	214	1.53	0.47	0.05	0.41	0.67	4
	0.40-0.50	12.7	0.9	86.1	1.6	58.4	5	5.3	72	444	380	176	1.51	0.43	0.05	0.39	0.65	9
	0.50-0.60	11.2	0.7	59.1	0.7	46.1	4.9	5.5	64	475	397	128	1.54	0.43	0.04	0.39	0.5	11
ESM	0-0.5	33.1	2.7	301	6.3	72.2	4.9	19.6	324	152	514	335	1.18	0.55	0.06	0.49	0.36	395
	0.5-0.10	25.5	2.2	283	2.5	49.9	4.8	22.2	186	161	493	346	1.27	0.52	0.07	0.44	0.43	494
	0.10-0.15	24.8	2.2	274	1.9	43.5	4.8	16.5	98	150	510	340	1.34	0.5	0.04	0.47	1.36	228
	0.15-0.20	23	2	255	0.8	25.1	4.8	8.9	84	181	495	325	1.34	0.48	0.03	0.45	0.51	72
	0.20-0.25	19.1	1.6	204	3.3	27.9	4.7	10	82	168	498	334	1.38	0.51	0.04	0.46	1.05	72
	0.25-0.30	19.3	1.5	184	1.3	16.6	4.8	8.8	74	212	440	348	1.34	0.48	0.04	0.43	0.88	86
	0.30-0.35	20	1.6	191	1.2	24.3	4.8	8.1	68	202	421	377	1.42	0.46	0.03	0.42	0.88	37
	0.35-0.40	15.4	1.2	143	1	18.8	4.7	7	62	253	433	315	1.41	0.46	0.05	0.41	0.71	127
	0.40-0.50	14.3	1.1	126	0.6	19.9	4.8	7.3	60	250	416	333	1.44	0.44	0.05	0.39	0.48	328
	0.50-0.60	13.1	0.9	96.1	0.3	18.2	4.8	5.5	58	261	417	322	1.5	0.43	0.05	0.37	0.26	211
EA	0-0.5	20.8	2	215	4	23.6	4.7	8.3	280	87	473	440	1.21	0.54	0.11	0.43	0.43	281
	0.5-0.10	20.9	2	211	1.4	33.2	4.7	8.8	198	84	472	445	1.25	0.56	0.03	0.54	0.82	25
	0.10-0.15	21.1	2.1	219	0.4	39.6	4.9	8.8	140	85	470	445	1.27	0.54	0.02	0.52	0.34	22
	0.15-0.20	21.2	2	222	1.5	40.6	4.8	7	88	82	483	436	1.35	0.53	0.02	0.52	0.94	14
	0.20-0.25	16.9	1.6	195	1.1	39.9	4.8	8.5	82	48	472	480	1.29	0.52	0	0.52	0.9	3
	0.25-0.30	13.1	1.3	242	0.8	37.5	4.8	11.5	78	50	502	448	1.18	0.57	0.02	0.55	0.92	20
	0.30-0.35	11.9	1.2	167	1	40.6	4.6	19	86	48	512	440	1.17	0.59	0.03	0.56	0.72	76
	0.35-0.40	10.8	1.1	164	1.3	32	4.7	12	92	50	489	461	1.2	0.58	0.03	0.55	0.79	194
	0.40-0.50	10.8	1.1	150	2.2	47.2	4.6	6.4	88	54	452	494	1.22	0.56	0.02	0.54	1.33	107
	0.50-0.60	9.4	1.1	145	1.3	34.5	4.8	6.8	84	43	429	528	1.2	0.57	0.02	0.55	0.77	71

[a] Total nitrogen (TN) and total carbon (TC) determined using dry combustion; [b] Alkaline hydrolysable nitrogen (AH-N) determined using direct steam distillation; [c] Extracted with 2*M* KCl; [d] pH determined on a 1:2 (m:v) soil-to-water suspension; P and K extracted with Melich-1; [e] Determined using pipette method; [f] Soil bulk density (BD), total porosity (TP), macro porosity (MacP), and micro porosity (MicP) determined using sand column; [g] Penetration resistance (PR) determined using an electronic bench penetrometer at saturated samples; [h] Hydraulic conductivity (HC) determined using constant head permeameter method

Table 2 Analysis of variance (ANOVA) *p* values for the quantification of ^{15}N content in rice leaves as affected by N application depth and sampling time for each soil site, and ^{15}N recovery by rice above ground biomass and roots biomass as affected by soil site and N application depth

Soil site	Source of Variation	df	^{15}N content
			········ *p value* ········
ASM	N application depth	4	<0.001
	Sampling time	3	<0.001
	N application depth vs Sampling time	12	<0.001
			········ *p value* ········
ESM	N application depth	4	<0.001
	Sampling time	3	<0.001
	N application depth vs Sampling time	12	<0.001
			········ *p value* ········
EA	N application depth	4	<0.001
	Sampling time	3	<0.001
	N application depth vs Sampling time	12	<0.001
Plant part	Source of Variation	df	^{15}N recovery
			········ *p value* ········
Rice above ground biomass	Soil site	2	<0.001
	N application depth	4	<0.001
	Soil site x N application depth	8	<0.001
			········ *p value* ········
Rice roots biomass	Soil site	2	0.013
	N application depth	4	<0.001
	Soil site x N application depth	8	0.178

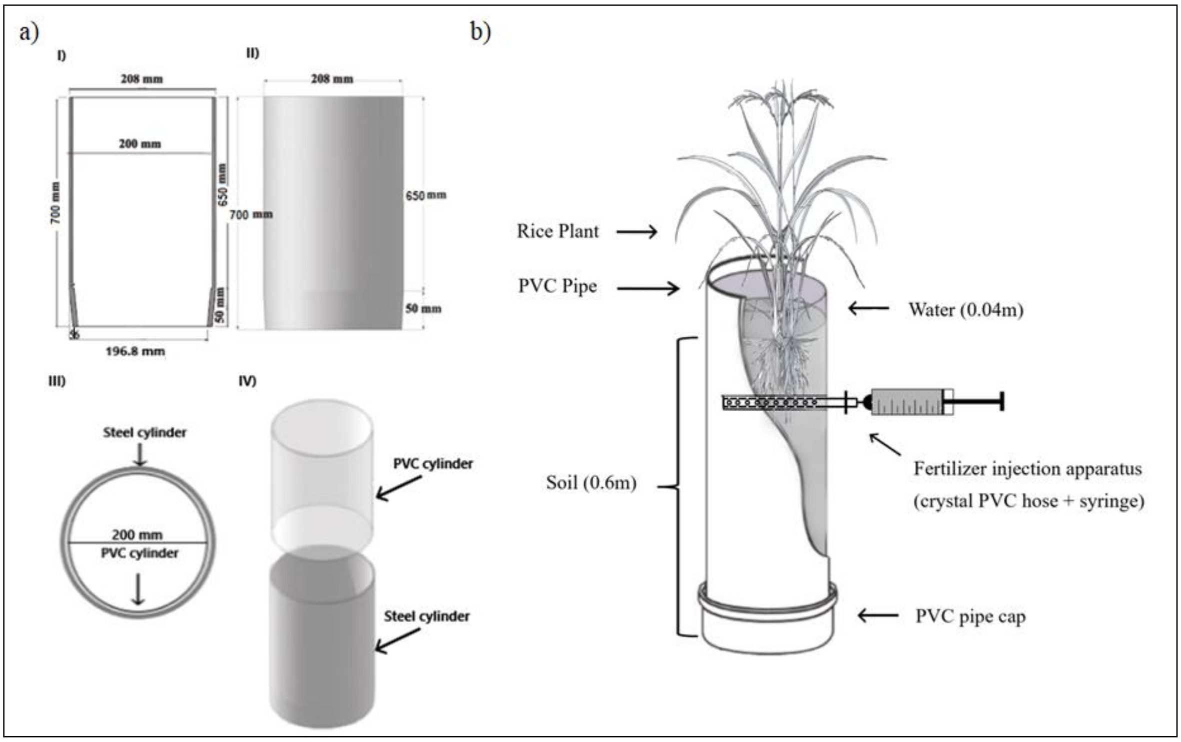

Fig. 1 Details of the monolith sampler (a): frontal section I), frontal view II), top view III), and projection of monolith sampler and PVC cylinder IV); and (b) ^{15}N labeled N injection apparatus in soil column. Adapted from Refatti et al. (2014)

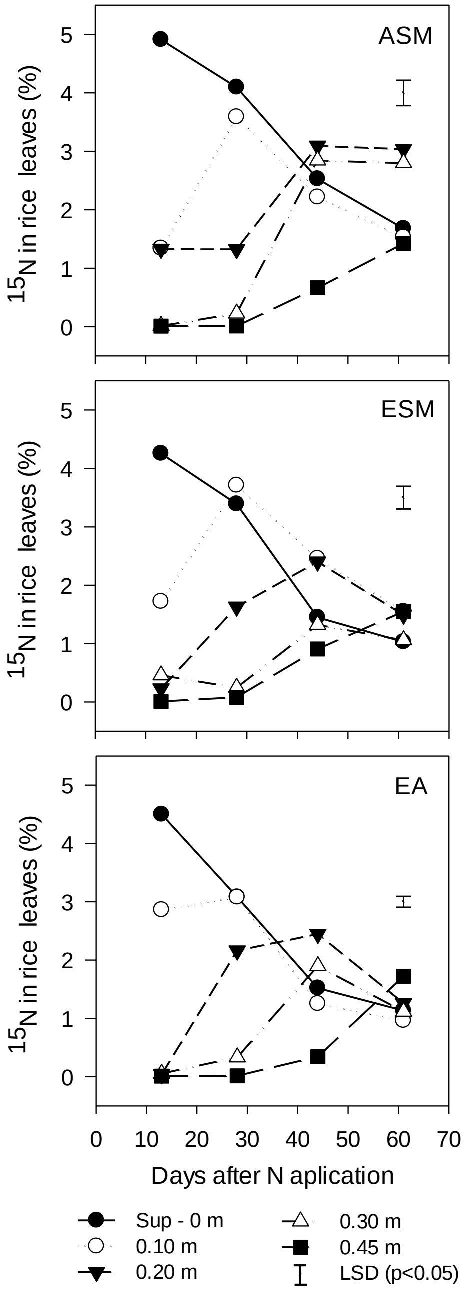

Fig. 2 Atom % ^{15}N excess in rice least fully elongated leaves over time in three paddy soils – (a) Alfisol soil from Santa Maria – ASM, (b) Entisol soil from Santa Maria - ESM and (c) Entisol soil from Agudo – EA - with N application at soil surface (0-m) and at 0.10, 0.20, 0.30 and 0.45-m deep. Vertical bars represent Fisher's least significant difference (LSD) value ($p<0.05$) for the interaction ($p<0.001$): Nitrogen application depth vs Leaves sampling time and is equal to 0.44, 0.39, and 0.19% for ASM, ESM and EA soil sites, respectively

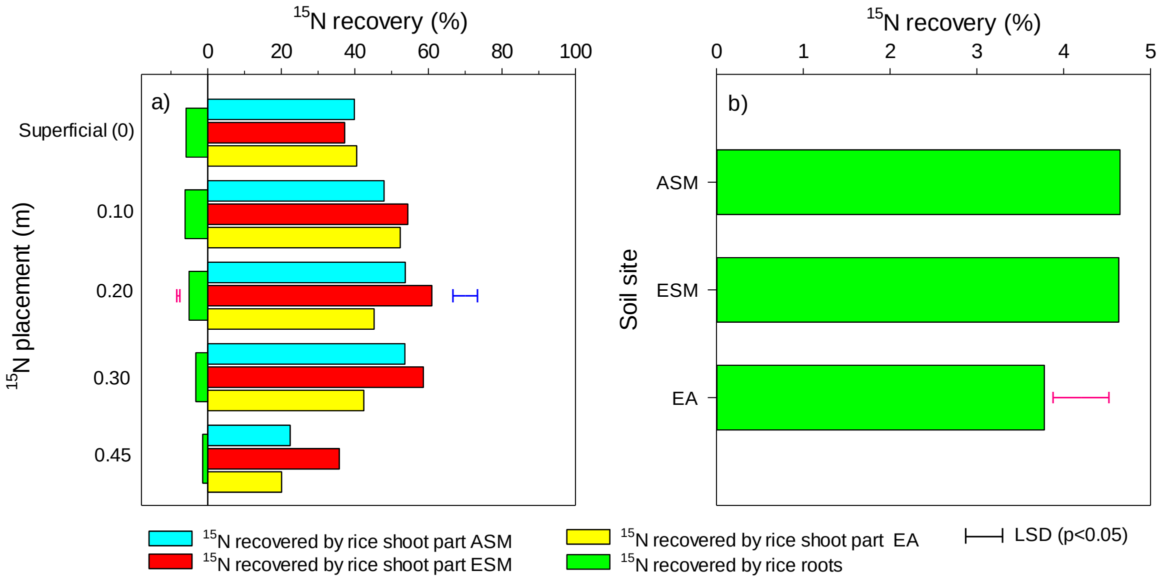

Fig. 3 (a) ^{15}N recovery by rice aboveground biomass and average of ^{15}N recovered by rice roots in three paddy soils (Alfisol soil from Santa Maria - ASM, Entisol soil from Santa Maria - ESM and Entisol soil from Agudo - EA) with N application at soil surface (0-m) and at 0.10, 0.20, 0.30 and 0.45-m deep. (b) Average of ^{15}N recovered by rice roots in three soil sites. Horizontal bars represent Fisher's least significant difference (LSD) value ($p<0.05$) for the interaction ($p<0.001$): Soil site vs N application depth (LSD equal to 6.70%) - blue bar; and for ^{15}N recovered by rice roots (red bar) as a function of (a) N position (LSD equal to 0.83%) and (b) soil site (LSD equal to 0.64%

5.4 ESTUDO IV

Available nitrogen in paddy soils depth: influence on rice root morphology and plant nutrition[1]

Abstract

Nitrogen (N) is a key nutrient for rice crop and its availability in soil profile can influence the plant root morphology and plant nutrition. The objective of this study was to evaluate if N from greater soil depths would increase rice root growth, N uptake and influence plant nutritional status. A greenhouse study was developed using undisturbed samples from three locations (Alfisol soil from Santa Maria – ASM, Entisol soil from Santa Maria – ESM, and Entisol soil from Agudo – EA) with enriched ^{15}N application at different depths (0, 10, 20, 30, and 45 cm), and rice was grown under flooded conditions. At rice heading, chlorophyll content (SPAD units) and photosynthesis rate were measured at flag leaves and rice biomass production, root morphology, total N and ^{15}N contents were evaluated. Higher dry mass and N uptake was observed with N application at near soil surface layers. Most rice N uptake was derived from soil, which was more evident when N fertilizer was injected at 45 cm deep (on average 74%). Highest root growth was observed at the near surface layers with constant decrease in depth. The effective rice rooting system was up to 60 cm at ASM and ESM soils and up to 45 cm for EA soil. Rice roots from deeper layers presented lower diameter, as there are mainly high branched roots and root hairs. The N application at 30 and 45 cm depths provided N later in the season, resulting in plants with lower dry mass but with higher N content in aboveground biomass, which culminated in higher chlorophyll content, photosynthesis rate, and carboxylation efficiency by Rubisco. Soils with available N in profile and with no physical impedance for root growth contribute to rice nutrition and likely causes plants to function more optimally in photosynthates supply.

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[1] Artigo elaborado de acordo com as normas da revista Journal of Plant Nutrition and Soil Science: *https://onlinelibrary.wiley.com/page/journal/15222624/homepage/forauthors.html*

Keywords: *Oryza sativa* L. N recovery. ^{15}N. Photosynthesis. Soil fertility. Lowland soils

1 Introduction

Nitrogen (N) is one of the most limiting nutrients for rice crop production. Plant growth requires a large amount of N, and the symptoms of an insufficient N supply to plants include lower chlorophyll content, photosynthesis activity, biomass production, root growth, a premature senescence and a decreased in grain yield and quality (*Fageria* and *Baligar*, 2005). Thus, the ability of soil suppling N is a key process for plant nutrition, grain yield, efficient mineral N fertilizer use, and the economic sustainability of rice cropping systems. Therefore, a better understanding in soil labile N forms and its availability for plant uptake is crucial for a better N fertilizer management as well as prevent environmental risks due to N losses.

Labile organic N forms, such as amino acids and amino sugars, extracted as alkaline hydrolyzable N (AH-N) have been reported to have a distinct behavior in paddy soils depth, reaching high concentration in depths up to 60 cm (*Roberts* ate al., 2011). Previous research also reported that the nonexchangeable (or clay fixed) NH_4^+, more specifically the recently adsorbed (plant-available) NH_4^+ pool, plays an important role in the N turnover of paddy soils and N nutrition of lowland rice (*Schneiders* and *Scherer*, 1998), and its release and solubility is strongly coupled to plant root vicinity (*Mengel* et al., 1990; *Scherer* and *Ahrens*, 1996), soil moisture content (*Mengel*, 1981), and redox potential (*Schneiders* and *Scherer*, 1998). However, the nonexchangeable NH_4^+ is coupled to clay minerals and its content as percent of total N usually increases in depth (*Nieder* et al., 2011). Thus, the availability of nonexchangeable NH_4^+ for plant uptake would also be dependent on the root growth in soil layers with this N form.

Plants have evolved various strategies to modulate their root uptake capacity and compensate lower nutrient availability in soil (*Nacry* et al., 2013). In particular, plant root system is very plastic and can adjust / regulate its architecture based on nutrient rich compartments (*Lima* et al., 2010), which can greatly impact the N acquisition from soil (*Nacry* et al., 2013). Some nutrients such as P and N, mainly in inorganic forms, have been reported to regulate plant root growth and influence the total soil volume explored by the plant. The NO_3^- and NH_4^+ extra-cellular concentrations are important external signals that are sensed locally by the roots (*Tsay* et al. 2011), while for the overall N status of the plant, several molecules, including hormones, sugars, and nutrients themselves or their metabolites, function as systemic internal signals about local conditions, from root to shoot and vice versa (*Liu* et al., 2009), informing the roots of the plant N demand. Therefore, these signaling mechanisms allow the plant to perceive the local N availability and stimulate both the expression of N transporters and the elongation of lateral roots in response to N presence (*Gojon* et al., 2009).

Plant nutritional status, such as chlorophyll content and photosynthesis rate vary over the plant season and is expressively affected by nutrient availability. *Sikuku* et al. (2016) reported that total chlorophyll (SPAD units) and net photosynthesis increased significantly with an increase in N levels in rice crop. Up to 75% of leaf N is found in the chloroplasts and consequently, lower photosynthesis rates under conditions of N limitation are often attributed to reduction in chlorophyll content and Rubisco activity (*Toth* et al., 2002). More than 90% of crop biomass is derived from photosynthetic products (*Makino*, 2011), thus a close relationship between leaf N content, maximum leaf photosynthetic rate, plant biomass, and yield can be observed in many plant species. Regulating mechanisms such as reducing shoot growth and nutrient remobilization are plant strategies to compensate nutrient starvation and keep photosynthates production. However, there is yet no information about the effect of N available in depth on rice root growth and morphology, or its contribution for plant nutrition. We hypothesize that N available in greater soil depths increases rice root length, contributes to plant N uptake, and promotes a higher chlorophyll content and photosynthesis rate. This study aims to evaluate if N form greater soil depths would increase rice root growth, N uptake and influence plant nutritional status.

2 Materials and Methods

2.1 Soil sites characteristics

Soil samples were collected form three fields commonly cropped with rice in Southern Brazil - Alfisol soil from Santa Maria – ASM (29°43'3.15"S; 53°42'21.67"W), Entisol soil from Santa Maria - ESM (29°43'8.38"S; 53°42'21.84"W) and Entisol soil from Agudo – EA (29°40'6.16"S; 53°18'50.22"W) - representing a variety of physical and chemical properties. Soil properties at the top 20 cm of ASM, ESM, and EA sites, are respectively: TC (22.6, 26.6, and 21.0 g kg^{-1}) and TN (1.9, 2.2, and 2.0 g kg^{-1}) determined using dry combustion at elemental analyzer (Thermo Scientific, Flash EA 1112, Milan, Italy); AH-N (120, 179, and 217 mg kg^{-1}) determined using direct steam distillation (*Roberts* et al., 2009); NO_3^- (2, 3, and 2 mg kg^{-1}) and NH_4^+ (89, 48, and 34 mg kg^{-1}) extracted with 2 mol L^{-1} KCl and determined using salicylate colorimetric techniques (Mulvaney 1996); sand (419, 161 and 84 g kg^{-1}), silt (379, 503, and 479 g kg^{-1}), and clay (203, 336, and 441 g kg^{-1}) determined using pipette method (*Donagema* et al., 2011); soil bulk density (1.5, 1.3, and 1.3 Mg m^{-3}) determined according *Reinert* and *Reichert* (2006); penetration resistance (0.5, 0.7, and 0.6 Mpa) determined using a Marconi-MA 933 electronic bench penetrometer at saturated samples (*Tormena* et al., 2007); and hydraulic conductivity (28, 297, and 85 mm h^{-1}) determined using constant head permeameter method (*Libardi*, 2005). Details of the undisturbed soil samples (0 to 60 cm) collection procedure, soils physical and chemical characteristics in depth, ^{15}N injection in depth and plant growth are described in *Drescher* et al. (2019).

2.2 Plant growth and fertilizer application

Experiment was conducted in 3x5 factorial arrangement (three soils and five N placement depths), in a complete randomized block design, with three replications in the greenhouse of the Soil Science Department at the Federal University of Santa Maria. Four rice plants from the cultivar ‘IRGA 424RI’ were growth in flooded conditions (i.e., with a 4 cm water layer maintained for the duration of the trial) in each soil column. To assess the N uptake by rice plant from various placement depths, 10-mL of a solution containing 196 mg N (rate equivalent to 62 kg N ha^{-1} – commonly used in the first N application in the standard two-way split N application) as 6.0 atom% ^{15}N-labeled ammonium sulfate ($(NH_4)_2SO_4$) was applied superficially (i.e., 0 cm deep) and injected at 10, 20, 30 and 45 cm depths when rice plants reached V3 stage. After N injection, 10-mL of distillated water was injected to evacuate all ^{15}N solution from the injection apparatus. Fertilizer injection was performed only after the establishment of a permanent flood to ensure there was no downward movement of water in the soil profile or upward flow of the ^{15}N-labeled solution via capillary action.

2.3 Plant tissue sampling and analysis

When rice plants were at the beginning of heading (70 days after rice transplanting and 61 days after ^{15}N addition), gas exchange measurements - using an open system infrared gas analyzer (IRGA) (LI-6400XT LI-COR, Inc., Lincoln, NE, USA) - and Minolta SPAD-502 chlorophyll meter values were taken at three rice plants per soil column. Therefore, two flag leaves of each plant were selected, and measurements taken in the intermediate leave position. Net photosynthetic rate (PhotoR), stomatal conductance (SC), intercellular CO_2 concentration (IC), transpiration rate (TR), water use efficiency (WUE) and instantaneous carboxylation efficiency (Photo/IC) were determined with IRGA at an ambient with CO_2 concentration of 400 µmol mol^{-1} at 20–25 °C, 50 ± 5% relative humidity and a photon flux density of 1000 µmol m^{-2} s^{-1}.

After SPAD meter and IRGA evaluations, rice aboveground biomass was collected, oven dried at 65 °C until constant mass, and dry matter production was determined for each soil column. After collecting plant aboveground biomass, the soil column was opened, and rice roots samples were collected in one half of the longitudinal section (i.e., corresponding to two rice plants) at the 0-10, 10-20, 20-30, 30-45, 45-60 cm depths. The roots were separated from soil by washing under running water, using sieves with 2 and 1mm openings. Once separated from the soil, rice root samples were washed with distillated water, remaining plant residues and impurities separated manually, and samples were stored in plastic bags under refrigeration (-20 ±5°C). Thereafter, root morphology (i.e., total root length (cm cm^{-1} soil deep), surface area (cm^2 cm^{-1} soil deep), volume (cm^3 cm^{-1} soil deep), average diameter (mm), and root length per diameter class (i.e., $0 < L \leq 0.125$, $0.125 < L \leq 0.25$, $0.25 < L \leq 0.50$, $0.50 < L \leq 1.00$, $1.00 < L \leq 1.50$, and $L > 1.50$ mm) was analyzed with WinRhizo Pro 2013 software coupled to an EPSON Expression 11000 scanner equipped with additional light (TPU), with a resolution of 600 dpi (Régent Instr. Inc.). After root morphology analysis, root samples were oven dried at 65 °C until constant weight and root dry matter production per soil layer (i.e., equivalent to four rice plants) was determined.

After drying, rice aboveground and root biomass was milled (particle size <1mm) to determine total N content in an elemental analyzer (Thermo Scientific, Flash EA 1112, Milan, Italy) and ^{15}N abundance by mass spectrometry (IRMS) (Thermo Scientific, Delta V Advantage, Bremen, Germany) coupled to an elemental analyzer (Thermo Scientific, Flash EA 1112, Milan, Italy). Total N uptake, N derived from fertilizer (NDFF), and N derived from soil (NDFS) were obtained from TN and ^{15}N content in plant tissue and dry matter production of each treatment. Nitrogen uptake, ^{15}N atoms excess in plant tissue, NDFF, and NDFS were calculated by the following equations:

$$\text{Total N uptake (mg)} = \frac{\text{\% N in sample x Dry mass production (g)}}{100} \text{x} 1000 \qquad \text{(Eq. 1)}$$

$$\text{\%atom } ^{15}\text{N in excess in sample} = \text{\%atom } ^{15}\text{N in sample} - 0.3663\% \qquad \text{(Eq. 2)}$$

$$\text{NDFF (mg)} = \text{Total N uptake (mg) x} \frac{\text{\%atom } ^{15}\text{N in excess in sample}}{\text{\%atom } ^{15}\text{N in excess in fertilizer}} \qquad \text{(Eq. 3)}$$

$$\mathrm{NDFS\ (mg)} = \mathrm{N\ uptake\ (mg)} - \mathrm{NDFF (mg)} \qquad \text{(Eq. 4)}$$

2.4 Statistical analysis

The experiment was a randomized block design with a factorial arrangement. Each treatment was replicated three times with replicate being a random effect in the ANOVA model. Normality test (Lilliefors and D'Agostino) was performed before running ANOVA. Root length, root surface area, and root volume data were transformed (square root and log(x)) to fit normality assumptions prior running ANOVA. Thereafter, ANOVA was conducted to determine the influence of N application depth, soil layer, and their interaction (fixed effects) on root morphology variables. ANOVA was also conducted to determine the influence of soil site, N application depth, and their interaction (fixed effects) on rice dry mass production, total N and ^{15}N concentration, N uptake, NDFF, NDFS, PhotoR, SC, IC, TR, WUE, Photo/IC and SPAD meter chlorophyll values. Means were compared using Fisher's least significant difference (LSD) at the 0.05 probability level. All statistical analyses were performed with BioEstat version 5.0 (Ayres et al. 2007) and Sisvar version 4.0 (Ferreira, 2011) softwares.

3 Results

3.1 Nitrogen uptake form different soil depths

There was a significant soil site vs N application depth interaction ($p<0.05$) for rice shot dry mass (DM) production, ^{15}N concentration, N uptake, NDFF and NDFS, while total N was affected only by the main factors (Table 1). Rice root biomass had no significant soil site vs N application depth interaction for plant variables and were affected only by one or both main effects. Otherwise, for total plant tissue (shoot plus root biomass) there was a significant soil site vs N application depth interaction for DM, N uptake, NDFF and NDFS (Table 1). Plant variables without interaction but with a significant difference for one or both main effects had the main effects means compared and are presented in Table 2.

((Table 1))

Rice dry mass production (shoot, roots, and total dry mass) was higher at EA soil, followed by ESM and ASM soils, respectively (Table 2). The N fertilizer application at soils surface and at the 10 cm depth resulted in higher dry mass production for all soils. As greater the N fertilizer injection depth, lower was the dry mass production by rice roots and aboveground biomass, and consequently, total dry mass production per soil column. Total N content at rice shoot was significantly higher (0.90%) at ESM soil, with rice roots following the same trend, although, with no difference from ESM soil (0.71% at both soils). For N position depth, total N content had difference only at rice shoot, with greater values (0.95 and 0.94%) for 30 and 45 cm depths. Otherwise, the ^{15}N content at rice shoot was higher at ASM soil (2.15, 2.14, and 1.98%) with N application at 30, 20 and 10 cm depth, respectively, while ESM soil had higher content (1.90 and 1.86%) at 30 and 20 cm and EA soil presented the greatest values (1.57 and 1.52%) for 20 and 10 cm depths, respectively. For rice roots, ASM and ESM soils had the highest (1.11 and 1.02%, respectively) ^{15}N content, and the near surface N fertilizer application resulted in greater ^{15}N content in rice roots. Most of the rice plants N uptake was observed at the shoot, with greatest values (448, 425 and 405 mg N col^{-1}) at the EA soil when N fertilizer was applied at soil surface and at the 30 and 10 cm depths, followed by ESM and ASM soils. The N uptake by rice roots accounted only for a small portion of total N uptake, and the greatest values (59, 54 and 53 mg N col^{-1}) were observed with N application at 10 cm, soil surface, and at 20 cm depth, respectively. Thus, rice total N uptake (shoot plus roots) followed the same trend as rice shoot N uptake.

((Table 2))

The proportion of rice shoot N uptake that is derived from fertilizer was higher when N was applied at 10, 20 and 30 cm depths for all soils (Table 2). The ASM soil had the highest proportion of NDFF (105 mg N col^{-1}, representing 36% of N uptake by shoot) at both 20 and

30 cm depths. Otherwise, the EA soil presented the lowest proportion of NDFF and most of the N uptake was derived from soil (277 and 369 mg N col^{-1}, representing 88 and 82% of total N uptake by shoot) when N was applied at 45 cm and at soil surface. Nonetheless, for rice root biomass ASM and ESM soils had the highest proportion of NDFF (9 mg N col^{-1}, representing 19 and 17% of total N uptake by rice biomass, respectively) (Table 2) and the N application at soil surface and at 10 cm depth had the largest proportions of NDFF among N application depths. Overall, the content of NDFF in total plant tissue was low and followed the same trend as NDFF at aboveground biomass, with larger proportion when N was applied at 10, 20 and 30 cm depths for ASM and ESM soils, while EA soil had more NDFF with N application at 10 and 20 cm depths. Most of the rice N uptake was derived from soil, which was more evident when N fertilizer was injected at 45 cm deep for all soils (321, 243 and 215 mg N col^{-1} for EA, ASM, and ESM soils, which represents 82, 74 and 66% of NDFS, respectively) (Table 2).

3.2 Rice root morphology

Significant N application depth vs soil layer interaction ($p<0.01$) was observed for rice root morphology variables. Overall, the highest root length, root surface area, and root volume was observed at the near surface layers with constant decrease in depth for all soils, independently of N application depth (Table 3). On an average the soil top 20 cm accounted for 90, 91, and 90 %; 84, 87, and 87 %; 94, 92, and 90%, of the total root length, root surface area, and root volume at ASM, ESM and EA soils, respectively. Although, by looking at these properties for each N application depth and soil type, it is possible to verify significant changes. For example, there was an increase in rice root length, root surface area and root volume at the 45- to -60 cm when N was applied at 45 cm depth compared to the intermediate layers (i.e., 10, 20, and 30 cm) at ASM soil. On the other hand, EA soil had no root development at the 45- to -60 cm, but it was also possible to verify a significant increase in root length, surface area and volume at the 20- to -30 cm depth with N application at greater depths. The average root diameter followed a similar trend as root length, surface area and volume at ASM soil, while at ESM and EA soils there was an increase in average diameter at the 20- to -30 cm layer when N was applied at 45 cm depth. Stands out that, independent of soil site and N application depth, rice roots from deeper soil layers had the lowest root average diameter. This characteristic can be clearly observed at the rice root length distribution in diameter classes presented in figure 1. For ASM and EA soils there was a larger proportion (more than 60%) of total root length in the two lowest diameter classes (i.e., $0 < L \leq 0.125$ and $0.125 < L \leq 0.25$ mm) at the 0- to -10 and at 45- to -60 cm layers, except for the 45- to -60 cm layer when N was applied 10 cm deep for ASM soil, while the intermediately layers presented a slightly reduction in root length. On the other hand, for ESM soil, the proportion of root length distribute in the two lowest diameter classes was larger (around 70%) and more evenly between soil layers, regardless of N application depth.

((Table 3))

((Figure 1))

3.3 Rice nutritional status

Significant N application depth vs soil site interaction ($p<0.005$) was observed for SPAD meter values (Table 1). Fertilizer application at 45 and 30 cm resulted in the highest SPAD meter values for all soils (35.7 and 35.8, 34.3 and 32.8, 33.0 and 32.3, for ASM, ESM, and EA soils, respectively) after 61 days of N fertilizer application (Figure 2). The SPAD meter values showed a constant decrease as fertilizer application was closest to soil surface, with an exception for the EA soil, where no significant difference ($p<0.05$) was observed between superficial N application and at the 30 and 45 cm depths. The rice plants physiological variables had no significant soil site vs N application depth interaction and were affected only by one or by both main effects (Table 1). The PhotoR, SC, WUE, Photo/IC in rice leaves after 61 days of N fertilizer application followed the same trend as the SPAD meter values. The highest values of these variables (25.248 and 24.129 µmol CO_2 m^{-2} s^{-1}; 0.985 and 0.916 mol H_2O m^{-2} s^{-1}; 2.316 and 2.303 mol CO_2 mol H_2O^{-1}; 0.0787 and 0.0752 µmol m^{-2} s^{-1} Pa^{-1}) were observed when N fertilizer was injected at 30 and 45 cm depths, respectively, and lower values observed at the near surface layers (Table 4). The IC and the TR were not affected by the main effects, while SC also presented difference between soil sites, with higher value (0.919 mol H_2O m^{-2} s^{-1}) for ESM soil. Drives attention the fact that both chlorophyll content (SPAD meter values) and physiological variables (i.e., PhotoR, SC, WUE, Photo/IC) measured at rice heading, were higher as greater the N application depth.

((Figure 2))

((Table 4))

4 Discussion

4.1 Nitrogen uptake form different soil depths

The highest rice dry mass production observed at EA soil, followed by ESM and ASM soils, might be related to soil N availability, as there was a higher AH-N content along the soil profile at EA and ESM soils. Subsoil nutrient levels, especially N, can influence plant growth. Previous research has indicated that a significant level of organic and inorganic N and relatively high rates of N mineralization can occur at depths up to 60 cm (*Kemmitt* et al., 2008), and therefore be available to plant uptake. Thus, soils with differences in nutrient availability and physical properties influencing root growth, likely causes different plant growth patterns and biomass production.

The higher dry mass production by rice plants when N fertilizer was applied near soil surface (i.e., 0 and 10 cm depth), can be ascribed to higher N availability it a depth where plants could effectively absorb the nutrient at the beginning of rice vegetative stages (V3/V4). At this stage, rice plants require a large amount of the nutrient for cell multiplication and elongation

(*Nario* et al., 2003) and tillering (*Yoshida*,1981). These treatments provided more N to rice plants in a responsive physiological stage, which may have also resulted in a better root growth and higher root activity (*Xu* et al., 2015). Therefore, rice plants could explore greater soil depths to absorb other nutrients and uptake them more efficiently, which resulted in a higher number of tills, shoot growth and dry mass production. On the other hand, total N and ^{15}N in rice tissue was higher when N was placed in deeper layers, probably due to a lower rice dry mass production, resulting in a lower nutrient dilution effect in plant tissue (*Fageria* and *Baligar*, 2005). Moreover, the low contribution of rice roots for total N uptake observed in this study can be ascribed to roots lower dry mass production and the fact that plant shoot part is the main N sink.

The larger proportion of NDFF that was observed when N was applied at 10, 20 and 30 cm depths for ASM and ESM soils and at 10 cm for EA soil (Table 1) might be related to ammonium (NH_4^+) absorption to soil cation exchange sites and protection against lost. Sorption and desorption of NH_4^+ is a paramount mechanism controlling retention and release of N, therefore affecting the N bioavailability for plants and its leaching from the soil (*Fen* et al., 1982; *Phillips* 1999). Furthermore, the addition of N fertilizer below the soil surface may have also led to an increase in the NDFF as there was no dilution in the flood water and potentially less immobilization (*Roberts* et al., 2013, *Yao* et al., 2018). Thus, NH_4^+ was protected and available for plant uptake when rice roots reached these layers, resulting in a higher contribution of N from fertilizer. On the other hand, when N fertilizer was applied at 45 cm there was a higher contribution of soil N in rice N uptake, probably due to the difficulty of plants access the fertilizer in deeper layers at the beginning of vegetative stages. *Norman* et al. (1992), suggest that rice crop uses preferentially fertilizer N early in the season, while later in the season, when the plant has developed an extensive root system, N uptake would be primarily from soil N pools. However, at this treatment, plants roots needed to explore greater soil depths in other to uptake fertilizer N, therefore soil N had greater contribution in plant nutrition. Previous research using a ^{15}N tracer has reported that N uptake by the crops can be originated primarily from the soil rather than fertilizer sources (*Schindler* and *Knighton*, 1999; *Kadiyala* et al., 2015), reinforcing the importance of available soil N near soil surface for better plant growth. Thus, the lower NDFF when N fertilizer was applied at soil surface can also be related to the higher mineral N and AH-N content at soil superficial layers, as result of deposition and decomposition of crop residues and organic N mineralization from SOM. The larger amount of available organic and inorganic-N at soil superficial layers might have also resulted in a dilution effect, leading to higher overall N uptake, but with a lower percentage of fertilizer-N uptake.

4.2 Rice root morphology

The majority of rice root length, root surface area, and root volume observed at the near surface layers, with constant decrease in depth for all soils, observed in this study is in accordance to previous research (*Beyrouty* et al., 1987). In their study, the authors pointed out that rice roots tend to increase in depth throughout the growing season, with the maximum penetration depth occurring near harvest. The total rice root length increases as the shoot grows (*Yoshida*, 1981). For EA soil, the maximum rooting depth was up to 45 cm, while for ASM and ESM soil rice roots increased in depth, reaching up to 60 cm. These results corroborate with *Beyrouty* et al. (1987) findings for silt loam soils in Arkansas, who reported rice root growth up to 72 cm near harvesting. However, *Yoshida* (1981) stated that in anaerobic environment, such as paddy soils with impaired water percolation, rice roots seldom exceed a maximum depth of about 40 cm, contrasting our results at ASM and ESM soils. *Beyrouty* et al. (1987) reported that an increase in rice roots growth in depth could be related to the fact that the minirhizotron tubes used in their study could encouraged roots to grow alongside the tube face, and to a higher partial pressure of oxygen, resulted from a continuous downward water percolation. We believe that those were not major effects in our study, as the PVC tubes were sealed at the bottom, and root growth was evenly distributed through the soil column (visible at root sampling) and there were no roots at the 45- to -60 cm layer at EA soil. These results indicate that rice growth in depth is controlled not only by genetic ability and environmental conditions (*Yoshida*, 1981), but also by soil properties, such as N availability in greater depths as well as soil physical properties such as penetration resistance, pore size and connectivity, and hydraulic conductivity. Thus, if the soil has no physical properties limiting root growth, it is likely that a proportion of rice roots may exceed the soil top 40 cm and feed the plant with nutrients form greater depths.

The rice roots in greater soil depths are probably from higher branching orders and root hairs. Nutrient rich areas, especially N, stimulates root growth and increases root branching order, as it can be observed by the root length increase in the two lowest diameter classes (i.e., $0< L \leq 0.125$ and $0.125< L \leq 0.25$ mm) at ASM and ESM soils with N application at 45 cm depth (figure 1). Previous research has indicated that plants react to a gradient in nutrient concentration in the soil by favoring root growth in the areas with higher nutrient availability (*Robinson* 1994; *Forde* 2002). The study of *Mounier* et al., (2014) shows the ability of *Arabidopsis thaliana* plants increase lateral root growth according to an increase in NO_3^- concentration. Moreover, *Lima* et al., (2010) reported that NH_4^+ presence in the external medium is perceived as a signal molecule by roots and can regulate lateral root development. In their study, localized NH_4^+ supply was found to mainly increase initiation of second- and third-order lateral roots leading to highly branched bushy root system. *Lima* et al., (2010) also stated that local NH_4^+ supply to *Arabidopsis thaliana* plants increases lateral root initiation and higher-order lateral root branching, whereas the lateral roots elongation is stimulated mainly by NO_3^-, therefore these ions act in a complementary manner. Therefore, the increase in rice root length, surface area, and volume observed in our study at the depths with $(NH_4)_2SO_4$ application can be resulted by a stimulus of NH_4^+ presence, as well as NO_3^- that might be originated via nitrification process at rhizosphere aerobic zone.

Despite of the largest proportion of rice root growth been at the soil superficial layers, we highlight that the rice rooting system in greater depths is constituted mainly by fine roots from high branching order and root hair, which account only for a small proportion of plant root biomass but represents an expressive rot length and surface area. These results reinforce the importance to consider the hole plant rooting depth for soil N availability indexes calibration, as fine roots and root hair (responsible for nutrient absorption) explore and uptake N form greater soil depths.

4.3 Rice nutritional status

The chlorophyll content (SPAD units), photosynthesis rate, and instantaneous efficiency of carboxylation by Rubisco values at the beginning of rice heading (70 days after rice transplanting and 61 days after ^{15}N addition) followed similar trends. The higher N content in rice aboveground biomass observed with N fertilizer application at 30 and 45 cm depths (Table 2) must have resulted in an increase in chlorophyll content, and therefore higher SPAD meter values (figure 2), PhotoR, and Photo/IC values (Table 4) at these treatments. The photosynthesis rate increases with an increasing N content in rice leaves as N is an essential component of both chlorophyll and Rubisco, the enzyme facilitating photosynthesis (*Hidayati* et al., 2016). Rubisco represents the majority of N invested in photosynthesis (*Zhong* et al., 2017). It can excess a quarter of leaf N and account for as much as half of soluble protein (*Parry* et al., 2013). Chlorophyll levels are closely related to the photosynthesis rate because they provide the photosynthetic apparatus which allows plants to absorb energy from light and transfer it to the chlorophyll *a* (*Porra* et al. 1993). Thus, a high N content in the leaf tissue allows the plant to have more chlorophyll and Rubisco, triggering a higher rate of photosynthesis (*Osaki* et al. 1995), which can be observed in this study at treatments with N application at greater depths. Soils that have available N pools in profile can provide N to rice lather in the season, allowing leaves to remain green during grain filling. This process enables photosynthesis and carboxylation efficiency by Rubisco to remain high, which is favorable for high yield due to the increase of photosynthate supply to seeds (*Fu* and *Lee*, 2009).

Stomata control water and gas exchange between leaves and ambient air. Trade-off between water loss and gas exchange is crucial for plants to maintain photosynthetic capacity under water deficiency condition (*Manzoni* et al., 2013). Although partial closure of stomata is an essential plant regulatory process for avoiding water loss and maintain water balance in plant, stomatal closure increases the resistance of CO_2 diffusion from ambient air to intercellular airspace as well as from intercellular airspace to carboxylation site (*Zhong* et al., 2017). However, the flooded soil condition in the experiment exceeded rice plant water supply needs. Therefore, N application depth had little effect on WUE, only the N fertilizer application at 10 cm depth resulted in lower WUE and differed from other treatments, which might be a random effect since other plant physiological variables (i.e., TR) had no difference between treatments. Similar to IC and TR, stomatal conductance had no influence of N application depth, but was affected by soil site, with higher values for ESM and EA soils. This effect can be related to lower soil N availability, dry matter production, total N content in shoot and N uptake at ASM soil site, which may have affected water balance in plant, resulting in partial stomatal closure and lower stomatal conductance.

The ^{15}N concentration in rice biomass and the higher NDFF observed with N application at greater depths is supported by the results from root growth and by previous study that estimate 86% of rice total root length in the top 36 cm of the soil profile when the rice crop is at the mid-tillering growth stage, growing rapidly, and actively taking up N and other nutrients (*Beyrouty* et al., 1987). As rice plants were growing, roots reached deeper layer and absorbed N from greater depths, increasing the N content in rice leaves and aboveground biomass. Thus, N application at 30 and 45 cm deep provided N later in the season and resulted in plants with lower dry mass but with higher N content in shoot at the three soils, and consequently in higher SPAD meter values as chlorophyll concentration in a leaf is closely correlated with leaf N concentration (*Gholizadeh* et al., 2017). These results reinforce that soils with higher available N forms in soil profile and with no physical impedance to root growth contribute to rice plant nutrition later in season and likely causes plants to function more optimally in photosynthates supply and in converting most of the tillers into productive ones.

5 Conclusions

The highest ^{15}N content and NDFF that was observed when N was applied at 10, 20 and 30 cm depths for all soils and is related to soil physical properties and NH_4 absorption to cation exchange sites and protection against lost. The N located bellow 20 cm contributes to plant nutrition, therefore it should be considered in future studies with calibration of soil-based N tests for rice. The N availability in depth affected rice root morphology, increasing root length, surface area and volume, while reducing root diameter. The effective rice rooting system was up to 60 cm at ASM and ESM soils and up to 45 cm for EA soil. The results of this study showed that the N available at near soil surface layers provide N at the beginning of rice physiologic stages, resulting in a higher dry mass production. On the other hand, N from greater soil depths (i.e., 30 and 45 cm) are probable used lather in season, resulting in lower dry mass production, but higher chlorophyll content (SPAD meter units), photosynthesis rate, and carboxylation efficiency by Rubisco, which ultimately affects the photosynthates supply to the grain and yield.

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Table 1: Analysis of variance (ANOVA) *p* values for the quantification of rice plant variables: dry mass production (DM), total N and ^{15}N concentration, N uptake, N derived from fertilizer (NDFF), N used from soil (NDFS), photosynthesis rate (Photo), stomatal conductance (SC), intercellular CO_2 concentration (IC), transpiration rate (TR), water use efficiency (WUE), instantaneous efficiency of carboxylation by rubisco (Photo/IC), and SPAD meter chlorophyll (SPAD) means as influenced by soil site and N application depth.

Source of Variation	df	Plant variable						
		DM	Total N	^{15}N content	N Uptake	NDFF	NDFS	-
		……… *p* value ………						
		Rice shoot biomass						
Soil site	2	<0.001	0.011	<0.001	<0.001	<0.001	<0.001	-
N application depth	4	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	-
Soil site x N application depth	8	<0.001	0.450	<0.001	<0.001	<0.001	<0.001	-
		Rice roots						
Soil site	2	<0.001	0.009	<0.001	0.086	0.013	0.009	-
N application depth	4	<0.001	0.067	<0.001	<0.001	<0.001	0.054	-
Soil site x N application depth	8	0.021	0.950	0.080	0.301	0.178	0.332	-
		Total rice plant						
Soil site	2	<0.001	-	-	<0.001	<0.001	<0.001	-
N application depth	4	<0.001	-	-	<0.001	<0.001	<0.001	-
Soil site x N application depth	8	<0.001	-	-	<0.001	<0.001	<0.001	-
		Photo	SC	IC	TR	WUE	Photo/IC	SPAD
		……… *p* value ………						
Soil site	2	0.843	0.04	0.175	0.528	0.834	0.932	0.27
N application depth	4	0.003	0.013	0.058	0.515	0.011	0.002	<0.001
Soil site x N application depth	8	0.118	0.154	0.066	0.611	0.956	0.063	0.005

Table 2: Rice dry mass (DM) production, total N and ^{15}N concentration, N uptake, N derived from fertilizer (NDFF), N derived from soil (NDFS) and its compartmentalization in rice plants in three paddy soils (Alfisol soil from Santa Maria - ASM, Entisol soil from Santa Maria - ESM and Entisol soil from Agudo - EA) with different N application depths.

Soil site	ASM	ESM	EA		ASM	ESM	EA		ASM	ESM	EA		ASM	ESM	EA		ASM	ESM	EA		ASM	ESM	EA	
Plant variable		DM		Av		Total N		Av		^{15}N		Av		N Uptake		Av		NDFF		Av		NDFS		Av
N depth (cm)		g col^{-1}				%				%				mg N col^{-1}				mg N col^{-1}				mg N col^{-1}		
Rice shoot part biomass																								
0	37.5	42.2	62.9					0.72	1.84	1.38	1.06		255	318	448		78	73	79		177	245	369	
10	41.2	44.2	52.7					0.78	1.98	1.64	1.52		285	394	405		94	107	103		191	287	302	
20	35.6	43.0	39.6					0.87	2.14	1.86	1.57		296	385	338		105	119	89		191	266	249	
30	30.8	39.0	44.3					0.95	2.15	1.90	1.17		292	362	425		105	115	83		187	248	342	
45	25.1	27.2	34.3					0.94	1.17	1.58	0.74		225	280	316		44	70	39		181	210	277	
Av†					0.81	0.90	0.85																	
LSD(p<0.05)‡		6.3				0.06		0.08		0.25				41.7				13.1				38.9		
Rice roots biomass																								
0	7.4	8.3	10.9									1.32				54				12				42
10	8.0	8.1	10.7									1.23				59				12				47
20	6.9	7.7	7.7									1.11				53				10				43
30	6.5	7.4	7.3									0.76				50				6				44
45	5.0	5.2	7.2									0.44				40				3				37
Av					0.71	0.71	0.62		1.11	1.02	0.79						9	9	7		39	43	37	
LSD(p<0.05)		1.3				0.06				0.12		0.15				7.2		1.3		1.6		4.8		6.2
Total rice plant biomass																								
0	44.9	50.5	73.8										300	374	510		90	85	90		210	288	420	
10	49.1	52.4	63.4										341	452	468		107	118	115		234	335	353	
20	42.6	50.7	47.3										348	444	388		116	131	96		232	313	292	
30	37.3	46.4	51.6										341	415	473		113	123	87		229	292	386	
45	30.1	32.3	41.5										262	316	363		46	73	42		215	243	321	
Av																								
LSD(p<0.05)		6.7												41.8				14.2				39.9		

† Average; ‡ Fisher's least significative difference (LSD) value ($p<0.05$) for the interaction: Soil site vs Nitrogen application depth; and for main effects of soil site and N application.

Table 3: Rice root morphology variables at three paddy soils (Alfisol soil from Santa Maria - ASM, Entisol soil from Santa Maria - ESM and Entisol soil from Agudo - EA) with N application at soil surface, and at 10, 20, 30, and 45 cm depths.

N position (cm)	ASM					ESM					EA				
	Soil layer (cm)					Soil layer (cm)					Soil layer (cm)				
	0-10	10-20	20-30	30-45	45-60	0-10	10-20	20-30	30-45	45-60	0-10	10-20	20-30	30-45	45-60
	Rice plants root length (cm cm^{-1} deep)														
0	13587_{bA}	5080_{bB}	1203_{aC}	804_{aD}	423_{aE}	12348_{abA}	7703_{aB}	1630_{bC}	1034_{aD}	523_{aE}	20171_{bA}	4546_{bB}	1038_{abcC}	1003_{aC}	-
10	26930_{aA}	8837_{aB}	1254_{aC}	519_{bD}	192_{cE}	12968_{aA}	5823_{cB}	1948_{abC}	919_{aD}	316_{bE}	24556_{aA}	7784_{aB}	1461_{aC}	955_{aC}	-
20	8717_{cA}	2448_{cB}	903_{bC}	285_{cD}	27_{eE}	11711_{bA}	4886_{dB}	1243_{cC}	654_{bcD}	364_{abE}	13379_{dA}	4126_{bB}	719_{cC}	235_{bD}	-
30	15440_{bA}	2570_{cB}	605_{cC}	243_{cD}	44_{dE}	10032_{cA}	6558_{bB}	1043_{cC}	589_{cD}	270_{bE}	16723_{cA}	4556_{bB}	846_{bcC}	237_{bD}	-
45	6897_{cA}	2511_{cB}	1326_{aC}	312_{cD}	227_{bE}	8664_{dA}	3309_{eB}	2194_{aC}	868_{abD}	287_{bE}	23629_{aA}	5058_{bB}	1237_{abC}	374_{bD}	-
	Rice plants root surface area (cm^2 cm^{-1} deep)														
0	1206_{bA}	446_{bB}	133_{aC}	71_{aD}	35_{aE}	1175_{aA}	616_{aB}	130_{bcC}	86_{aD}	41_{aE}	1666_{cA}	437_{bB}	142_{abC}	96_{aD}	-
10	2131_{aA}	681_{aB}	113_{abC}	46_{bD}	16_{cE}	1140_{aA}	506_{bB}	148_{bC}	76_{abD}	27_{abE}	2104_{aA}	688_{aB}	174_{aC}	85_{aD}	-
20	793_{cA}	251_{cB}	90_{bC}	28_{cD}	2_{eE}	1041_{bA}	444_{cB}	108_{cdC}	62_{bcD}	31_{abE}	1317_{dA}	360_{cB}	90_{cC}	20_{bD}	-
30	1280_{bA}	262_{cB}	70_{cC}	24_{cD}	4_{dE}	928_{cA}	556_{bB}	99_{dC}	52_{cD}	22_{bE}	1434_{dA}	418_{bcB}	110_{bcC}	24_{bD}	-
45	636_{cA}	227_{cB}	118_{aC}	28_{cD}	17_{bE}	824_{dA}	291_{dB}	181_{aC}	73_{abD}	23_{bE}	1838_{bA}	413_{bcB}	138_{abC}	36_{bD}	-
	Rice plants root volume (cm^3 cm^{-1} deep)														
0	8.66_{bA}	3.13_{aB}	1.18_{aC}	0.49_{aD}	0.23_{aE}	9.08_{aA}	3.98_{aB}	0.84_{bC}	0.57_{aD}	0.26_{aE}	11.05_{bA}	3.47_{bB}	1.55_{abC}	0.74_{aD}	-
10	13.48_{aA}	4.19_{aB}	0.82_{bC}	0.33_{bD}	0.10_{cE}	8.08_{bA}	3.53_{abB}	0.90_{bC}	0.50_{abD}	0.18_{aE}	14.40_{aA}	4.90_{aB}	1.65_{aC}	0.61_{aD}	-
20	5.82_{cA}	2.04_{bB}	0.72_{bC}	0.22_{cD}	0.01_{eE}	7.42_{bcA}	3.23_{bB}	0.75_{bC}	0.48_{abD}	0.21_{aE}	10.51_{bcA}	2.58_{cB}	0.92_{cC}	0.15_{bD}	-
30	8.52_{bA}	2.15_{bB}	0.65_{bC}	0.20_{cD}	0.03_{dE}	6.96_{cA}	3.82_{aB}	0.75_{bC}	0.37_{bD}	0.15_{aE}	9.80_{cA}	3.09_{bcB}	1.17_{bcC}	0.22_{bD}	-
45	4.69_{cA}	1.64_{bB}	0.84_{bC}	0.20_{cD}	0.11_{bE}	6.32_{dA}	2.05_{cB}	1.19_{aC}	0.50_{abD}	0.15_{aE}	11.47_{bA}	2.70_{cB}	1.23_{bcC}	0.28_{bD}	-
	Rice plants roots average diameter (mm)														
0	2.03_{abA}	1.75_{aA}	0.85_{aB}	0.70_{aB}	0.52_{aB}	1.38_{bA}	1.27_{aA}	0.89_{aB}	0.98_{aB}	0.50_{aC}	1.33_{bA}	1.10_{aAB}	0.88_{cB}	1.07_{aAB}	-
10	1.77_{bcA}	1.11_{cB}	1.15_{aB}	0.85_{aBC}	0.50_{aC}	1.38_{bA}	1.24_{aAB}	0.96_{aC}	1.05_{aBC}	0.41_{aD}	1.57_{bAB}	1.26_{aB}	1.90_{aA}	0.85_{abC}	-
20	2.25_{aA}	0.98_{cB}	0.95_{aB}	0.84_{aB}	0.23_{aC}	1.85_{aA}	1.16_{abB}	0.83_{aC}	0.91_{aBC}	0.54_{aD}	2.08_{aA}	1.04_{aB}	1.21_{bcB}	0.32_{cC}	-
30	1.49_{cA}	1.17_{bcA}	1.10_{aA}	0.64_{aB}	0.29_{aB}	1.75_{aA}	1.11_{abB}	0.91_{aBC}	0.84_{aC}	0.35_{aD}	1.63_{bA}	1.04_{aBC}	1.27_{bAB}	0.69_{bC}	-
45	2.20_{aA}	1.50_{abB}	1.14_{aB}	0.57_{aC}	0.25_{aC}	1.22_{bA}	0.93_{bB}	1.06_{aAB}	0.81_{aB}	0.34_{aC}	1.36_{bA}	0.65_{bB}	1.24_{bA}	0.31_{cB}	-

Lower case letters within columns indicate the difference ($p<0.05$) among N position, while upper case letters within rows indicate the difference ($p>0.05$) between soil layers.

Table 4: Photosynthesis rate (Photo), stomatal conductance (SC), intercellular CO_2 concentration (IC), transpiration rate (TR), water use efficiency (WUE) and instantaneous efficiency of carboxylation by rubisco (Photo/IC) means in rice leaves as influenced by N position depth and soil site (Alfisol soil from Santa Maria - ASM, Entisol soil from Santa Maria - ESM and Entisol soil from Agudo - EA).

N application depth (cm)	Photo	SC	IC	TR	WUE	Photo/IC
	µmol CO_2 m^{-2} s^{-1}	mol H_2O m^{-2} s^{-1}	µmol CO_2 mol^{-1}	mmol H_2O m^{-2} s^{-1}	mol CO_2 mol H_2O^{-1}	µmol m^{-2} s^{-1} Pa^{-1}
0	21.994	0.797	320.357	10.558	2.098	0.0687
10	21.412	0.847	325.79	11.063	1.952	0.0659
20	22.649	0.759	315.492	10.037	2.296	0.0718
30	25.248	0.985	321.27	11.018	2.316	0.0787
45	24.129	0.916	320.848	10.577	2.303	0.0752
$LSD_{(p<0.05)}$†	2.006	0.105	-	-	0.235	0.00639
Standard Error	0.695	0.0468	2.282	0.457	0.0813	0.00221
Soil site						
ASM	22.938	0.785	318.114	10.412	2.21	0.0722
ESM	23.343	0.919	322.82	10.967	2.162	0.0724
EA	22.978	0.879	321.321	10.572	2.207	0.0716
$LSD_{(p<0.05)}$	-	0.105	-	-	-	-
Standard Error	0.538	0.0363	1.768	0.354	0.063	0.00171

† Fisher’s least significative difference (LSD) value ($p<0.05$) for the main effects of soil site and nitrogen application depth.

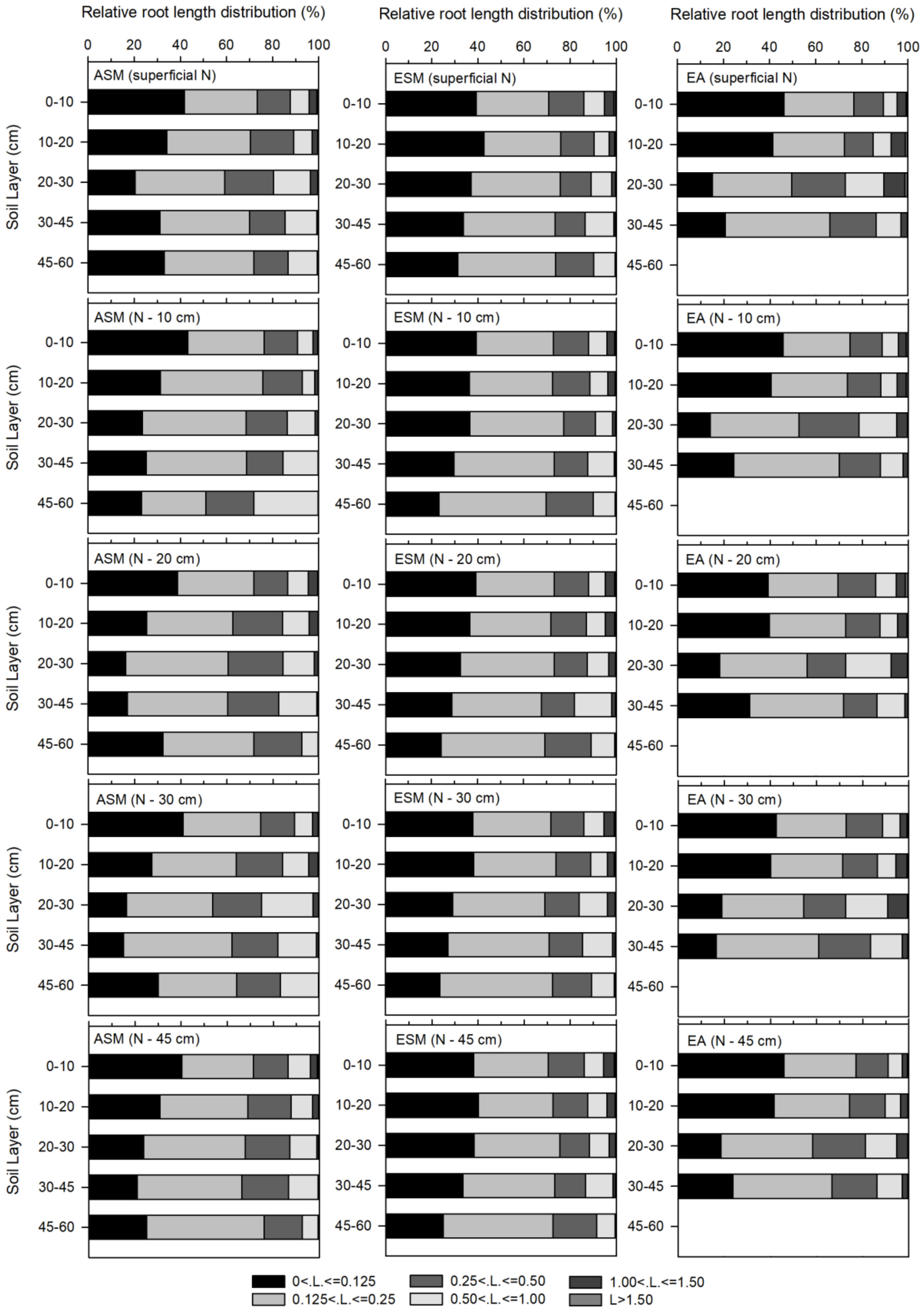

Figure 1: Relative rice root length (L) distribution in diameter classes (0- to -0.125, 0.125- to- 0.25, 0.25- to- 0.50, 0.50- to -1.00, 1.00- to -1.50, and above 1.50 mm) at three paddy soils (Alfisol soil from Santa Maria - ASM, Entisol soil from Santa Maria - ESM and Entisol soil from Agudo - EA) with N application at soil surface, and at 10, 20, 30, and 45 cm depths.

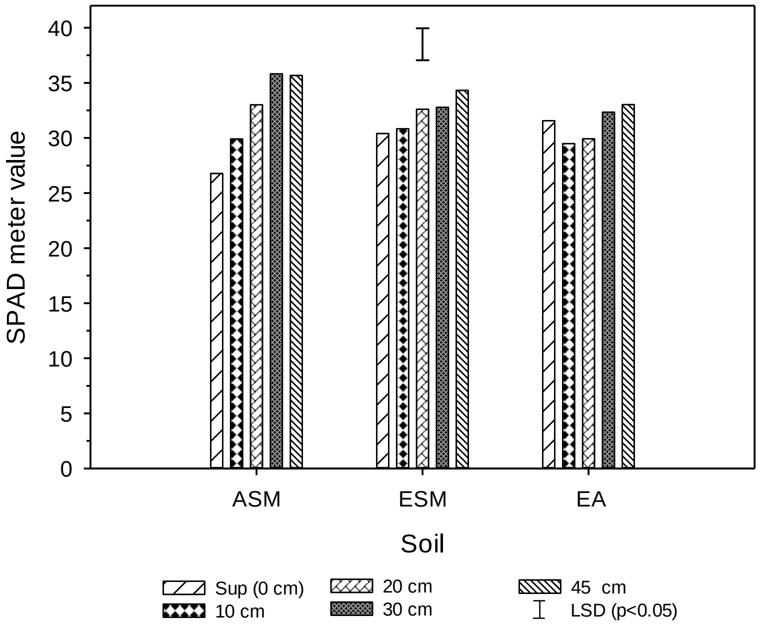

Figure 2: SPAD meter values in rice flag leaves as influenced by nitrogen application depth (soil surface and 10, 20, 30, and 45 cm deep) and soil (Alfisol soil from Santa Maria - ASM, Entisol soil from Santa Maria - ESM and Entisol soil from Agudo - EA). Vertical bar represents Fischer's least significant difference (LSD) value ($p<0.05$) (equal to 2.90) for the interaction ($p<0.005$): Soil site vs N application depth.

5. DISCUSSÃO GERAL

O N é um nutriente chave para a produção sustentável da cultura do arroz irrigado, ao mesmo tempo em que consiste de um elemento de natureza muito dinâmica. Rütting et al. (2018) abordam este comportamento com expertise e musicalidade ao citar "*we have just one world, but we live in different ones*" (parte da letra da música Brothers in Arms da banda Dire Straits) dizendo que o mesmo se aplica ao N; uma vez que, trata-se de um elemento limitante na produção e alimentos em muitos locais no planeta, ao mesmo tempo em que, seu manejo ineficiente e suas formas reativas causam graves impactos ambientais. Este comportamento se evidencia ainda mais nos solos cultivados com arroz irrigado, "um mundo à parte" em que ocorrem ciclos de umedecimento e secagem de solo, alterando a população microbiana do solo, a dinâmica de decomposição os resíduos culturais e da MOS, a transformação de elementos no solo, bem como o ciclo deste nutriente. Este comportamento, aliado à demanda por uma produção de alimentos de maneira sustentável, vêm inquietando pesquisadores e motivado o desenvolvimento de trabalhos visando aprimorar o manejo do N para diversas culturas, em especial para o arroz irrigado. Neste intuito, foram propostos e realizados os estudos anteriormente abordados em detalhes, numa tentativa de trazer respostas para algumas das lacunas do conhecimento sobre a dinâmica do N neste sistema de produção, fatores que afetam sua dinâmica no solo e a sua utilização pela planta.

Os resultados do Estudo I demonstram que os teores totais de CF-NH_4 e do CF-NH_4 recuperado pelo N-STaR apresentaram ampla variação entre os solos e profundidades avaliadas no Arkansas. O CF-NH_4 é um importante compartimento de N para os solos de várzea, podendo corresponder até 28% do teor de N total do solo, sendo sua disponibilidade maior em solos siltosos, possivelmente devido à adsorção nas arestas dos argilominerais (NH_4^+ recentemente adsorvido) e menor proteção à solução extratora. A capacidade de troca do NH_4^+ fixo pela argila (tido como não trocável) pelos íons Na^+ da solução extratora do N-STaR, indicam que este mesmo efeito pode ser causado pela rizosfera das plantas (MENGEL et al., 1990; SCHERER; AHRENS, 1996). Através da liberação ácidos orgânicos pela exsudação radicular e da depleção do NH_4^+ da solução do solo e adsorvido nos complexos de troca da CTC, as plantas podem favorecer a migração / dessorção do NH_4^+ da entrecamada de argilominerais (NIEDER, et al., 2011). Além disso, o alagamento do solo desencadeia reações de redução, podendo solubilizar

Ca^{+2} e Mg^{+2} para a solução, íons estes, que podem expandir a entre camada de argilominerais (STEVENSON; DHARIWAL, 1959) e liberar o NH_4^+ adsorvido.

O procedimento de preparo da amostra de solo, secagem e moagem interferem no teor total de N extraído pelo método N-STaR, bem como o ter o de N extraído é afetado pela base utilizada durante da destilação (NaOH ou KOH). Ao mesmo tempo em que a secagem e a moagem das amostras conferem maior uniformidade de partículas para a análise, estes processos podem causar uma superestimava da real quantidade de N disponível no solo, possivelmente por uma maior exposição de superfícies dos argilominerais à solução extratora. Além disso, a solução de NaOH, devido à presença de ions Na^+, expande as lâminas dos argilominerais e extrai maior quantidade do NH_4^+ fixo em relação à solução de KOH, cujos os íons K^+ colapsam a entrecamada (STEVENSON; DHARIWAL, 1959), afetando a quantidade de AH-N extraída. Deste modo, estes fatores precisam ser considerados em futuros trabalhos visando a calibração do índice AH-N para que consiga uma boa capacidade preditiva do método. De maneira similar, é necessário investigar com mais detalhes a importância do compartimento de CF-NH_4 nos solos cultivados com arroz irrigado e a real capacidade das plantas acessarem esta forma de N e a sua contribuição para a nutrição da cultura.

Por sua vez, os resultados do Estudo II indicam que os teores de AH-N nos solos cultivados com arroz no Sul do Brasil apresentam ampla variação entre solos e em profundidade, comparado com os teores na camada de 0-20 cm amostrada para fins de recomendação de adubação e calagem (SBCS, 2016; SOSBAI, 2018). Comportamento este, também evidenciado no estudo de Roberts et al. (2011) para os solos do Arkansas, nos Estados Unidos. Além disso, o Estudo II evidencia que o teor de AH-N no perfil pode estar fortemente relacionado com as frações granulométricas mais finas (silte e argila < 50 µm), o que pode ser observado em mais detalhes na figura 1.

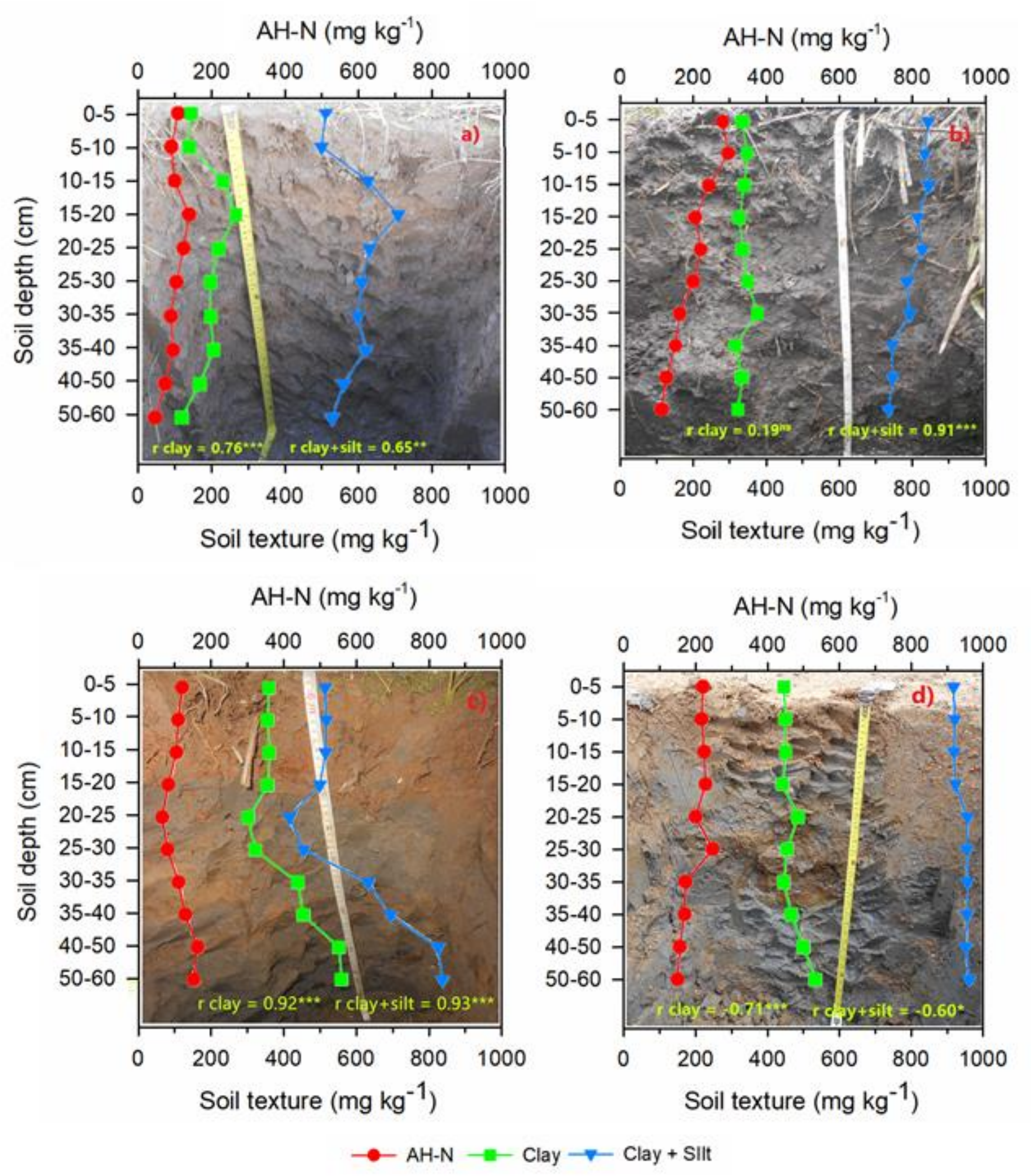

Figura 1 - Distribuição do teor de AH-N no perfil de quatro solos cultivados com arroz irrigado na Depressão Central do Rio Grande do Sul e sua relação com as frações granulométricas do solo. (a) solo 6, (b) solo 9, (c) solo 13 e (d) solo 15 do Estudo II.

É possível verificar um aumento dos teores de AH-N na camada de 15-20 cm no solo ilustrado na figura 1a e aos 25-30 cm no solo da figura 1d, camadas estas que coincidem com a presença de Fe precipitado (visíveis à campo no momento da coleta dos solos e também ilustrados nas figuras 1a e 1d), decorrente dos fenômenos de oxirredução do solo. Isso indica que, a presença de frações lábeis de N no perfil do solo pode estar relacionada à solubilização da MOS e compostos nitrogenados em virtude da redução do solo com o alagamento, conforme abordado por Maie et al. (2004). Por sua vez o solo ilustrado na figura 1b apresenta uma redução do teor de AH-N proporcional com o aumento da profundidade do solo, enquanto que a figura

1c ilustra um acréscimo no teor de AH-N em profundidades maiores que 35 cm. Nas figuras 1a, 1c e 1d é possível verificar um aumento dos teores de argila nas camadas com incremento de AH-N, logo, outra possibilidade poderia ser um aumento dos teores de AH-N em detrimento do CF-NH_4 nestas camadas, visto que uma fração deste compartimento é extraída com a solução de NaOH. Contudo, esta não é a causa para o comportamento evidenciado, visto que poucos solos avaliados no Estudo II apresentaram teores de CF-NH_4 no perfil, e sua correlação com o teor de AH-N é baixa (r= 0.18). Neste sentido, presença de AH-N no perfil dos solos avaliados está mais relacionada com o teor de N total, verificado pelo efeito direto desta variável sobre o AH-N.

Os resultados obtidos no Estudo II também indicam que que o teor de N total de camadas mais profundas do solo é composto por uma proporção expressiva de frações de N mais lábeis, possivelmente lixiviadas no perfil em virtude do alagamento e solubilização de compostos nitrogenados. A proporção do N total ocupada pelo AH-N é muito variável entre os solos avaliados e entre as profundidades de amostragem, o que indica que este é um compartimento de N mais dinâmico e que pode ser mais facilmente afetado por práticas de manejo do solo e da área, mesmo quando os teores de N total não sofrem mudanças expressivas. Este comportamento reforça o potencial deste índice predizer a disponibilidade de N para a cultura do arroz, uma vez que é mais sensível que os teores de N total e MOS (DRESCHER et al., 2016).

A presença de teores expressivos e a capacidade de extração do CF-NH_4 pelo N-STaR nos solos do Arkansas, e, a presença de quantidades expressivas de AH-N em profundidades de até 60 cm nos solos cultivados com arroz irrigado do Sul do Brasil, geram a dúvida sobre a real capacidade do arroz acessar o N presente em camadas mais profundas do solo, bem como se existe alguma mudança na arquitetura radicular das plantas em virtude da localização do N no perfil. Os resultados obtidos no Estudo III demonstra claramente a capacidade das plantas de arroz recuperarem efetivamente o N disponível até 45 cm de profundidade. Além disso, o arroz consegue absorver rapidamente o N das camadas superiores nos estágios iniciais de desenvolvimento, enquanto a absorção do N de camadas mais profundas é mais lenta, contribuindo de maneira mais expressiva para a nutrição da planta nos estágios próximos ao florescimento. A maior recuperação do N (Estudo III) e a maior proporção de NDFF (Estudo IV) foi observado com a aplicação do N nas camadas intermediárias (10, 20 e 30 cm de profundidade) para todos os solos avaliados. A adição do N, na forma de $(NH_4)_2SO_4$ nestas camadas permitiu que o NH_4^+ fosse adsorvido ao solo, ficando protegido às perdas e disponível quando o sistema radicular das plantas atingiu estas camadas. Cabe ressaltar que a aplicação do fertilizante nitrogenado em subsuperfície pode ser uma estratégia para aumentar a eficiência de utilização de N pela cultura, conforme apontado por Khatum et al. (2015) e Wu et al. (2017).

A capacidade de recuperação do N no perfil está atrelada às propriedades físicas do solo. Solos que apresentam baixa resistência à penetração e maior macroporosidade, conectividade e continuidade de poros e, consequentemente, maior condutividade hidráulica, conforme observado no solo ESM, favorecem o crescimento radicular em profundidade bem como a absorção de N via fluxo de massa. Além disso, a presença de N em camadas de solo mais profundas estimula o crescimento radicular, aumentado a quantidade de raízes finas e pelos radiculares (LIMA et al., 2010; MOUNIER et al., 2014), aumentando a área superficial e o volume de solo explorado. Neste estudo, os tratamentos com aplicação de N em camadas mais profundas (30 e 45 cm), apresentaram uma redução significativa na produção de massa seca. Este comportamento ocorreu em virtude de uma carência do nutriente nas fases inicias de desenvolvimento da cultura, como no perfilhamento, resultando em um menor número de colmos e menor crescimento da parte aérea das plantas. Contudo, uma vez que as raízes das plantas atingiram as camadas onde o N foi aplicado, houve um aumento no teor de N no tecido, que foi distribuído para um menor número de colmos. Este comportamento proporcionou aos tratamentos com aplicação de N aos 45 e 30 cm maior teor ^{15}N nas folhas, N total na parte aérea, teor de clorofila (SPAD), bem como as maiores taxas fotossintéticas e a maior eficiência de carboxilação pela Rubisco ao final do experimento (início do florescimento). Cabe ressaltar que o N localizado em maiores profundidades no solo é utilizado pelas plantas de maneira mais expressiva próximo ao florescimento, contribuindo para a síntese de clorofila produção de fotossintatos que serão translocados para as sementes. Esses resultados podem ajudar a explicar a falta de resposta a adubação nitrogenada da cultura de arroz em solos com presença de N lábil em maiores profundidades.

Os resultados destes trabalho também ajudam a entender a necessidade de uma amostragem distinta para a calibração do método N-STaR e o estabelecimento de protocolos de recomendação específicos para solos siltosos e arenosos (amostragem na camada de 0-45 cm) (ROBERTS et al., 2012) e para solos argilosos (amostragem na camada de 0-30 cm) (FULFORD et al., 2013) no Arkansas, visto que o teor de AH-N e a capacidade de recuperação do N de maiores profundidades estão relacionado com as propriedades físicas do solo. Deste modo, cabe ressaltar que há necessidade de levar em consideração estes fatores no processo de correlação e calibração do AH-N ou outros índices de disponibilidade de N para a cultura do arroz irrigado no Sul do Brasil, de modo que se consiga uma correta predição da disponibilidade de N e um aprimoramento do atual sistema de recomendação do fertilizante nitrogenado.

Diante deste cenário, visando uma melhor eficiência do uso do N pela cultura do arroz irrigado e uma boa recuperação do N disponível no solo, há necessidade de considerar as propriedades do solo e o sistema de produção como um todo, uma vez que, existem duas frentes opostas: ter uma boa recuperação do N no perfil do solo e manter a eficiência do sistema de irrigação da lavoura. É comum a presença de camadas de solo adensadas nas áreas cultivadas com arroz irrigado, resultantes da ação dos implementos de preparo do solo, bem como naturalmente, servindo de barreira para evitar a perda da água de irrigação por percolação no perfil, proporcionando um sistema de irrigação eficiente e com menores custos ao produtor. Por outro lado, busca-se aumentar a eficiência de utilização do N pela cultura e uma maior recuperação do N disponível no perfil do solo. Embora esses processos sejam antagônicos, é possível traçar estratégias para aprimorar a eficiência de uso do N pelo arroz e o sistema de recomendação de adubação nitrogenada da cultura. De acordo com os dados obtidos neste estudo, para alguns solos existem quantidades de N-lábil expressivas até 60 cm de profundidade, ao mesmo tempo em que as plantas conseguem utilizar efetivamente o N disponível até os 45 cm de profundidade. Além disso, o maior crescimento radicular do arroz ocorreu até os 30 cm de profundidade que, aliado a elevada eficiência de recuperação do N e às maiores proporções de NDFF com a aplicação do N aos 10, 20 e 30 cm de profundidade, podem indicar que a camada de amostragem de até 0-30 cm poderia ser mais representativa em termos de disponibilidade de N para a cultura e trazer avanços para o sistema de recomendação de adubação da cultura.

Ademais, conforme apontado em trabalhos anteriores (KHATUM et al., 2015; WU et al., 2017), houve maior recuperação do N, NDFF e uma contribuição efetiva na nutrição das plantas com a aplicação do fertilizante nitrogenado em profundidade (aos 10, 20 e 30 cm), indicando um uso mais eficiente do N. Contudo, existe a dificuldade de implementação desta estratégia de manejo para lavouras comerciais. Para contornar este problema, uma alternativa poderia ser a aplicação de uma dose única de N (exceto para expectativas de produtividade muito altas, com elevadas doses de N) em solo seco, antes do alagamento, de modo que o fertilizante seja incorporado ao solo com a infiltração da água de irrigação; outra alternativa, voltada para o sistema pré-germinado, poderia ser a aplicação do N em profundidade antes do alagamento do solo, de modo que este possa ficar adsorvido ao solo e protegido contra a perda por volatilização. Para ambas as estratégias apontadas seria necessária a utilização de N na forma amoniacal, conforme as atuais recomendações técnicas par cultura, e esperar-se-ia uma melhor resposta com a utilização de inibidores de nitrificação, uma vez que minimizaria as perdas por desnitrificação. Contudo, há necessidade de desenvolver novos estudos para verificar a viabilidade prática, econômica e ambiental destas práticas de manejo, bem como investigar a melhor profundidade de amostragem do solo para indicar a disponibilidade de N do solo e calibrar efetivamente o índice AH-N, de modo que seja aprimorado não apenas o manejo da adubação nitrogenada da cultura do arroz, mas o sistema de produção como um todo, essenciais para uma agricultura eficiente, rentável e mais sustentável.

6. CONCLUSÕES GERAIS

O N fixado pela argila representa 24,8 e 28,1% do teor de N total nas camadas de 0-15 e 15-30 cm, respectivamente, sendo que o método N-STaR estima que apenas uma pequena proporção (<15%) deste compartimento de N seja prontamente disponível as plantas. Estes resultados indicam que CF-NH_4 é um importante compartimento de N do solo, principalmente para os solos argilosos do Arkansas, podendo ser um potencial destino do N adicionado via fertilizantes e uma fonte primária de N para o arroz.

A condição de umidade do solo e o tipo de base utilizada no processo de destilação influencia a quantidade de AH-N extraída, sendo que o processo de secagem e moagem das amostras e a destilação com NaOH aumenta a quantidade de N extraída pelo N-STaR. Deste modo, estes fatores precisam ser levados em consideração em futuros trabalhos de correlação e calibração do método, uma vez que podem afetar o índice N-STaR e a quantidade de N a ser aplicada.

A distribuição do teor de AH-N no perfil do solo é distinta entre solos e pode apresentar incrementos em profundidade. Embora as frações granumétricas do solo (argila e argila + silte) estejam fortemente correlacionas com o teor de AH-N para alguns solos, o N total do solo é a variável que determina a distribuição destas formas de N no perfil.

O AH-N não é um compartimento constante de N no solo. A proporção de N total ocupada pelo AH-N é muito variável entre os solos avaliados e entre as profundidades de amostragem, o que indica que este é um compartimento de N mais dinâmico e que pode ser mais facilmente afetado por práticas de manejo do solo, mesmo quando os teores de N total não sofrem mudanças expressivas.

A cultura do arroz irrigado consegue utilizar efetivamente o N disponível em profundidades de até 45 cm e a recuperação é afetada pelas propriedades físicas do solo, especialmente a condutividade hidráulica. Os maiores valores de recuperação de ^{15}N e NDFF foram observados quando o fertilizante foi aplicado aos 10, 20 e 30 cm de profundidade, o que indica que a aplicação do N em subsuperfície pode ser uma estratégia para aumentar a eficiência de utilização do nutriente pela cultura.

A disponibilidade de N em profundidade afetou a morfologia radicular do arroz, aumentando o comprimento das raízes, a área de superficial e volume radicular, bem como reduziu o diâmetro médio das raízes. O N localizado nas camadas superficiais do solo é rapidamente absorvido pela cultura do arroz, sendo utilizado pela planta nos estágios fenológicos mais responsivos à adubação, resultando maior perfilhamento e produção de massa seca. Por outro lado, o N localizado em maiores profundidades (30 e 45 cm) é absorvido mais lentamente, contribuindo para a nutrição das plantas nas fases de desenvolvimento reprodutivo, o que se evidencia pelo aumento do teor de ^{15}N nas folhas e pelo maior teor de N no tecido, teor clorofila (unidades SPAD), taxa fotossintética e eficiência de carboxilação pela Rubisco no florescimento da cultura.

O N localizado em camadas superiores à 20 cm de profundidade contribui para a nutrição das plantas. Portanto, camadas de amostragem do solo mais profundas devem ser consideradas em estudos futuros envolvendo a predição e calibração de índices de disponibilidade de N para a cultura do arroz irrigado por alagamento.

7. PERSPECTIVAS DE ESTUDOS FUTUROS

i) Investigar a magnitude e importância do NH_4 fixo pela argila em solos com predomínio de argilominerais 2:1 e sua contribuição para a nutrição das plantas;

ii) Verificar o efeito da base (NaOH e KOH) utilizada no processo de destilação sobre o teor de AH-N extraído nos solos cultivados com arroz irrigado no Sul do Brasil;

iii) Avaliar a profundidade de amostragem do solo para uma correta predição do N disponível parra o arroz e calibração do método AH-N para a cultura do arroz;

iv) Verificar a possibilidade da utilização do índice AH-N para predição da disponibilidade de N para outras culturas e sistemas de produção;

v) Buscar estratégias de aplicação do fertilizante nitrogenado na cultura do arroz irrigado para melhorar a eficiência de utilização do N pela planta.

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