UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE AGRONOMIA
PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA

LÓREN PACHECO DUARTE

DESVENDANDO INTERAÇÕES ENTRE O FORNECIMENTO DE NITROGÊNIO E ÁGUA POR MEIO DE RELAÇÕES ALOMÉTRICAS NO AZEVÉM

Porto Alegre
2024

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Bacharela em Zootecnia

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Dissertação apresentada como requisito para obtenção do Grau de Mestre em Zootecnia, na Universidade Federal do Rio Grande do Sul.

Orientador: Paulo Cesar de Faccio Carvalho

Coorientadora: Taise Robinson Kunrath

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“Palavras são,

na minha nada humilde opinião,

nossa mais inesgotável fonte de magia.

São capazes de formar grandes sofrimentos

mas também de remediá-los.”

Alvo Dumbledore

DESVENDANDO INTERAÇÕES ENTRE O FORNECIMENTO DE NITROGÊNIO E ÁGUA POR MEIO DE RELAÇÕES ALOMÉTRICAS NO AZEVÉM [1]

Autora: Lóren Pacheco Duarte
Orientador: Paulo Cesar de Faccio Carvalho
Coorientadora: Taíse Robinson Kunrath

RESUMO

Os eventos climáticos extremos, cada vez mais frequentes, são impulsionados pela intensificação do ciclo hidrológico global, representando uma ameaça crescente à sustentabilidade e à estabilidade dos agroecossistemas. O objetivo deste estudo foi avaliar a relação entre o consumo de água e nitrogênio (N) em diferentes doses de N e o estresse fisiológico em campos encharcados por períodos mais longos ou mais curtos. Para atingir esse objetivo, foram analisadas pastagens de azevém puro em parcelas de campo sob três diferentes doses de N (0, 75 e 150 kg N ha^{-1}) e dois níveis de água: irrigado (maior tempo de encharcamento) e não irrigado (menos tempo/encharcamento normal). O delineamento experimental foi blocos inteiramente casualizados com parcelas subdivididas e fatorial cruzado, com três repetições. A taxa de acúmulo e a produção total de matéria seca do azevém demonstraram significância para as doses de N, para a quantidade de N total absorvida (N_{ABS}) e o uso eficiente da água (UEA), teor de N nas plantas, e o índice de nutrição nitrogenada (INN). Somente o NNI se mostrou significante para os diferentes tempos de encharcamento do solo. Os Δ de solo foram significativos para as doses de N para amônio e N orgânico. Sendo somente o N orgânico significativo para os diferentes tempos de encharcamento do solo. O Δ N total do solo somente diferenciou entre camadas de solo. Nas condições ambientais de encharcamento do solo em áreas onde essa atividade não é normal o azevém apresentou baixos teores de N absorvido. O azevém, quando exposto a um solo encharcado, obteve sua máxima resposta produtiva e fisiológica na dose de 75 kg de N ha^{-1}, independente do tempo de exposição ao encharcamento do solo.

Palavras-chave: doses de nitrogênio; mudanças climáticas; encharcamento de solo; subtropical; manejo *rotatínuo*.

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[1] Dissertação de Mestrado em Zootecnia – Plantas Forrageiras, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil. (73 p.) Março, 2024.

DESVENDANDO INTERAÇÕES ENTRE O FORNECIMENTO DE NITROGÊNIO E ÁGUA POR MEIO DE RELAÇÕES ALOMÉTRICAS NO AZEVÉM [2]

Author: Lóren Pacheco Duarte
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ABSTRACT

Extreme weather events, increasingly frequent, are driven by the intensification of the global hydrological cycle, posing a growing threat to the sustainability and stability of agroecosystems. The objective of this study was to evaluate the relationship between water and nitrogen intake in different doses of N and physiological stress under longer and shorter waterlogged fields. To achieve this aim, we analyzed pure ryegrass pasture in field plots under three different doses of N (0, 75, and 150 kg N ha^{-1}), and two water levels: irrigated (longer waterlogging) and non-irrigated (shorter/normal waterlogging). The experimental design was blocks completely randomized with split-plot and crossed factorial with three replications. The grass management followed the "*Rotatínuos*" management concept. The accumulum rate and total crop biomass of ryegrass demonstrated significance for the N doses, the amount of total N absorbed (N_{upt}) and the water use efficient (WUE), N content in plants, and the nitrogen nutrition index (NNI). Only the NNI was significant for the different soil waterlogging times. Soil Δ were significant for N doses for ammonium and organic N. Only organic N is significant for the different soil waterlogging times. Total soil Δ N only differed between soil layers. In environmental conditions of soil waterlogging in areas where this activity is not normal, ryegrass showed low levels of absorbed N. When exposed to waterlogged soil, ryegrass obtained its maximum productive and physiological response at a dose of 75 kg of N ha^{-1}, regardless of the time of exposure to waterlogging.

Keywords: doses of nitrogen; climate changes; soil waterlogging; subtropical; *rotatinuos* management.

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[2] Dissertação de Mestrado em Zootecnia – Plantas Forrageiras, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil. (73 p.) Março, 2024.

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CAPÍTULO I

1. INTRODUÇÃO

O manejo eficaz dos recursos hídricos e nitrogenados é crucial para otimizar o crescimento e a produtividade das plantas, especialmente em condições ambientais desafiadoras. O azevém (*Lolium multiflorum* Lam.) é uma gramínea forrageira amplamente cultivada em muitas regiões do mundo devido à sua rápida taxa de crescimento, alta qualidade nutricional (Yavuz, et al., 2017) e capacidade de adaptação a diferentes condições climáticas (Abraha et al., 2011). No entanto, para maximizar o potencial produtivo do azevém, assim como de qualquer gramínea, é essencial compreender as interações complexas entre o fornecimento de nitrogênio e água (Quemada & Gabriel, 2016), dois fatores fundamentais para o seu desenvolvimento.

Esse manejo mais eficaz vem tendo que quebrar mais barreiras, pois eventos climáticos extremos, cada vez mais frequentes, são impulsionados pela intensificação do ciclo hidrológico global, representando uma ameaça crescente para a sustentabilidade e a estabilidade dos agroecossistemas (Jägermeyr et al., 2021). Tais fenômenos representam uma ameaça à segurança alimentar; no entanto, avaliações globais dos impactos causados pelo encharcamento do solo em espécies forrageiras são raras. Além disso, a exposição frequente a condições climáticas extremas (IPCC, 2015) impactará como as plantas acessam o fertilizante nitrogenado, comprometendo potencialmente o crescimento e a produtividade (Swarbreck et al., 2019).

O uso indiscriminado de recursos não renováveis já levou a uma diminuição na eficiência do uso de nutrientes na agricultura (Lemaire et al., 2009). O nitrogênio é essencial para várias funções vitais das plantas (Plett et al., 2020). No entanto, as plantas têm capacidade limitada de absorver o N aplicado, utilizando tipicamente apenas 40-50% da quantidade fornecida, ou até menos (Raun and Johnson, 1999; Kronzucker et al., 2000; Coskun et al., 2017). Ainda, com limitação significa que a resposta do crescimento das plantas à água e ao N, é maior do que sua resposta a cada fator isoladamente, e implica que estratégias para maximizar o crescimento das plantas devem garantir que ambos os recursos estejam igualmente disponíveis.

Os eventos de instabilidade climática estão se tornando cada vez mais frequentes, impactando diretamente a duração da exposição das plantas ao estresse e, consequentemente, afetando diretamente a relação solo-planta (Liu et al., 2023).

Esses mesmos autores concluíram também que as penalidades de rendimento causadas pelo encharcamento podem aumentar historicamente de 3-11% para 10-20% até 2080, com as penalidades refletindo tanto a duração quanto o *timing* do encharcamento em relação ao estágio da cultura. Assim, o desenvolvimento de estudos para aprimorar a compreensão dos fatores envolvidos na eficiência de utilização de água e fertilizantes nitrogenados torna-se essencial para o manejo que atenda aos requisitos de sustentabilidade. Tais entendimentos podem servir de base para o estabelecimento de diretrizes no manejo de fertilização nitrogenada sob condições de estresse por encharcamento.

2. REVISÃO BIBLIOGRÁFICA

2.1 Dinâmica do nitrogênio na interface solo-planta

2.1.1 Do solo a planta

O nitrogênio (N) é um dos elementos absorvidos em maiores quantidades pelas plantas. Esse macronutriente tão importante no sistema solo-planta-atmosfera é bem complexo, principalmente por possuir várias formas e por consequência, vários processos envolvidos em seu ciclo. Há alguns meios de entrada desse nutriente no sistema, assim como também várias formas de perdas e/ou saídas. A maneira mais básica e primária, por deposições atmosféricas (a atmosfera é um ambiente com abundância de N), por fixação biológica, onde a planta é capaz de captar o seu próprio adubo nitrogenado da atmosfera; por mineralização de resíduos orgânicos humificados e em contato com o solo, sejam eles de fonte vegetal ou animal; e por fim mas talvez a mais emblemática de todas as formas, a adubação por ação antrópica, que pode ser com adubos chamados de “químicos” ou “formulados”, que nada mais são do que adubos advindos de processos industriais.

A disponibilidade do N no solo para as culturas é influenciada por diversos fatores, como clima, temperatura, uso atual e histórico da terra dentro outros tantos. Mas em direção ao sistema solo-planta, os dois principais fatores são a quantidade de N presente no início de ciclos e a mineralização ao longo do tempo (Sadras & Lemaire, 2014; Reetz, 2017). A quantidade inicial de N disponível depende das aplicações de fertilizantes e da quantidade de resíduos orgânicos incorporados ao solo, assim como o histórico do local (Souza et al., 2009). Quanto à mineralização do N, ela é diretamente afetada pela relação carbono:nitrogênio (C:N) dos resíduos orgânicos, que podem ser resíduos vegetais ou animais. Resíduos com alta relação C:N (>30:1) tendem a imobilizar o N do solo, pois os microrganismos utilizam o N como fonte de energia para degradar esse carbono. Logo, a população de microrganismos aumenta e indisponibiliza esse N em maior quantidade. Com o passar do tempo, esse carbono vai sendo degradado e os microrganismos passam a diminuir sua atividade também, ocasionando a mineralização do N que estava tornando o disponível para as plantas. Já os resíduos com baixa relação C/N não requerem tanta imobilização do N, acelerando assim o processo de decomposição e mineralização do N (Aita & Giacomini, 2003).

Sendo assim, a dinâmica do N está diretamente ligada à matéria orgânica do solo. Em solos tropicais e subtropicais, onde há naturalmente menos acúmulo de matéria orgânica devido a condições que favorecem a rápida decomposição, como altas temperaturas e umidades (Lal, 2020), o N torna-se um fator crítico na produtividade das culturas, especialmente para plantas forrageiras gramíneas, que precisam de N como forma dependente de produção.

A absorção de N pelas culturas é um processo chave nos moldes produtivos solo-planta. Este processo afeta o crescimento e determina não só a quantidade, mas também a qualidade das culturas, atuando como um processo chave na ciclagem de N no solo, sendo responsável por 50% a 60% do destino do N no solo em ecossistemas agrícolas (Ladha et al., 2016). O N é um fator crítico na produtividade das culturas, sendo o mais dinâmico entre os macronutrientes. Aproximadamente 95% do nitrogênio no solo está na forma orgânica, com os 5% restantes na forma mineral, que é a parte absorvida pelas plantas (Galloway et al., 2004).

O fertilizante nitrogenado pode ser dividido em amônio (NH_4^+), nitrato (NO_3^-) e fertilizante N amida. NH_4^+ é fácil de ser adsorvido pelos coloides do solo, fácil também de ser oxidado em NO_3^-, facilmente volátil em ambiente alcalino e alta concentração de NH_4^+ é tóxica para as plantas. NO_3^- é facilmente solúvel em água, move-se mais rapidamente no solo e possui um ânion carregado negativamente, que não pode ser absorvido pelos coloides do solo e é facilmente absorvido pelas plantas. Mas, NO_3^- também é facilmente reduzido ao estado gasoso pela desnitrificação no solo e por consequência, sendo perdido. A maioria dos solos agrícolas são deficientes em N, e a aplicação racional de fertilizantes nitrogenados pode aumentar significativamente a biomassa das plantas e o rendimento das culturas.

As plantas podem absorver e utilizar as formas inorgânicas de N do solo. O NO_3^- é a principal forma de N absorvida e assimilada pela maioria das plantas cultivadas (em solos bem arejados), mas o NH_4^+ pode ser importante em solos não revolvidos ou não fertilizados (Paungfoo-Lonhienne et al., 2012). A ureia (através da excreção de urina de animais ou fertilizante de ureia) pode, pelo menos temporariamente, ocorrer no solo em concentrações elevadas e pode ser utilizada pelas plantas (Kraiser et al., 2011; Witte, 2011). Essas formas de N são retiradas do solo diretamente pelas raízes ou por micorrizas associadas às raízes e depois transferidas para as demais partes da planta.

2.2 Relação planta *x* água

2.2.1 *A importância da água para a planta*

A água desempenha um papel fundamental na regulação fisiológica das plantas, sendo indispensável para várias funções vitais. A água atua como solvente primário dentro das células vegetais, facilitando o transporte de nutrientes e minerais dissolvidos na fase líquida do solo, para todas as partes da planta por meio dos vasos do xilema (Chavarria et al., 2012). Atua também no processo de fotossíntese, onde a água está envolvida na fotólise das moléculas de água durante as reações dependentes de luz.

Rutherford & Boussac (2004) definiram a fotólise como um processo fotoquímico que ocorre no interior das folhas, no qual uma substância é decomposta por meio da absorção de energia luminosa. Na fotólise da água, moléculas de água são decompostas em oxigênio (O_2) e hidrogênio (H^+) na presença de luz, durante a fase de reações dependentes de luz da fotossíntese. Durante a fotólise da água, a energia luminosa é absorvida pelos pigmentos fotossintéticos, como a clorofila, nas membranas dos tilacoides dos cloroplastos (Rutherford & Boussac, 2004). Essa energia é utilizada para separar as moléculas de água (H_2O) em íons H^+ e íons hidroxila (OH^-), processo esse que libera O_2 como subproduto. Os íons de hidrogênio gerados são utilizados na síntese de ATP e NADPH, que são moléculas de alta energia usadas para impulsionar as reações químicas da fase de fixação de carbono da fotossíntese (Zeiger et al, 2015).

A fotólise da água é um processo crucial para a produção de oxigênio e para a geração de energia química necessária para a fixação do carbono na fotossíntese. É um dos principais passos na conversão de energia luminosa em energia química durante o processo fotossintético das plantas. Esse processo gera oxigênio e fornece elétrons que são utilizados na síntese de carboidratos, sendo assim a base para a produção de energia das plantas. Basicamente, através da energia da radiação solar, a água que está no cloroplasto, dentro da folha, sofre lise e separa gás o H e o O_2. Além disso, a água mantém a turgidez celular, garantindo a integridade estrutural das células e dos tecidos vegetais (Cosgrove, 2018). Essa pressão de turgor é crucial para sustentar a rigidez das plantas, facilitar o crescimento ereto e apoiar várias funções celulares.

Segundo Wahid et al. (2007), ao facilitar o resfriamento dos tecidos vegetais, a transpiração ajuda a evitar o superaquecimento e mantém condições fisiológicas adequadas para o crescimento das plantas. Ainda, a água desempenha um papel crítico na capacidade das plantas de resistir a estresses ambientais, como seca, salinidade e temperaturas extremas (Dos Santos et al., 2022). A habilidade das plantas de regular o conteúdo de água e modular a abertura dos estômatos em resposta a esses estresses é essencial para sua sobrevivência e sua adaptação.

Em resumo, a água é indispensável para todas as funções fisiológicas das plantas e serve como reguladora central do crescimento, do desenvolvimento e da adaptação a diferentes condições ambientais. Sua disponibilidade e utilização eficiente são fundamentais para garantir a saúde e a produtividade das plantas.

2.2.2 Alterações na relação ‘planta *x* água’ em solos enxarcados

Dentre todos os estresses que a planta pode sofrer, a seca e as inundações são consideradas os principais fatores que restringem o rendimento das culturas agrícolas porque têm efeitos negativos na fotossíntese, no teor de proteínas, na expansão das folhas e na respiração/transpiração (Chaves et al., 2003; Liao e Lin, 2001). Isso ocorre devido a vários fatores (Liu et al., 2020) (Fig. 1) que influenciam a disponibilidade e a absorção de importante nutriente pelas plantas. Um dos principais efeitos do excesso de água no solo é a redução da disponibilidade de oxigênio para as raízes das plantas (Bartholomeus et al., 2008), o que pode levar à hipoxia ou anoxia. Esse ambiente anaeróbico diminui a atividade metabólica das raízes, afetando negativamente sua capacidade de absorção delas.

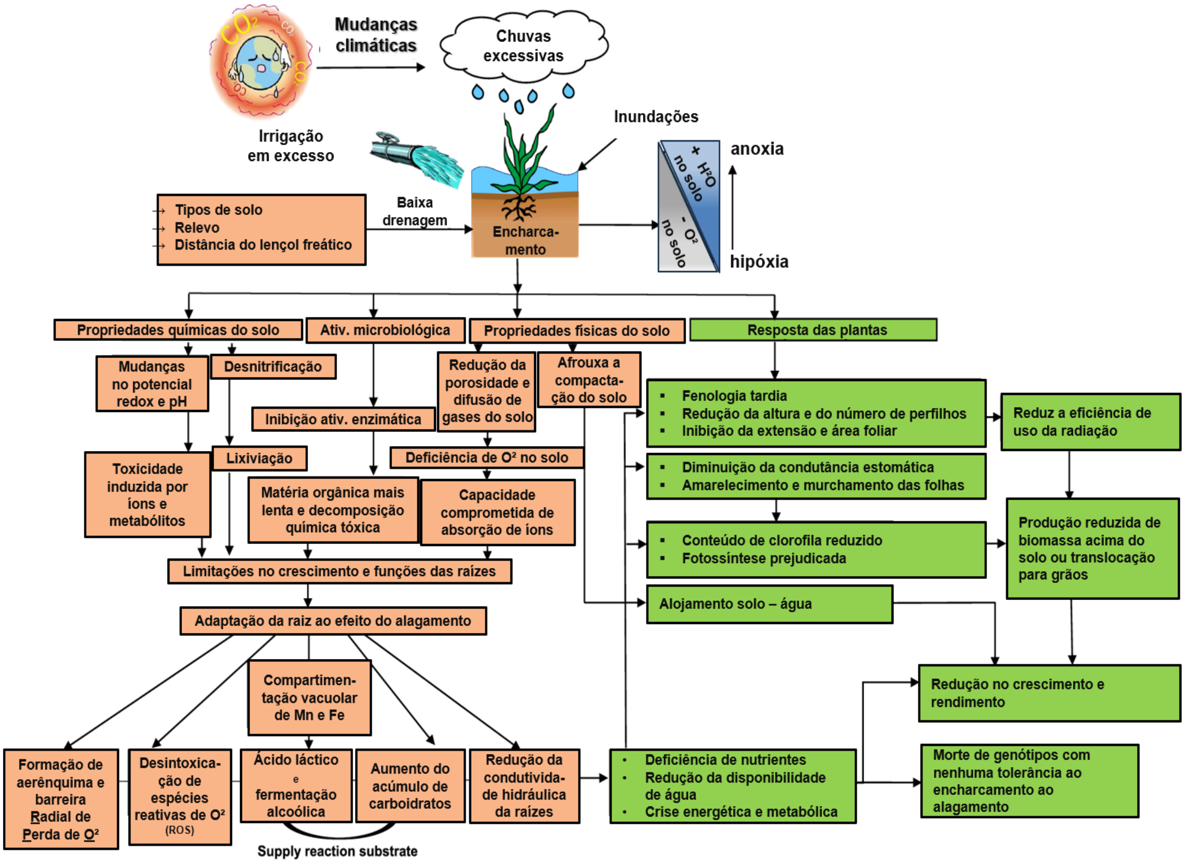

Figura 1. Mecanismos fisiológicos, morfológicos, físicos e químicos implicados nas respostas do crescimento das plantas ao encharcamento e/ou alagamento. Fonte: adaptado de Liu et al. (2020).

Gao et. al (2020), em um experimento com alfafa (*Medicago sativa*), descobriram que ela foi capaz de absorver mais N em condições de seca do que em condições de inundação. Ainda, os mesmos autores afirmaram que em condições de alagamento, a alfafa teve aumento da taxa de fotossíntese, ou seja, o N que foi absorvido, mesmo que baixo, foi advindo da fixação biológica de N. Esse fato foi corroborado pela diminuição significativa de raízes em ambientes alagados, em relação à parte aérea. O N desempenha um papel crucial no crescimento das plantas, sendo essencial para regular a assimilação de carbono. No entanto, sua disponibilidade é amplamente influenciada pelo estado hídrico da planta. Por exemplo, estudos demonstraram uma redução significativa, entre 30% e 60%, na absorção total de N em plantas submetidas ao estresse de inundação das raízes. Adicionalmente, a absorção de N é menos afetada em plantas com alta tolerância a inundações (Gastal e Lemaire, 2002; Kreuzwieser et al., 2002).

2.3 Uso de recursos

Em 2008, Lemaire et al., falavam que os custos com o suprimento de N para uma cultura eram relativamente baixos em relação ao potencial de colheita que ele gera, mas que esse cenário poderia se modificar caso não fossem adotadas práticas de manejo mais eficientes de N, pois a demanda por N seria ainda maior, o que geraria não somente um aumento por unidade de nutriente nitrogenado, mas também alguns riscos ambientais e sociais, como a eutrofização de corpos d'água (Hwang et al., 2020), que consiste na chegada do nutriente a importantes fontes de água, o que é extremamente danoso à saúde humana (Temkin et al., 2019). Ainda, a relação entre o preço do fertilizante nitrogenado e as emissões de dióxido de carbono (CO_2) está relacionada à própria produção desses fertilizantes. O processo de fabricação de fertilizantes minerais nitrogenados geralmente envolve a fixação de N do ar, que é a síntese de Haber-Bosch, uma reação que consome uma quantidade significativa de energia, fazendo a síntese da amônia (Bicer, et al., 2017).

Ao considerar as emissões de CO_2 provenientes da produção de fertilizantes, Menegat et al. (2022) defendem a ideia de balizar o preço desses produtos com base na equivalência dessas emissões. Isso significa que o preço do fertilizante deve refletir não apenas os custos tradicionais de produção, mas também as externalidades ambientais, como as emissões de gases de efeito estufa associadas à sua fabricação. Aliado a isso, as mudanças climáticas vêm exigindo mais adaptações e ainda mais eficiências em usos, principalmente em recursos não renováveis. Ainda está a se descobrir como adaptar-se a tantas mudanças, mas o que se sabe, é que o uso precisa ser repensado e reusado, pois o solo nos mostra que isso é possível, principalmente com o uso de N.

3. HIPÓTESES

Dado o exposto, o presente estudo hipotetiza que: **a)** a dinâmica alométrica da relação entre o consumo de água e nitrogênio em plantas de azevém é influenciada pelo tempo de exposição ao estresse de encharcamento no inverno subtropical, e **b)** sob condições de encharcamento do solo, o azevém não responde à dose de nitrogênio recomendada para resposta máxima de produtividade (150 kg ha^{-1}).

4. OBJETIVO

O objetivo desse trabalho foi, portanto, avaliar a relação entre o consumo de água e N em plantas de azevém submetidas a diferentes doses de N e estresse de encharcamento.

CAPITULO II [3]

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[3] Article prepared according to the norms of the journal *Agriculture, Ecosystems & Environment* (Appendix A).

UNRAVELING INTERACTIONS BETWEEN DIFFERENT NITROGEN DOSES AND WATER SUPPLY THROUGH ALLOMETRIC RELATIONSHIPS IN ANNUAL RYEGRASS

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ABSTRACT

Extreme weather events, increasingly frequent, are driven by the intensification of the global hydrological cycle, posing a growing threat to the sustainability and stability of agroecosystems. The objective of this study was to evaluate the relationship between water and nitrogen intake in different doses of N and physiological stress under longer and shorter waterlogged fields. To achieve this aim, we analyzed pure ryegrass pasture in field plots under three different doses of N (0, 75, and 150 kg N ha^{-1}), and two water levels: irrigated (longer waterlogging) and non-irrigated (shorter/normal waterlogging). The experimental design was completely randomized with split-plot and crossed factorial with three replications. The grass management followed the "*Rotatínuos*" management concept. The accumulum rate and total crop biomass of ryegrass demonstrated significance for the N doses, the amount of total N absorbed (N_{upt}) and the water use efficient (WUE), N content in plants, and the nitrogen nutrition index (NNI). Only the NNI was significant for the different soil waterlogging times. Soil Δ were significant for N doses for ammonium and organic N. Only organic N is significant for the different soil waterlogging times. Total soil Δ N only differed between soil layers. In environmental conditions of soil waterlogging in areas where this activity is not normal, ryegrass showed low levels of absorbed N. When exposed to waterlogged soil, ryegrass obtained its maximum productive and physiological response at a dose of 75 kg of N ha^{-1}, regardless of the time of exposure to waterlogging.

KEYWORDS: rates of nitrogen; climate changes; soil waterlogging; subtropical; *rotatinuos* management.

1. INTRODUCTION

Extreme weather events, increasingly frequent, are driven by the intensification of the global hydrological cycle, posing a growing threat to the sustainability and stability of agroecosystems (Jägermeyr et al., 2021). These phenomena represent a threat to food security; however, global assessments of the impacts caused by waterlogging on forage species are rare. Furthermore, this frequent exposure to extreme weather conditions (IPCC, 2015) will impact how plants access nitrogen fertilizer, potentially compromising growth and productivity (Swarbreck et al., 2019). This scenario, coupled with the increasing world population and a growing demand for food stability, demands the development of new knowledge, technologies, and practices that enable sustainable intensification.

The indiscriminate use of non-renewable resources has led to a decrease in the efficiency of nutrient use in agriculture (Lemaire et al., 2009). An example of this is that, in the last fifty years, global food production has tripled (Lemaire et al., 2015), while the application of nitrogen (N) fertilizers has increased eightfold (Subbarao et al., 2013). N is essential for various vital functions of plants, including the capture and transformation of solar energy through chlorophyll, the development of structure, nucleotides, and enzymes (Plett et al., 2020). However, plants have a limited capacity to absorb applied N, typically utilizing only 40-50% of the supplied amount, or even less, as observed in previous studies (Raun and Johnson, 1999; Kronzucker et al., 2000; Coskun et al., 2017). Under waterlogged conditions, much of the unabsorbed N can be leached, particularly in the form of nitrate (NO_3^-) (Subbarao et al., 2009), resulting in significant pollution to water bodies (Rabalais et al., 2002; Wang et al., 2019; Thompson et al., 2020).

The application of irrigation is an available management tactic aimed at improving nitrogen use efficiency (N_{upt}) and reducing its losses. In cropping systems subject to droughts or water shortages, adapting N management to water constraints can help mitigate N losses, resulting in increased N_{upt} (Quemada & Gabriel, 2016). However, in systems where waterlogging occurs, N management also needs to be adapted to reduce losses. And while waterlogging is one of the main abiotic stresses frequently faced by plants (Tewari & Mishra, 2018) and directly affects the efficiency of fertilizer use, such as N, knowledge of the impacts of soil waterlogging on crop growth is still limited (Li, Y. et al., 2019; Kotz, M. et al., 2022; Peng, B. et al., 2020; Webber, H. et al., 2020).

The events of climate instability are becoming increasingly frequent, directly impacting the duration of plant exposure to stress and, consequently, directly affecting the soil-plant relationship (Liu et al., 2023). These same authors also concluded that yield penalties caused by waterlogging may historically increase from 3-11% to 10-20% by 2080, with penalties reflecting both the duration and timing of waterlogging relative to crop stage. Thus, the development of studies to enhance understanding of factors involved in water and nitrogen fertilizer utilization efficiency becomes essential for management meeting sustainability requirements. Such understandings can serve as a basis for the establishment of guidelines in nitrogen fertilization management under waterlogging stress conditions.

Given the above, the present study hypothesizes a) the allometric dynamics of the water-nitrogen consumption relationship in ryegrass plants are influenced by exposure time to waterlogging stress in the subtropical winter, and b) under soil waterlogging conditions, ryegrass does not respond to the recommended dose for maximum nitrogen productivity response (150 kg ha^{-1}). The objective was therefore to evaluate the relationship between water-nitrogen consumption in ryegrass plants subjected to different N doses and waterlogging stress.

2. MATERIAL AND METHODS

2.1 *Site, climate, and soil description*

The experiment was conducted at Eldorado do Sul, in southern Brazil, between May and November of the year 2022, at the Federal University of Rio Grande do Sul's experimental farm (latitude 30º 05' S, longitude 51º 39' W, and 46 m of altitude) in the wintertime. Second Kottek et al. (2006), the site's climate is classified as humid subtropical (Cfa), following the climatological norms depicted in Figure 1, which was shown in comparison with weather all trial time.

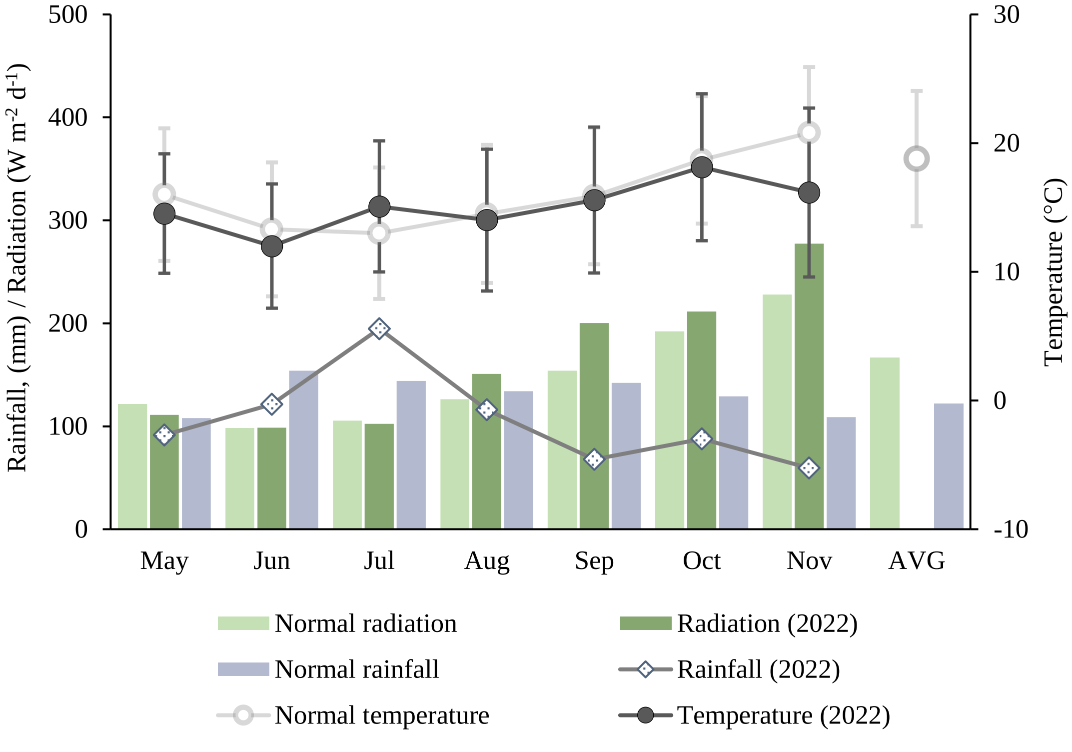

Figure 1. Annual average rainfall, solar radiation, and mean air temperature in the long-term climatic means between 1973 and 2016 at the Agronomy Experimental Station from the Federal University of Rio Grande do Sul. AVG is the average of the normal by all months of the study.

Four months before the start of the experiment and soil sampling for initial soil fertility assessment, six tons of limestone and 160 kg ha^{-1} of P_2O_5 were applied. Both were mechanically applied and incorporated into the 0-20 cm soil layer. The soil was an Acrisol (26% clay, textural class 3) which at the beginning of the trial had a pH of 5.6; the phosphorus and potassium were 13.9 (medium) and 152.3 (very high) respectively; the organic matter was 2.3 (low); the calcium and magnesium content were 3.2 (medium) and 1.8 (high), respectively; the S content was 4.8 (medium). All the interpretations were done based on CQFS-RS/SC (2016).

2.2 *Experimental design and treatments*

The experimental design was blocks completely randomized with split-plot and crossed factorial with three replications. The main plots (irrigated and non-irrigated) were randomly assigned. Within each plot, the subplots (N doses) were randomly assigned. It was in one area that had four thousand square meters, including the plots (experimental unit, with sixty square meters each) and the passageways. The treatments were three doses of N (0, 75, and 150 kg N ha^{-1}), and two water levels: irrigated (longer waterlogging duration) and non-irrigated (shorter/normal waterlogging duration). The blocks were allocated based on differences in terrain. The pasture utilized was the pure ryegrass plots (*Lolium multiflorum* [Lam] *cv* Bolt). It was seeded at a density of 45 kg ha^{-1}, with pure and viable seeds, on May 22. When the ryegrass emitted the third leaf, the role N was applied to the treatments. The form of N used was ammonium nitrate, in a combined fertilizer type which also provided 4% of calcium (Ca) and 2% of sulfur (S).

2.3 Plot managements

2.3.1 *Height measures*

The pasture height of each plot was monitored twice each week (from sowing to flowering) by reading 30 points using a sward stick, following the methodology established by Barthram (1985). The plots were maintained with mechanical mowing for homogenization, each time they reached a height of 20 cm, simulating the “entry height of grazing animals” (Amaral et al., 2012). Then, homogenization was conducted by mechanical mowing of the plot, removing 40% of the initial pasture height, as recommended by the "*Rotatínuos*" management concept (de Faccio Carvalho, 2013). The grass was mechanically mowed to simulate the effects of grazing by removing biomass to did not ‘replenish’ soil nutrients with dry matter returns. So, after mowing, all the biomass was taken out of the plot. The remaining pasture residue in the plot after homogenization was 12 cm. When the pasture in a specific plot reached the cutting height, before complete homogenization, aerial biomass cutting occurred (item 2.3.1).

*2.3.2 **Above-ground plant measures and analyses***

Above-ground plant biomass was harvested inside a 25 × 25 cm quadrat randomly placed three times in each plot, cut by an automatic scissor, and placed inside a paper bag. Samples were dried for 4 days at 55 ◦C (or until they reached a constant weight), weighed, ground through a 0.85mm sieve, and analyzed for total N in the grass. The entire weighed dryer was used to calculate the total biomass productive.

Of the three sample cuts performed in the plot, one was subdivided into two parts: A - upper stratum, corresponding to the homogenization height (40% of the pasture initial height); and B - lower stratum, located close to the ground, as shown in the figure 2. The harvested biomass followed the same process as the others. The sum of the upper stratum, which increased within each cut time, subtracting the residue from the previous cut and divided by the number of days between each cut, resulting in the forage accumulation rate. Automatically, stratum B corresponds to the direct residue values of the plot. After weighing, the weights of strata A and B were combined, and the sample was unified again to be incorporated into the analyses of N content in plants, as described earlier.

Figure. 2. Representative scheme of how the cuts were made within each experimental plot to analyze the N and plot height of homogenization by mechanical mowing.

The aerial biomass samples from the plot cuts will be analyzed for N content in their tissues. Two composite samples from the three cuts will be analyzed: two collected close to the ground and the third one from the combined A and B strata. N levels will be determined after digesting the plant tissue, involving warm digestion of the sample in a mixture of sulfuric acid and hydrogen peroxide with a catalyzing mixture. Nutrient content determination was carried out by steam distillation (TE-0363, Tecnal, Piracicaba, SP, Brazil), based on the methodology of Tedesco et al. (1995).

2.3.3 *Soil sampling and analyses*

In each experimental plot, on May 17th, a soil sample composed of three sub-samples was collected to characterize the initial soil fertility and determine the levels of total nitrogen (N), nitrate (NO_3^-), and ammonium (NH_4^+). For this collection, a soil auger was used in the layers of 0 to 10 cm and 10 to 20 cm, while a Dutch auger was employed in the layers of 20 to 40 cm and 40 to 60 cm, covering the zone of greatest root development. After collection, the samples were quickly frozen to halt microbial activities. The soil was initially analyzed for the mineral nitrogen fraction (NH_4^+ and NO_3^-+ NO_2^-) using the steam distillation method described by Bremner & Keeney (1966) and adapted by Tedesco et al. (1995). Total nitrogen was evaluated by the Kjeldahl method (1983), adapted by Tedesco et al. (1995), using a steam distillation apparatus (TE-0363, Tecnal, Piracicaba, SP, Brazil). The entire collection and analysis procedure was repeated after the pasture cycle to determine the remaining N levels in the soil, following the methodology described by Tedesco et al. (1995), and the analysis results were transformed to stock (kg ha^{-1}) using bulk density (BD) for different layers (0-10: 1.52 ± 0.11; 10-20: 1.70 ± 0.05; 20-40: 1.65 ± 0.06; and 40-60: 1.58 ± 0.08 g cm^{-3}).

2.4 Irrigation management and field UV moisture management

In the experimental area, after four months of soil management for limestone incorporation and phosphorus correction, trenches were opened to collect undisturbed soil samples. Six trenches were opened in total, three on the irrigated side and three on the non-irrigated side. Undisturbed soil samples were collected from each trench (two sides) at depths of 0-5, 5-10, 10-20, 20-30, 30-50, 50-70, and 70-90 cm. These undisturbed soil samples were subjected to the tension table method (TEIXEIRA, 2017), obtaining gravimetric moisture content (UG, in g g^{-1}) at saturation point, and field capacity (FC, in g g^{-1}) at 10 kPa tension. At the end of the methodology, samples were dried at 105ºC to determine the bulk density (BD, in g cm^{-3}). Soil volumetric moisture content (UV, in cm^3 cm$^{3\ -1}$) was obtained by multiplying UG by BD. The permanent wilting point (PWP, in g g^{-1}) was estimated based on soil clay content in the 0-20 cm layer, using pedotransfer functions according to KLEIN et al. (2010). Total available soil water (TAW, in g g^{-1}) was considered as the difference between FC and PWP. The ready plant available water (PAW, in g g^{-1}) was considered as 30% of TAW.

Field UV was collected per experimental plot. Field UV was measured forty-three times during the ryegrass cycle, representing an average of 4 days between measurements. UV readings were obtained using the Hydro Farm M1010 moisture sensor. The amount of water to be irrigated was calculated for each UV value below FC down to PWP in the 0-20 cm layer. The decision to irrigate or not was based on the average UV value of the irrigated experimental area corresponding to ARD. The methodology used for decision-making and irrigation management is described in KLEIN (2012). The average flow rate of the irrigation system was 8 mm h^{-1}, measured in the field using 10 rain gauges spread across the irrigated experimental area. The FC that was maintained in the soil is in the figure 3.

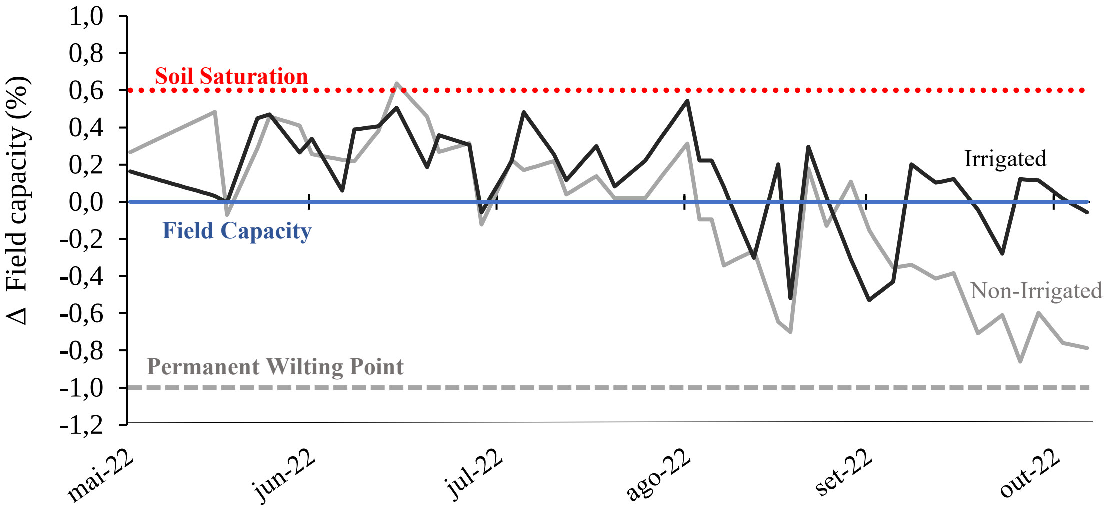

Figure. 3. Δ Field capacity in relationship with all measures of soil moisture during all the time of the field experiment. The soil saturation (SS) and permanent wilting point (PWP) were pointed on to verify the conditions that the ryegrass faced by the soil moisture dynamic. Δ means the difference between the field capacity reference and field capacity values real by the field.

2.5 *Estimation of crop transpiration*

The evapotranspiration was estimated using the FAO Penman-Monteith method, as described by Allen et al. (1998). The reference evapotranspiration estimation method relied on climatic data, which were obtained from the meteorological station located in the experimental area. After calculating the reference evapotranspiration (ET_{ref}), the crop evapotranspiration (ET) is then determined, which is obtained by multiplying ET_{ref} by the crop coefficient (Kc) for each period. A water stress coefficient (Ks), also by Allen et al. (1998), based on water deficit in field capacity (FC), was generated from the soil moisture readings for each treatment in the field and included in the calculation of ET.

2.6 Determination of water use efficiency and N grass status

The water use efficiency was calculated from the crop biomass divided by the evapotranspiration:

$$\mathrm{WUE} = \mathrm{W/ET} \qquad (1).$$

As described by Lemaire et al. (2008), the critical N uptake, the Ncrit, i.e., the minimum N uptake for maximum biomass (W), is given by the relationship:

$$N_{crit} = a \times W^{-b} \qquad (2)$$

where a is the critical N uptake when $W = 1tha^{-1}$ and b is the allometric ratio between the relative N uptake rate and the relative biomass accumulation rate (Lemaire et al. 2007). The coefficients a and b are respectively 4.8 and 0.32 for temperate grasses (Lemaire & Gastal, 1997; Lemaire et al., 2008). Therefore, the Nitrogen Nutrition Index (NNI) can be calculated on any plant sampling date and for any water-N supply treatment to quantify the effects of these treatments on the nitrogen status of the crop (Lemaire et al., 2008):

$$\mathrm{NNI} = N_{act} / N_{crit} \qquad (3)$$

where N_{act} is the actual concentration of N in biomass, and N_{crit} is a critical concentration below which the accumulation of W decreases.

2.7 *Statistical analyses*

Using mixed models with the Emmeans package, two-way analysis of variance was employed to identify significant effects of N doses and water supply. Block and plot were tested as random effects within each model, and the Akaike information criterion (AIC) was used to determine the best option. To quantify these effects, Tukey's test at a 5% significance level was performed using the LmerTest and Lme4 packages with the Emm and Cld functions. The analyzed variables were subjected to the Shapiro-Wilk normality test and the Bartlett test to verify the homogeneity of variances. When a variable did not meet the H0 assumptions (N plant %, accumulation rate, and Soil Δ total N), a Box-Cox test was conducted, and data transformation was performed using the LAMBDA function. Normality and homogeneity of variance tests were then repeated. The independence of the residuals of all variables was visually analyzed using the GGplot package and a Shapiro-Wilk analysis for model residuals. For correlation and regression analyses, the Hmisc and Corrplot packages were used, respectively, with the Rcorr and Corr functions. All analyses were performed with R software (R Core Team, 2022), and all graphs were generated with SigmaPlot 14.0.

3. RESULTS

The accumulation rate of forage and the total biomass production of ryegrass (Fig. 4b and 4a) showed significant results for N doses ($P<0.001$). However, both variables did not present significant difference for the effect of exposure time to waterlogging ($P>0.05$). Treatment 0N showed a daily accumulation of 15.5 ± 3.4 kg DM ha^{-1} day^{-1}, which was significantly lower than in treatments 75N and 150N, where the daily forage accumulation was 31.4 ± 2.6 kg DM ha^{-1} day^{-1} and 31.3 ± 2.3 kg DM ha^{-1} day^{-1}, respectively. The same response dynamics occurred for total biomass production (Fig. 4b). Total biomass production was lower for treatment 0N (2.031 ± 559 kg DM ha^{-1}) when compared to doses of 75N and 150N. There is no difference in total biomass production of ryegrass when we increase the dose from 75N to 150N, corresponding, respectively, to productions of 4.136 ± 349 and 4.771 ± 413 kg DM ha^{-1}.

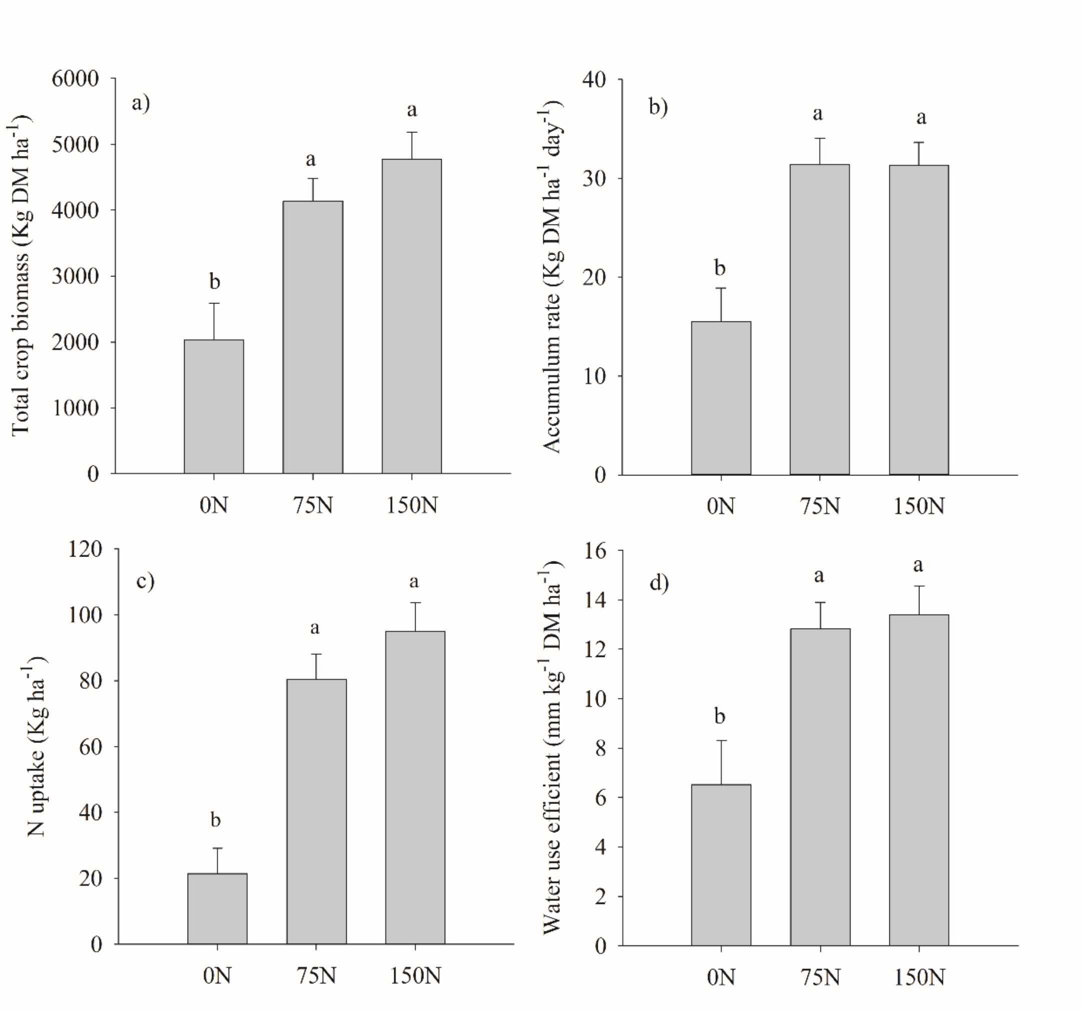

Figure 4. Crop biomass, accumulum rate, N uptake, and, water use efficiency, all of them within three rates of nitrogen applied as ammonium nitrate. All data were analyzed by ANOVA 5% of significance level, and the means were compared by the Tukey test at 5% significance level.

There was no effect of exposure time to waterlogging stress ($P>0.05$) on the total absorbed nitrogen (N_{upt}) and water use efficiency (WUE) (Fig. 4c). However, these variables were significantly influenced by different N doses ($P<0.01$). Ryegrass plants of the treatments 75N and 150N absorbed a total of 80.4 ± 7.7 and 95.0 ± 8.6 kg of N ha^{-1}, respectively, with no significant difference between them. On the other hand, the treatment without nitrogen fertilization (0N) absorbed 21.4 ± 7.76 kg of N ha^{-1}, which was significantly lower than the other treatments. Similarly, treatments 75N and 150N did not differ in WUE, recording 12.8 ± 1.08 and 13.4 ± 1.16 mm kg^{-1} DM ha^{-1}, respectively. Both values were higher than that of the

0N treatment, where water use efficiency was 6.52 ± 1.79 mm kg^{-1} DM ha^{-1}.

The linearity of the relationships between total crop biomass, evapotranspiration, and N uptake was tested and is presented in Figure 5 and Table 1. The irrigated 75N treatment showed an increment of 10.9 ± 0.8 kg DM ha^{-1} per mm of ET, with a strong coefficient of determination ($R^2 = 0.93$) and significant linearity ($P<0.001$). For the 150N irrigated dose, there was an increment of 13.3 ± 0.7 kg DM ha^{-1} per mm of ET, with 96% of the crop biomass being explained by ET and significant linearity ($P<0.01$). However, in the non-irrigated treatment (Fig. 5b), with 0N, it was not possible to predict total crop biomass linearly by ET ($P>0.05$). For the non-irrigated 75N treatment, linearity was significant ($P<0.001$), with a strong coefficient of determination ($R^2=0.95$) and a prediction of total crop biomass of 11.8 ± 0.8 kg DM ha^{-1} per mm of ET. In the linear equation ($P<0.001$, $R^2=0.90$) of the non-irrigated 150N treatment, an increment of 8.75 ± 0.7 kg DM ha^{-1} per mm of ET was observed.

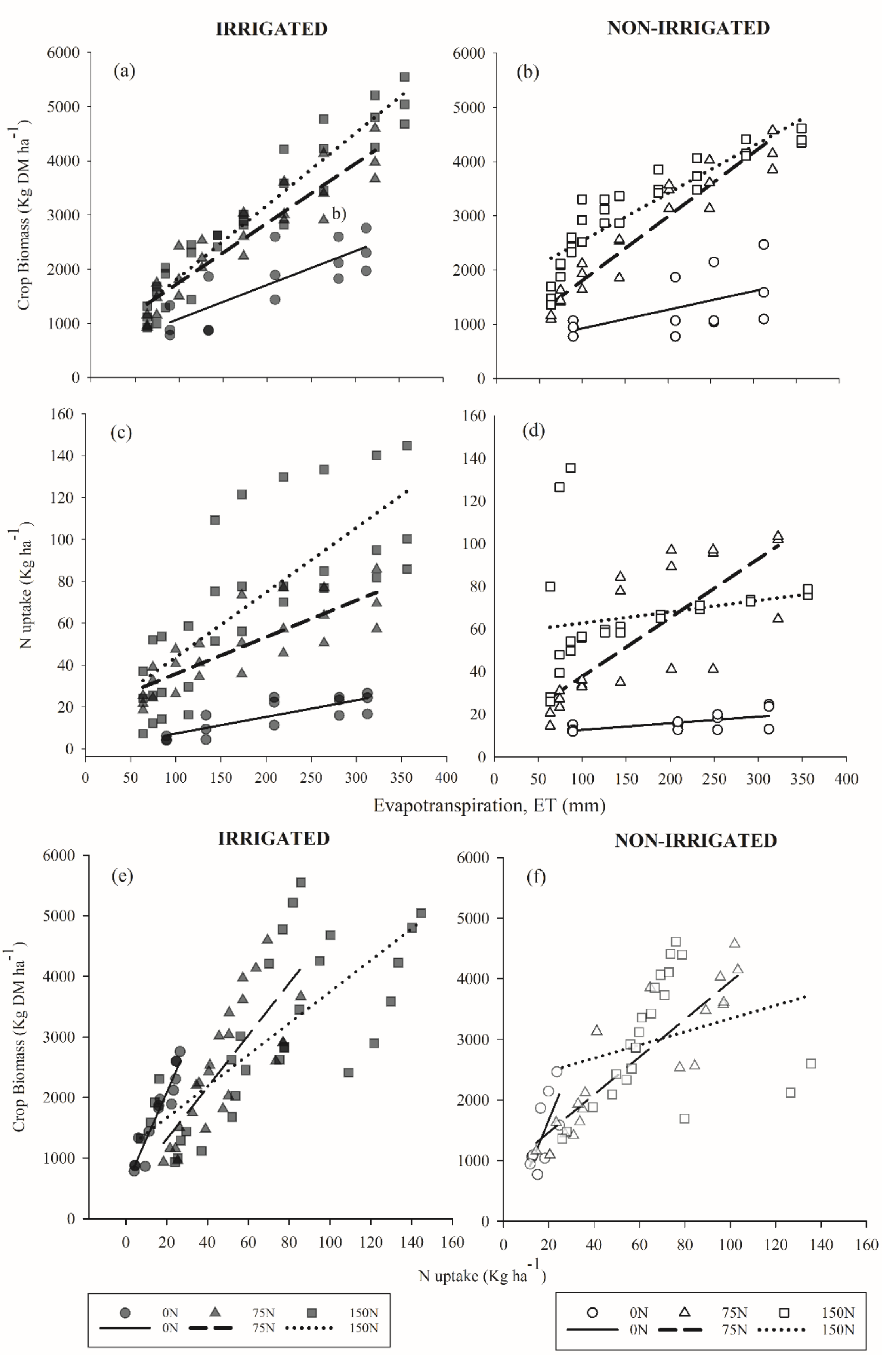

Figure 5. Regression between crop biomass (W) and evapotranspiration (ET), in irrigated (dark points) **(a)** and non-irrigated (open points) **(b)** both with 0 (circles), 75 (triangles) and, 150 (square) kg N ha^{-1};

regression between N uptake (N_{upt}) and evapotranspiration (ET), in irrigated (dark points) **(c)** and non-irrigated (open points) **(d)** both with 0 (circles), 75 (triangles) and, 150 (square) kg N ha^{-1}; and, the regression between crop biomass (W) and N uptake (N_{upt}), in irrigated (dark points) **(e)** and non-irrigated (open points) **(f)** both with 0 (circles), 75 (triangles) and, 150 (square) kg N ha^{-1}. Regression equations reported in Table and the value of P indicates the risk of non-linearity of the regression.

In Figure 5c, the irrigated 75N dose ($P<0.001$) showed that for each mm of ET, plants absorbed 0.17 ± 0.02 kg N ha^{-1} with a coefficient of determination of $R^2=0.81$. The 0N and 150N treatments did not exhibit linearity ($P>0.05$). Conversely, when evaluating doses under shorter exposure time to waterlogging stress (Figure 5d), the 75N dose did not exhibit linear significance ($P>0.05$). However, the 150N dose showed linear significance ($P<0.001$), albeit with only 20% of Nupt explained by ET. The 0N dose was linear ($P<0.01$) with a coefficient of determination of $R^2=0.59$. In the absence of nitrogen fertilization (0N), linearity was significant ($P<0.001$), and 95% of crop biomass could be explained by Nupt (Figure 5e). For 0N, every kg of N absorbed by the plants represented an increment of 77.0 ± 6.6 kg DM ha^{-1}. For the 75N dose, there was no linear explanation ($P>0.05$). In the 150N treatment, there was linearity significance ($P<0.01$), and for each unit of N absorbed, there was an increment of 26.0 ± 4.2 ($R^2=0.75$). In the absence of irrigation (shorter exposure time to waterlogging) (Figure 5f), linearity was not significant for the treatment without nitrogen fertilization ($P>0.05$). For the 75N dose, there was significance ($P<0.01$), and 88% of crop biomass could be explained by Nupt, with an increment of 31.08 ± 3.7 kg DM ha^{-1} for every kg of N absorbed. For 150N, linearity was present ($P<0.01$), but the coefficient of determination was 0.27. Still, for the 150N dose, every kg of N absorbed resulted in 10.89 ± 8.1 kg DM ha^{-1} increment.

Table 1. Linear regression coefficients, slop, x-intercept, R2 values, and their respective *P* values.

Regression by	**Treatment**	**Slop**	**X-intercept**	**2**	**value**
Crop biomass *vs* ET			(kg DM ha^{-1} by each mm)		
	Irr, N0	462.44 (± 284.8)	6.24 (± 1.2)	.80	.128
	Irr, N75	667.31 (± 169.6)	10.93 (± 0.8)	.93	.001

	Irr, N150	529.48 (± 151.4)	13.26 (± 0.7)	.96	.002
	Non-Irr, N0	582.64 (± 406.2)	3.43 (± 1.7)	.52	.182
	Non-Irr, N75	625.73 (± 152.0)	11.80 (± 0.8)	.95	.001
	Non-Irr, N150	1665.84 (± 146.6)	8.75 (± 0.7)	.90	.001
Nupt *vs* ET			(kg N ha^{-1} by each mm)		
	Irr, N0	-0.656 (± 3.3)	0.07 (± 0.01)	.82	.848
	Irr, N75	18.27 (± 4.9)	0.17 (± 0.02)	.81	.001
	Irr, N150	12.89 (±9.9)	0.30 (± 0.04)	.77	.205
	Non-Irr, N0	9.63 (± 3.0)	0.03 (± 0.01)	.59	.009
	Non-Irr, N75	10.12 (± 9.3)	0.27 (± 0.04)	.78	.292
	Non-Irr, N150	57.42 (± 10.2)	0.05 (± 0.02)	.20	.001
Crop biomass *vs* N_{upt}			(kg DM ha^{-1} by each kg N ha^{-1})		
	Irr, N0	541.77 (± 117.6)	77.0 (± 6.6)	.95	.001
	Irr, N75	457.69 (± 370.8)	42.85 (± 7.2)	.78	.230
	Irr, N150	1142.18 (± 342.1)	26.0 (± 4.2)	.75	.002
	Non-Irr, N0	-173.85 (± 467.5)	92.01 (± 27.8)	.72	.718
	Non-Irr, N75	843.65 (± 239.2)	31.08 (± 3.7)	.88	.002
	Non-Irr, N150	2252.14 (± 573.5)	10.89 (± 8.1)	.27	.010

In the nitrogen dilution curve (Fig. 6a), it was demonstrated that, regardless of the N dose and exposure time to waterlogging stress, most values remained below the critical level curve of N, indicating compromised biomass accumulation. The nitrogen content absorbed in the aboveground biomass showed a significant effect of the applied nitrogen doses ($P < 0.01$), with the 0N treatment differing from 75N, while these two were considered equivalent to 150N. The average N content in ryegrass aboveground biomass was 1.06 ± 0.12% in the 0N treatment, 1.43 ± 0.10% in 75N, and 1.35 ± 0.93% in 150N. Meanwhile, the nitrogen nutrition index (NNI) (Fig. 6b) was influenced by the isolated effects of N doses and exposure time to stress ($P<0.05$). In the 0N treatment, the NNI was 0.22 ± 0.03, lower than that observed for 75N, which recorded an index of 0.34 ± 0.02. While the 150N dose (0.31 ± 0.03) did not show significant differences in relation to both. Additionally, the longer exposure period to waterlogging stress resulted in a lower index (0.29 ± 0.02) compared to the reduced exposure time (0.38 ± 0.02).

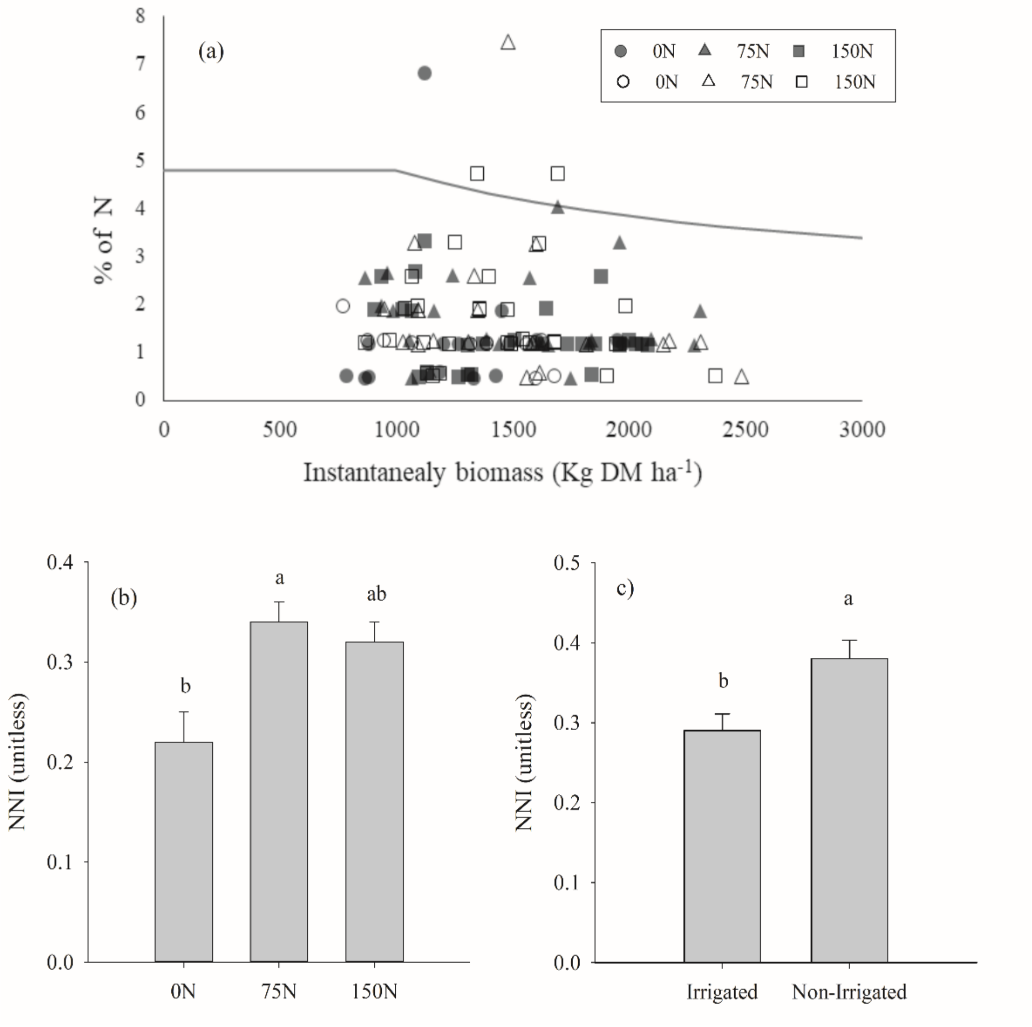

Figure 6. (a) Nitrogen dilution curve for C3 temperate grasses (Lemaire & Gastal, 1997, Lemaire et al., 2008), where filled symbols represent irrigated (●▲ ■) and empty symbols represent non-irrigated (○△ □) conditions, with 0N, 75N, and 150N denoting nitrogen doses, respectively for both; and (b) is the nitrogen nutrition index by N doses and (c) the nitrogen nutrition index for irrigated and non-irrigated conditions, both with ANOVA at 5% significance level and Tukey's test for mean comparison.

The results for total soil N change (ΔN) (Fig. 7a) was significant only by soil layers ($P<0.05$). The smallest variation in soil N was observed in the 0-10 cm layer, with a content of -0.18 ± 0.016 g N kg^{-1} of soil, while the 40-60 cm layer exhibited the largest variation in total nitrogen content, with ΔN of -0.11 ± 0.016 g N kg^{-1} of soil. The intermediate layers of 10-20 and 20-40 cm did not differ from any of the other layers, presenting values of -0.16 ± 0.01 and -0.13 ± 0.016 g N kg^{-1} soil, respectively. The Δ of ammonium contents in the soil (Fig. 7b) showed differences only for N doses ($P<0.05$), with 0N (0.015 ± 0.002 g N kg^{-1} soil) being higher than 75N, which had a value of 0.00882 ± 0.002 g N kg^{-1} soil. Meanwhile, the 150N dose resulted in 0.01351 ± 0.002 g N kg^{-1} soil, considered equal to the other treatments.

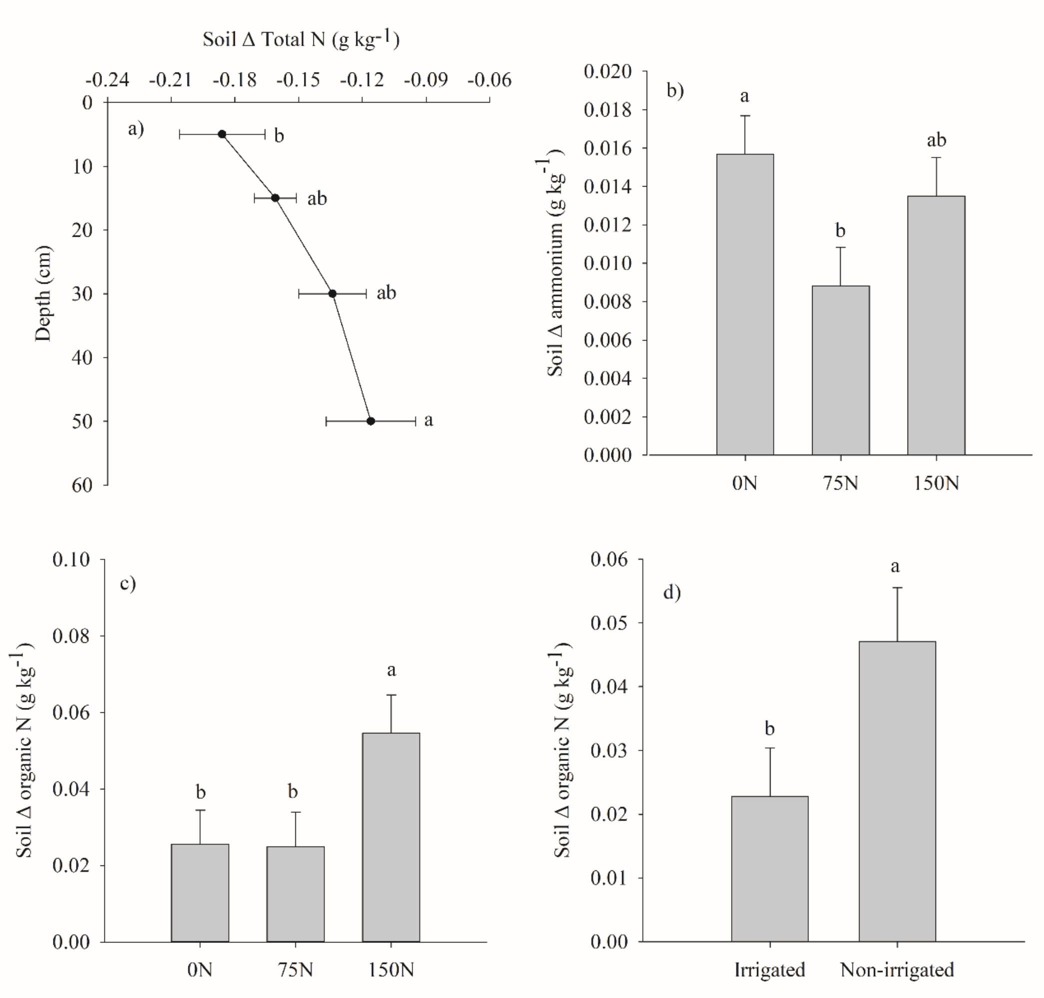

Figure 7. a) Soil Δ total N in different layers, b) soil Δ ammonium, c) soil Δ organic N, all with three doses of N, and also d) soil Δ organic N, irrigated or not. ANOVA was performed at 5% significance level and Tukey's test for mean comparison. Δ means the difference between the initial soil sample and the values of the final soil sample.

The response for soil organic N change (ΔN) (Fig. 7c) was significant for N doses ($P<0.05$). The 150N treatment, which ended with a Δ of 0.01351 ± 0.002 g N kg^{-1} soil, was considered significantly higher than 0 and 75N, which were considered equal and had Δ of 0.0255 ± 0.009 and 0.0249 ± 0.008, respectively. Additionally, the variation of organic N in the soil showed a significant difference concerning the exposure time to stress (Fig. 7d). The non-irrigated treatment recorded a higher average, with 0.0471 ± 0.0084 g N kg^{-1} soil, while the irrigated treatment reduced soil organic nitrogen by 52%, totaling 0.0228 ± 0.0076 g N kg^{-1}

soil.

4. DISCUSSION

The use of ryegrass is of paramount importance for productive systems in subtropical regions, either as a forage plant or as part of integrated arrangements with agriculture. To increase the efficiency of these systems, the environmental condition for N fertilization management should be taken into consideration as a primary factor in decision-making. In the conditions studied in this work with high rainfall incidence (consequently, also reducing solar radiation incidence), leading to soil waterlogging in uplands, when the N dose exceeded 75 kg of N ha^{-1}, there was no increase in productivity. In other words, ryegrass does not respond as expected to the recommended dose of 150 kg of N per hectare (CQFS, 2016) to achieve maximum productivity, as clearly observed from the nitrogen dilution curve. The results of forage accumulation rate and total crop biomass showed that, regardless of the duration of exposure to waterlogging stress, there were no productive responses from plants to fertilization with 150 kg N ha^{-1} when the soil moisture was above field capacity.

The recommendation for N fertilization for winter forage grasses is calculated considering the soil organic matter content, related to the desired dry matter production by the crop (CQFS, 2016). Ryegrass is a widely used forage grass in high relief areas such as lowlands. However, empirically, there is a belief in ryegrass adaptation to waterlogging. Nonetheless, continuous stress can reduce the crop's response, including factors like N fertilization management. Under the environmental conditions of this study, with a low range of soil organic matter (2.3%), 150 kg N ha^{-1} on average is recommended to achieve 6 ton DM ha^{-1} (CQFS, 2016). However, biomass production was below 6 ton DM ha^{-1}, revealing that using the 150N dose did not increase productivity compared to the 75N dose.

Following the same dynamics, N_{upt} and WUE were similarly affected, with no response to the application of 150N compared to 75N. Overall, the main response observed is related to the use of the 75N dose when the soil remains waterlogged for longer periods. Even though there is a response of ryegrass to N application, this response may be limited by excess water conditions; and under these specific conditions, the recommendation of nitrogen fertilization applied entirely at stage V3-V4 may be lower (75 kg N ha^{-1}) than the recommended dose (150 kg N ha^{-1}). This new possibility of nitrogen fertilization management recommendation in winter forage grasses reduces excess N application (given the lack of response of the crop at doses above 75N) when the soil is waterlogged or when there are forecasts for such conditions. This will benefit not only the rural producer in reducing production costs but also in reducing the use of non-renewable resources. Additionally, later, under better environmental conditions for nitrogen fertilization application, the remaining or part of the total dose not applied at stage V3-V4 can be applied during the ryegrass production cycle.

These results from the N dilution curve reflect the impairments in N uptake by plants related to both the duration and timing of waterlogging. Since both moisture conditions evaluated in this study persisted for more than half of the ryegrass cycle with soil moisture remaining above field capacity. Furthermore, this impairment had an effect on the NNI, where the best index responses were obtained under non-irrigation conditions and with the 75N dose. In contrast to what has been observed so far, under water deficit conditions, the NNI proves to be sensitive in demonstrating the effect of water shortage on nitrogen uptake by winter forage grasses (Gonzales-Dugo et al., 2005). Similarly, in this study, the NNI proved to be sensitive to changes in management and nitrogen fertilization under excess water conditions.

Nitrogen fertilization is of fundamental importance to achieve the maximum productivity of a crop. Temperate forages are highly responsive to N application (Lemaire and Gastal, 1997; Yu-lan 2013; Ertekin et al., 2020). However, the absorption and utilization of N by forage plants can be limited by environmental conditions during the crop's production cycle. In the case of waterlogged soil (Fig. 2), microbial respiration can lead to oxygen depletion, causing hypoxic stress for the roots (Colmer and Voesenek 2009; Sasidharan et al. 2017; Ploschuk et al., 2017). In this regard, the decrease in oxygen concentration in the root zone leads to a reduction in N uptake rate, even if there is availability of this nutrient in this zone. Additionally, according to Liu et al. (2020), flooding or waterlogging results in the inhibition of processes in the mesophyll, the transport of photoassimilates in the phloem, gas conductance, and consequently reduces the photosynthetic rate. All these factors tend to affect the soil-plant relationship, potentially reducing biomass production.

As for soil responses, it was expected that N doses could influence the mineral fractions of NO_3^- and NH_4^+ in the soil, given the N source in the fertilizer used. However, the changes found for total N in the soil indicated a decrease in the element's concentration. In the context of soil tillage and the implementation of conservation tillage systems, such as no-till farming, it is expected that there will be a decrease in the total soil N stock (Cantarella, 2007). This reduction arises from the soil organic matter being exposed to heightened microbial activity, particularly pronounced under aerated conditions after the initial soil preparation during the implementation of the conservation system (Bayer, Mielniczuk, & Martin-Neto, 2000).

Another issue regarding N fertilization is nitrogen losses through leaching (Sangoi et al., 2003). However, there was no evidence of transfers of NO_3^- and NH_4^+ forms from surface layers (0-5 and 5-10 cm) to subsurface layers of the soil (10-20, 20-40, 40-60, and 60-80 cm). However, the changes presented for NH_4^+ in the soil were positive (Fig. 7b) for the tested N doses, indicating an increase in this fraction in the soil regardless of the soil layer analyzed. The increase in the NH_4^+ form in the soil may reflect the constant mineralization of soil organic matter (Monteiro et al., 2014). However, the decrease in NH_4^+ content in the soil at the 75N dose compared to the 0N dose may correspond to a form of nitrogen that is still being mineralized. This decrease in the 75N dose may have occurred because the fertilization did not "satisfy" the plant's nitrogen uptake rate, causing the plant to rely on its own soil nitrogen mineralization rate. This does not occur in the 0N dose, where the plant is not in the same growth rate, nor in the 150N dose, where the plant does not access what is being mineralized because there is already abundant nitrogen. However, it is worth noting that compared to the total soil nitrogen, this increase in NH_4^+ availability in the soil represents only a small increase in relation to the total soil nitrogen.

The organic fractions of N in the soil are easily accessible reserves through mineralization by microorganisms. These fractions can be represented and referred to as the potentially mineralizable N of the soil. After the experimental period, these organic fractions increased, due to the transition of organic matter from stable fractions to labile fractions. Additionally, in the 0N and 75N doses, due to the continuous absorption and extraction of N by the plant, there was a lower delta in the organic fractions at these doses. In the 150N dose, the excess nitrogen may have been retained and stored in this labile fraction, as indicated by the negative delta for total soil nitrogen. Between irrigated and non-irrigated conditions, the increase in organic fractions where there was no irrigation may have occurred because of the longer period under aerobic conditions, where the process of formation of this organic nitrogen fraction prevailed (Liu et al., 2020).

In subtropical regions, hibernal meteorological conditions involve periods of large-scale rainfall, as depicted by the climatological normal shown in Figure 1, leading to periods that may have low solar radiation. These factors can impact the development of temperate crops. In low-lying areas (such as floodplains or lowlands), it is common to have flooded areas that limit the range of crops to only those that can withstand such conditions. However, with the incidence of climate change factors, in some years, excess water may persist, especially in subtropical zones (Gelcer et al., 2013; Penalba and Rivera, 2016; N. Júnior et al., 2019; N. Junior et al., 2020). Consequently, soils that typically do not flood begin to experience waterlogging effects, exposing plants to increasingly longer periods of water stress (Grimm, 2003; Penalba and Rivera, 2016; Moura et al., 2019) (see Figure 2).

Technical recommendations for crop fertilization management need to consider social, economic, and environmental risks, not just productivity. Environmental and social risks can be determining factors, as in a comprehensive systematic review, nitrate intake in drinking water has been associated with an increased risk of cancer in the population (Temkin et al., 2019). Even though there is consensus that field studies on waterlogging periods are extremely difficult to conduct, new studies on the effect of soil waterlogging time, together with N fertilization, are necessary, focusing on the intensity of crop exposure to water stress.

5. CONCLUSIONS

Os achados deste estudo destacam, pela primeira vez, que a dinâmica alométrica da relação entre o consumo de água e nitrogênio em plantas de azevém é sensível à duração da exposição ao estresse de alagamento durante o inverno subtropical. A prolongada permanência do solo em condições alagadas, simulando episódios mais frequentes e duradouros de eventos climáticos extremos, teve um impacto adverso na nutrição nitrogenada do azevém.

Sob condições de encharcamento, a aplicação de 150 kg de N por hectare não proporciona ganhos adicionais de produtividade no azevém em comparação com a aplicação de 75 kg de N por hectare. Essa falta de resposta evidencia que o azevém não é capaz de utilizar eficientemente todo o nitrogênio adicionado quando a umidade do solo está acima da capacidade de campo. Essas descobertas têm implicações significativas para os produtores rurais e para a gestão ambiental. A recomendação de aplicar doses mais elevadas de nitrogênio não se justificam em áreas propensas a alagamentos, o que pode resultar em desperdício de recursos e custos adicionais de produção. Portanto, é crucial reconsiderar as práticas de fertilização nitrogenada sob essas circunstâncias para garantir uma gestão eficaz dos recursos. Potencialmente, isso não apenas resultará na redução dos custos de produção para os agricultores, mas também na minimização dos impactos ambientais adversos derivados do excesso de aplicação de N.

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Data availability

Data will be made available on request.

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CAPÍTULO III

CONSIDERAÇÕES FINAIS

Os achados deste estudo destacam que mesmo que haja a resposta da cultura do azevém à aplicação de nitrogênio, essa resposta pode ser limitada pelas condições ambientais de excesso hídrico; e que nestas condições a recomendação de adubação nitrogenada aplicada totalmente em estádio V3-V4 pudesse ser menor (75 kg N ha^{-1}) do que a dose recomendada (150 kg N ha^{-1}). Assim sendo, esta nova possibilidade de manejo da adubação nitrogenada em gramíneas forrageiras hibernais diminui a aplicação em excesso de nitrogênio (visto a não resposta da cultura na dose acima de 75N) quando o solo está encharcado ou há previsões climáticas para tal. Assim beneficiará não somente o produtor rural na diminuição dos custos de produção, mas também em redução de uso de recursos não renováveis.

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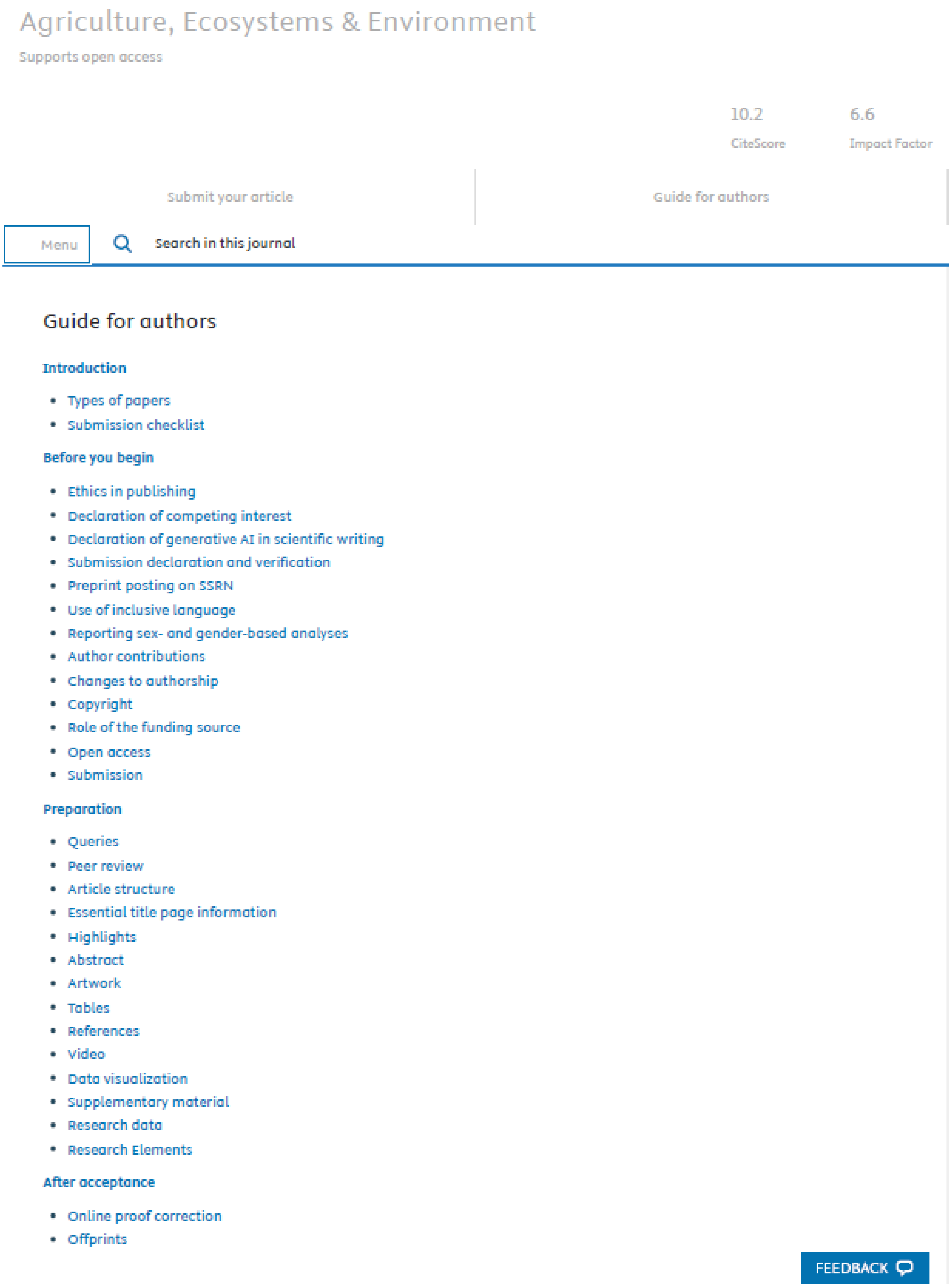
ANEXOS

ANEXO - A. Rules to elaborate and submit a manuscript for (journal)



Guide for authors

Introduction

- Types of papers
- Submission checklist

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- Declaration of generative AI in scientific writing
- Submission declaration and verification
- Preprint posting on SSRN
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- Reporting sex- and gender-based analyses
- Author contributions
- Changes to authorship
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Introduction

Agriculture, Ecosystems and Environment deals with the interface between agriculture and the environment. Preference is given to papers that develop and apply interdisciplinarity, bridge scientific disciplines, integrate scientific analyses derived from different perspectives of agroecosystem sustainability, and are put in as wide an international or comparative context as possible. It is addressed to scientists in agriculture, food production, agroforestry, ecology, environment, earth and resource management, and administrators and policy-makers in these fields.

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- Full postal address

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- All tables (including titles, description, footnotes)
- Ensure all figure and table citations in the text match the files provided
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Graphical Abstracts / Highlights files (where applicable)
Supplemental files (where applicable)

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The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

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VITA

Lóren Pacheco Duarte, filha de Neusa Billow Pacheco e Cleomar Rodrigues Duarte, nasceu aos 27 dias do mês de maio, do ano de 1995, na cidade de Rio Grande – RS. Foi criada pelos pais na cidade de Cerro Grande do Sul, onde cursou o ensino fundamental e o ensino médio na escola Mem de Sá. Durante todo o período de ensino médio, trabalhou meio período como auxiliar de lanchonete (garçonete, auxiliar de cozinha e atendente) e outro meio período como auxiliar de *petshop* (banho e tosa de animais). Após finalizar o ensino médio em 2013, manteve as atividades de trabalho até 2016, quando ingressou na Universidade Federal do Pampa (UNIPAMPA) na cidade de Dom Pedrito – RS, onde morou e cursou Zootecnia por um ano. No ano de 2017, transferiu os estudos em Zootecnia para a Universidade Federal do Rio Grande do Sul (UFRGS). Ainda em 2017, foi monitora voluntária no Departamento de Solos (disciplina de Fertilidade do Solo) e depois se voluntariou ao Grupo de Pesquisa em Ecologia do Pastejo (GPEP), junto ao Departamento de Plantas Forrageiras e Agrometeorologia (DPFA). Foi bolsista de iniciação cientifica (IC) de 2018 a 2019 pela Fundação de Pesquisa Agropecuária do RS (FEPAGRO). De 2019 a 2021, seguiu como bolsista de IC do GPEP/DPFA. Ainda em 2021, foi estagiária na Universidade Sueca de Ciências Agrárias (SLU) em Uppsala – SE. No início do ano de 2022, concluiu o curso de Zootecnia na UFRGS e mudou-se para Cuiabá – MT. Lá, iniciou atividades laborais na Papalotla Brasil®, onde atuou como representante técnica até o final do mesmo ano. Ainda no final do ano de 2022, ingressou como aluna especial no Programa de Pós-Graduação em Zootecnia pela UFRGS e, em 2023, como aluna oficial de mestrado acadêmico pelo mesmo Programa e mesma Universidade. Em março de 2024, sob orientação de Paulo Cesar de Faccio Carvalho e coorientação de Taíse Robinson Kunrath, submeteu essa dissertação para julgamento à obtenção do título de mestra.