

**UNIVERSIDADE ESTADUAL PAULISTA  
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VETERINÁRIAS DE JABOTICABAL**

**ELUCIDANDO OS MECANISMOS DE RESPOSTA DE GRAMÍNEAS  
FORRAGEIRAS AO CULTIVO EM SEQUEIRO E IRRIGADO NO ESTADO DE  
SÃO PAULO**

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## RESUMO

Os pastos constituem a principal fonte de alimento de custo relativo baixo para os ruminantes, mas a produção é limitada pela ocorrência da seca e consequente déficit hídrico no solo, o que tem impulsionado a expansão de sistemas irrigados e busca por cultivares forrageiras mais tolerantes à restrição hídrica. No entanto, o potencial dessas tecnologias é ainda pouco explorado, sendo pouco conhecidos os mecanismos fisiológicos e nutricionais envolvidos na tolerância das gramíneas forrageiras à seca, sua influência na microbiota do solo, bem como as respostas das gramíneas sob cultivo fertirrigado a nível de campo. Esta pesquisa buscou investigar a tolerância à seca e a resposta à irrigação por gotejamento subterrâneo nas forrageiras (*Urochloa brizantha* x *U. ruziziensis* cv. Mavuno, e *Megathyrsus maximus* cv. Zuri), bem como os impactos desses sistemas de cultivo na microbiota do solo. Foi desenvolvido um experimento de campo na área da Fazenda experimental da Unesp, Jaboticabal/SP em Latossolo Vermelho eutrófico, com tratamentos organizados em esquema fatorial 2x2, sendo duas espécies forrageiras (Mavuno e Zuri) e duas condições hídricas (sequeiro e irrigado por gotejamento). Observou-se que o cultivo em sequeiro limitou a produção das duas espécies forrageiras, mas o capim Mavuno foi mais tolerante mantendo maior integridade da membrana celular, níveis elevados de compostos fenólicos, melhor homeostase nutricional, maior atividade microbiana no solo e maior acúmulo de biomassa. Em contraste, o capim Zuri foi mais sensível, apresentando maiores perdas nos pigmentos fotossintéticos, na estabilidade da membrana e na eficiência do fotossistema II. A irrigação por gotejamento favoreceu o equilíbrio na homeostase C:N, C:P e N:P no tecido foliar dos capins Mavuno e Zuri, e beneficiou grupos microbianos no solo como bactérias esporulantes, leveduras e actinomicetos, com incremento no C total do solo cultivado com capim Mavuno. Consequentemente, a irrigação aumentou a produção de forragem em ~10 Mg ha<sup>-1</sup> para ambas gramíneas. Sob irrigação o capim Zuri contribuiu mais para a biomassa microbiana do solo, e favoreceu o acúmulo de C total do solo independente da condição hídrica, o que pode beneficiar a pastagem a longo prazo. Ambas as cultivares demonstraram desempenho otimizado sob irrigação, com aumento no teor de aminoácidos, folhas mais tenras e maior produtividade de forragem. Portanto, o capim Mavuno é recomendado para cultivo em sequeiro com boa tolerância ao déficit hídrico, e ambas gramíneas apresentam alta produtividade de forragem sob irrigação por gotejamento subterrâneo. Este estudo contribui para o desenvolvimento de sistemas pastoris eficientes e sustentáveis, adaptados às restrições hídricas previstas diante das mudanças climáticas.

**Palavras-chave:** déficit hídrico, microbiota do solo, nutrição de forrageiras, irrigação por gotejamento, pastagem de sequeiro

## 1. INTRODUÇÃO

Gramíneas forrageiras tropicais são amplamente cultivadas em pastagens para tornar a pecuária mais eficiente (Nehring, 2024), e são conhecidas por terem impulsionado a produção de forragem no Brasil, especialmente com o melhoramento genético do capim braquiária (*Uroclhoa* ssp.) e de forrageiras do gênero *Megathyrsus* (sin. *Panicum*). Porém, o déficit hídrico imposto pela seca agrícola anual em regiões tropicais com inverno seco provoca a sazonalidade de produção de forragem, que se concentra na época chuvosa (Tulu et al., 2023; Santos et al., 2024).

A seca afeta a comunidade microbiana do solo em pastagens (Toledo et al., 2023), processo influenciado pela espécie forrageira cultivada (Moreno et al., 2021). Embora o cultivo de gramíneas seja benéfico para a abundância microbiana no solo (Momesso et al., 2022), ainda não se conhece como a espécie de gramíneas forrageiras tropicais influenciam a microbiota do solo sob seca, e como essa microbiota corrobora para a tolerância dessas plantas a seca em condições de campo.

O déficit hídrico afeta também o equilíbrio hídrico foliar e o fluxo transpiratório das plantas (Mganga et al., 2023), prejudicando o acúmulo de nutrientes e o equilíbrio estequiométrico entre carbono (C), nitrogênio (N) e fósforo (P) no tecido foliar que é essencial para produção de massa seca (MS) (Olivera-Viciedo et al., 2024), e para o metabolismo vegetal em geral, podendo limitar a oferta de substrato orgânico para os microrganismos do solo (Pereira et al., 2021). No entanto, a maioria dos estudos avaliando pastagem e níveis de água no solo não investiga seus efeitos na homeostase estequiométrica elementar.

A pesquisa científica tem buscado elucidar a tolerância de diversas gramíneas ao déficit hídrico em ambiente controlado, indicando prejuízos desse estresse nas trocas gasosas, na integridade de membranas e pigmentos fotossintetizantes, e consequentemente na produção de forragem (Mastalerczuk & Borawska-Jarmułowicz, 2021; Zuffo et al., 2022; Oliveira et al., 2022). Sob restrição hídrica as plantas forrageiras dispõem de ajustes fisiológicos e bioquímicos como o acúmulo de compostos osmorreguladores e antioxidantes para a manutenção do equilíbrio hídrico celular e a mitigação do estresse oxidativo (Rocha et al., 2022). Porém, são escassos estudos em condições de campo que indiquem essas estratégias em

diferentes espécies forrageiras (Olivera-Viciedo et al., 2021), o que é fundamental para nortear a escolha da espécie adaptada ao déficit hídrico para favorecer a estabilidade da produção de forragem.

Para mitigar os prejuízos do déficit hídrico na produção de forragem, está consolidada a irrigação de pastagens aliada a adubações de manutenção (Jesus et al., 2021). A irrigação pode modificar a biomassa e a atividade de grupos de microrganismos do solo (Gong et al., 2015; Fu et al., 2021). Considerando que o acúmulo de matéria orgânica do solo (MOS) é favorecido pela deposição de raízes no solo e pela biomassa microbiana (Frene et al., 2022), a irrigação pode contribuir também para o acúmulo de C no solo de pastagem bem manejada (Li et al., 2024), embora possa haver em curto prazo um estímulo à respiração microbiana.

Tem sido preconizado o uso de sistemas mais eficientes como o gotejamento subterrâneo (Rocha et al., 2024), que minimiza perdas de água por evaporação e escoamento superficial em comparação a sistemas de aspersão (Allen & MacAdam, 2020). Apesar do custo-benefício do gotejamento subterrâneo ser um possível entrave à sua utilização em pastagens com forrageiras de clima temperado (Finger et al., 2015), seu uso pode ser viável para gramíneas tropicais que tem alto potencial de acúmulo de biomassa (Rocha et al., 2024). Porém, são escassas as evidências científicas de campo sobre a resposta fisiológica e produtiva de cultivares de *Megathyrsus* e *Uroclhoa* à irrigação por gotejamento subterrâneo, e seus impactos na microbiota do solo de pastagem.

Diante do exposto, este estudo investigou as seguintes hipóteses: i) a tolerância de diferentes espécies de gramíneas ao cultivo em sequeiro é influenciada pela homeostase C:N:P e pela microbiota do solo; ii) as espécies de gramínea apresentam diferentes tolerâncias ao déficit hídrico em função de seus mecanismos bioquímicos e fisiológicos; iii) a irrigação por gotejamento subterrâneo é capaz de mitigar os efeitos da seca, promovendo ganhos fisiológicos e produtivos, com impactos positivos na microbiota do solo de pastagem cultivada com gramíneas tropicais.

## 2. OBJETIVOS

Objetivou-se com a presente pesquisa:

- a. Elucidar a influência da espécie de gramínea forrageira cultivada na microbiota do solo de pastagem nos cultivos em sequeiro e irrigado;
- b. Compreender os mecanismos relacionadas à homeostase elementar C:N:P e aos aspectos fisiológicos que contribuem para a tolerância de forrageiras ao déficit hídrico na estação seca;
- c. Avaliar a resposta produtiva das forrageiras *M. maximus* cv. Zuri e *U. brizantha* x *U. ruziziensis* cv. Mavuno à irrigação por gotejamento subterrâneo;
- d. Fomentar a recomendação mais assertiva de espécies de gramíneas conforme a condição hídrica disponível, indicando o potencial de uso da irrigação por gotejamento em pastagem.

## 3. MATERIAIS E MÉTODOS

### 3.1 Localização e condições edafoclimáticas da área experimental

Foi realizado um experimento de campo na Fazenda experimental da Universidade Estadual Paulista (UNESP/FCAV), situada no município de Jaboticabal, Brasil, nas coordenadas 21° 14' 54" S e 48° 17' 06" W, a 560 metros de altitude, sendo caracterizada por um clima Aw, que apresenta chuvas no verão e um inverno seco.

O período experimental estendeu-se de abril a outubro de 2023, coincidente com a estação seca do ano. Dados meteorológicos foram obtidos de estação meteorológica instalada na universidade próximo à área experimental, sendo registrados o volume de chuvas, a evapotranspiração potencial, a temperatura do ar e a radiação solar global (Figura 1a, b). A evapotranspiração e a precipitação pluvial acumuladas durante o período experimental foram iguais a 595 e 184 mm, respectivamente.

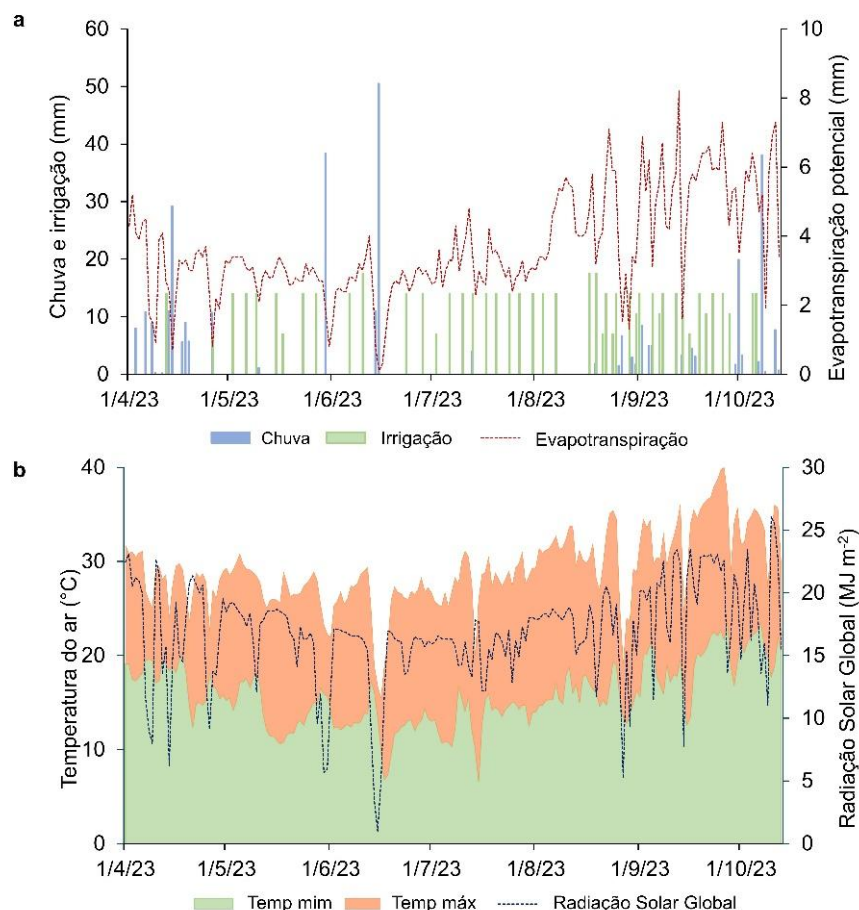


Figura 1. Dados observados durante o período experimental relativos à precipitação, irrigação, evapotranspiração (a), temperatura do ar e radiação solar (b) em Jaboticabal, Brasil.

Foram coletadas amostras de solo da área experimental para uma avaliação química inicial (Raij et al., 2001), que apresentou os seguintes resultados nas profundidades de 0-20 cm e 20-40 cm, respectivamente: pH de 5.8 e 6.0, matéria orgânica (MOS) = 22 g dm<sup>-3</sup>, fósforo (P) = 78 e 51 mg dm<sup>-3</sup>, potássio (K) = 5.5 e 3.6, cálcio (Ca) = 46 e 44, magnésio (Mg) = 15 mmolc dm<sup>-3</sup>, enxofre (S) = 3 e 4, boro (B) = 0.32 e 0.25, cobre (Cu) = 3.4 e 3.5, ferro (Fe) = 6 e 5, manganês (Mn) = 25.5 e 18.6, zinco (Zn) = 1.6 e 1.0 mg dm<sup>-3</sup>, H+Al = 21 e 18 mmolc dm<sup>-3</sup>, capacidade de troca catiônica (CTC) = 88 e 81 mmolc dm<sup>-3</sup>, e a saturação por bases ( $V = \text{Ca} + \text{Mg} + \text{K} / \text{CTC}$ ) = 76% e 77%. O solo foi classificado como Latossolo Vermelho eutroférico.

### 3.2 Tratamentos e implantação do experimento

O experimento seguiu um esquema fatorial 2 x 2, com duas espécies de gramíneas forrageiras Mavuno (*Urochloa brizantha* × *U. ruziziensis*) e Zuri (*Megathyrsus maximus*), e duas condições hídricas de cultivo (sem irrigação e irrigado). O delineamento utilizado foi em blocos casualizados, com quatro repetições.

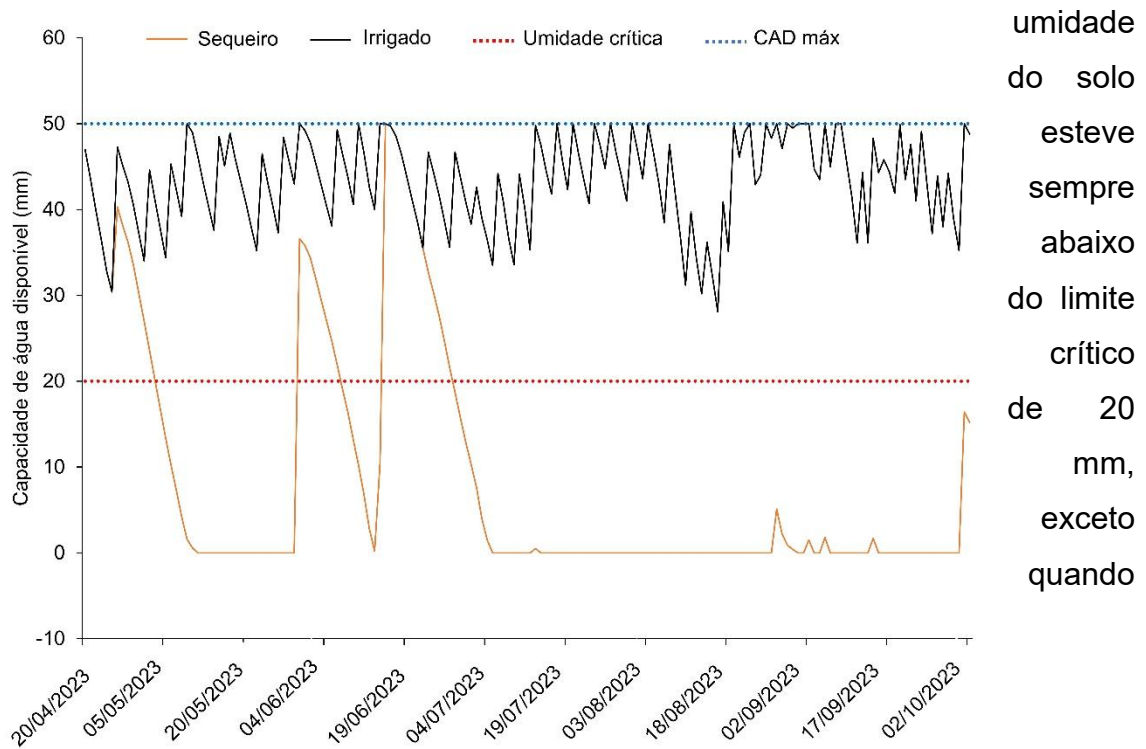
A implantação do experimento ocorreu em abril de 2022, após o preparo do solo por gradagem. Para as parcelas irrigadas, foi instalado um sistema de gotejamento subsuperficial. Os tubos gotejadores foram enterrados a 20 cm de profundidade, com espaçamento de 80 cm entre as linhas de cultivo (Rocha et al., 2024). Os emissores possuíam vazão de 1,45 L h<sup>-1</sup>, operando a 100 kPa e espaçados a cada 30 cm, garantindo uma distribuição uniforme da água de acordo com Bernardo et al. (2009).

As parcelas, com dimensões de 3 × 4,8 m, receberam semeadura manual das gramíneas, distribuindo-se sobre o solo uma quantidade equivalente a 10 kg ha<sup>-1</sup> de sementes puras viáveis. Após a germinação e crescimento inicial, foi realizado um corte para estimular o perfilhamento, realizado a 10 e 20 cm do solo para os capins Mavuno e Zuri, respectivamente, utilizando uma roçadora manual (Husqvarna 31R/330-2). Durante a fase de formação, o pasto foi irrigado por aspersão para garantir um estabelecimento homogêneo das plantas. Em abril de 2023, com a pastagem completamente formada, realizou-se o corte para uniformização das parcelas, deixando uma altura de 15 cm para Mavuno e 30 cm para Zuri (Barbosa et al., 2021; Sbrissia et al., 2020).

### 2.3 Aplicação dos tratamentos e adubações da pastagem

A partir do corte de uniformização foram aplicadas as distintas condições hídricas, sendo as parcelas em sequeiro mantidas sem irrigação, e as irrigadas foram submetidas à irrigação por gotejamento subsuperficial. A capacidade de armazenamento de água no solo (CAD) na camada de 0-40 cm foi determinada previamente a partir da umidade na capacidade de campo e no ponto de murcha permanente obtidos em curva de retenção de água no solo com amostras indeformadas (Klute, 1986), sendo igual a 50 mm. A lâmina e o turno de rega foram estabelecidos para repor totalmente as perdas de água por evapotranspiração,

com um fator de disponibilidade ( $f$ ) = 0.6, resultando em uma lâmina total aplicada de 577 mm durante o período experimental (Figura 2). Na condição de sequeiro a



ocorreram chuvas esporádicas no mês de junho.

Figura 2. Dados de capacidade de água disponível no solo durante o período experimental de abril a outubro de 2023 na área experimental localizada no município de Jaboticabal, Brasil. A condição de sequeiro é representada pela linha de cor amarela, e a condição irrigada pela linha de cor preta. As linhas horizontais

tracejadas indicam a capacidade máxima de água no solo (cor azul) e a umidade crítica do solo estabelecida para manejo de irrigação (cor vermelha).

A fertilidade inicial do solo foi considerada adequada ao cultivo das gramíneas, sendo necessárias apenas adubações de manutenção após cada ciclo de crescimento do pasto, repondo os nutrientes nitrogênio (N), fósforo (P), potássio (K) e enxofre (S) exportados na matéria seca (MS) em cada tratamento (Dubeux Jr. et al., 2007; Quaggio et al., 2022). Os fertilizantes utilizados incluíram ureia, cloreto de potássio, sulfato de amônio e fosfato monoamônico purificado para fertirrigação, enquanto no sistema de sequeiro, o fósforo foi aplicado na forma de superfosfato simples. A adubação nas parcelas irrigadas foi realizada por fertirrigação, e nas áreas de sequeiro os fertilizantes foram distribuídos manualmente a lanço.

### **3.3 Variáveis analisadas**

#### **Altura do pasto, perfilhos, densidade e produção de massa seca**

Dados relativos ao crescimento e produção de massa seca das forrageiras foram coletados em todo o período experimental. Os cortes do pasto foram feitos sempre que as forrageiras em condição hídrica adequada (irrigadas) atingiam altura recomendada para o pré-pastejo de 85 e 35 cm para os capins Zuri e Mavuno, respectivamente (Barbosa et al., 2021; Silva et al., 2020). Assim, foi realizado um corte na condição de sequeiro e cinco cortes no pasto irrigado. Imediatamente antes do corte do pasto era aferida a altura das plantas em cinco pontos por parcela, utilizando régua de manejo graduada.

A amostragem do pasto foi realizada entre 7 e 10 horas a.m., utilizando uma moldura com área de 2,25 e 1,0 m<sup>2</sup> nas parcelas dos capins Zuri e Mavuno, respectivamente, cortando o pasto contido nessa área utilizando uma tesoura de poda. A massa do estrato pastejável foi coletada, deixando uma altura de resíduo igual a 35 e 15 cm para Zuri e Mavuno, respectivamente. As amostras foram pesadas obtendo-se a massa fresca (MF), e retirou-se uma subamostra de 100 g de massa fresca, que foi acondicionada em saco de papel para secagem em estufa de circulação forçada de ar a  $65 \pm 5$  °C por 72 h. Com os teores de MS e a altura do pasto calculou-se a produção de massa de forragem (MF, Mg ha<sup>-1</sup> de MS), a densidade da massa de forragem (DMF, kg ha<sup>-1</sup> cm<sup>-1</sup> de MS). Após cada coleta de

biomassa fez-se a contagem dos perfilhos (vivos e mortos) contidos dentro de uma moldura de 635 cm<sup>2</sup> fixada em cada parcela (Bahmani et al., 2003), estimando por diferença o saldo de perfilhos vivos por metro quadrado.

No mês de setembro de 2023, no pico da estação seca foram analisadas as seguintes variáveis:

### **Conteúdo volumétrico de água no solo ( $\theta$ , cm<sup>3</sup> cm<sup>-3</sup>)**

O conteúdo volumétrico de água no solo foi determinado pelo método indireto do Refletometria no Domínio do Tempo (*Time Domain Reflectometry* - TDR) utilizando um sensor portátil de umidade do solo (HydroSence II), com hastes de 20 cm. As aferições foram realizadas no pico da estação seca no mês de setembro de 2023, realizando a sondagem em três pontos por parcela. A precisão da estimativa por esse sensor foi considerada adequada, com  $R^2 = 0.84$  ( $y = 1,0294x - 3,2562$ ) e desvio padrão de 1%, obtida ao comparar os dados do sensor com a umidade de amostras do solo coletadas nos mesmos pontos da aferição indireta (Gonzalez-Porras et al., 2024).

### **Potencial hídrico foliar ( $\Psi_w$ , MPa) e Conteúdo relativo de água na folha (CRA, %)**

Foi determinado o  $\Psi_w$  utilizando uma câmara de pressão Scholander (3000F01, Soil Moisture Equipment) utilizando o terço médio da última folha completamente desenvolvida (com lígula exposta). A avaliação foi realizada no mês de setembro, entre 3h e 5h a.m., inserindo o segmento da folha na câmara, e logo aplicando pressão crescente até observar pela parte externa da câmara a exsudação pela nervura central (Turner, 1981).

Para análise do CRA na folha foram coletadas amostras frescas (dez discos de 129 mm<sup>2</sup>) da última folha completamente desenvolvida, que foram pesados para obter sua massa fresca (MF). As amostras foram reidratadas em água deionizada por 6 h, e novamente pesadas para obter a massa túrgida (MT). O excesso de água foi retirado em papel toalha. Em seguida os discos foliares foram secos em estufa com circulação forçada de ar (TE-394/3-MP, Tecnal) a 80 °C por 24 h, obtendo-se a MS. Os valores de CRA foram obtidos pela equação:  $[(MF - MS)/(MT - MS)] \times 100$  (Barrs & Weatherley, 1962).

**Índice de firmeza foliar (N):** o índice de firmeza da folha foi medido com um penetrômetro digital com ponta de 8 mm, com capacidade para aplicar de 5 a 200 Newton  $\pm$  1 N (Impac, Modelo IP-90DI, São Paulo, SP, Brasil), seguindo o princípio metodológica de (Calbo et al., 2010). Foram realizadas seis aferições na porção média da última folha completamente desenvolvida de perfilho coletado aleatoriamente.

### **Estequiometria elementar na parte aérea das gramíneas**

Para analisar as razões estequiométricas C:N, C:P, N:P e C:Si na biomassa das forrageiras, primeiramente determinou-se os teores de C, N, P e silício (Si) na MS. Para os tratamentos irrigados a amostra utilizada foi composta pela reunião e homogeneização das amostras de todos os cortes realizados, e para os tratamentos em sequeiro foi retirada subamostra do material obtido no corte único das parcelas.

O teor de N foi obtido pelo método de Kjeldahl com determinação por titulação, utilizando extrato obtido por digestão sulfúrica da amostra (Bataglia et al., 1983). Determinou-se o teor de C por digestão em dicromato de potássio em ácido sulfúrico e titulação com sulfato ferroso amoniacal (Tedesco et al., 1995). O teor de P foi obtido após digestão nítrico-perclórica da amostra, seguida de reação colorimétrica com mistura de metavanadato e molibdato, e leitura do extrato em espectrofotômetro a 420 nm (Malavolta et al., 1997). Para determinar o Si, procedeu-se digestão alcalina da amostra em peróxido de hidrogênio a 120 °C, reação colorimétrica do extrato com molibdato de amônio, e leitura em espectrofotômetro a 410 nm (Kraska & Breitenbeck, 2010). Os teores dos elementos na massa seca foram expressos em g kg<sup>-1</sup>, e com eles foram calculadas as razões estequiométricas C:N, C:P, N:P e C:Si.

**Índice de extravasamento de eletrólitos:** coletou-se dez discos foliares (26 mm<sup>2</sup>) da última folha completamente desenvolvida no perfilho às 6h a.m. Os discos foram imediatamente levados ao laboratório em recipiente refrigerado, onde foram imersos em água deionizada por duas horas. Foi aferida a condutividade elétrica inicial da solução (CE<sub>1</sub>) utilizando um condutivímetro portátil (TSD-3). As amostras em água deionizada foram autoclavadas a 121 °C por 20 min, e após seu esfriamento, foi aferida a condutividade elétrica final da solução (CE<sub>2</sub>). O índice de

vazamento de eletrólitos foi calculado pela fórmula:  $CE_1 / CE_2 \times 100$  (Dionisio-Sese & Tobita, 1998).

**Compostos fenólicos totais:** utilizou-se 0,1 g de massa fresca da última folha completamente desenvolvida do perfilho das forrageiras. Após pesada, a amostra vegetal foi submetida à extração em metanol por 3h. Procedeu-se então uma reação colorimétrica com reagente Folin-Ciocalteu 2 N por 3 minutos, seguida da adição de carbonato de sódio 20%. Após 2 h a absorbância do extrato foi aferida em espectrofotômetro (B442, Micronal) a 765 nm (Singleton & Rossi, 1965). A concentração de fenóis totais foi obtida utilizando uma curva padrão com ácido gálico, e expressa como equivalente de ácido gálico por 100 gramas (GAE 100 g<sup>-1</sup>).

**Carboidratos solúveis e aminoácidos totais:** foi utilizado 1,0 g de material vegetal seco (massa do estrato pastejável) para extração em 10 mL de solução metanol-clorofórmio-água (MCA 60:25:15%) em tubos Falcon, que foram armazenados em geladeira por 48h. Retirou-se então 4 mL de sobrenadante no qual foi adicionado 1 mL de clorofórmio e 1,5 mL de água deionizada, levando à geladeira por 24h. Para determinar o teor de carboidratos solúveis totais, seguiu-se a reação de 0,5 mL de extrato com 0,5 mL de fenol 5% e 2 mL de ácido sulfúrico concentrado, e leitura em espectrofotômetro a 490 nm (DuBois et al., 1956). Na análise de aminoácidos, utilizou-se 1mL do extrato, adicionou-se 500 µL citrato de sódio 0.2 M, 200 µL solução ninhidrina 5% em éter monometílico de etilenoglicol, e 1 mL solução KCN 0,0002 M. O ensaio foi aquecido a 100 °C por 20 minutos e, em seguida resfriado por 10 minutos em água corrente. Adicionou-se 1 mL de álcool etílico 60%, seguindo-se leitura em espectrofotômetro a 570 nm (Yemm et al., 1955).

**Pigmentos clorofila total, feofitina total, carotenoides e flavonoides:** coletou-se aleatoriamente discos com massa de 0,025 a 0,030 g do terço médio da última folha desenvolvida do perfilho. O material vegetal foi inserido em tubos Eppendorf, adicionando em seguida 1,5 mL de acetona 80%. Os tubos foram armazenados no escuro, e após a despigmentação completa do material vegetal (aproximadamente 48h), procedeu-se as leituras do extrato em espectrofotômetro (DU640, Beckman, EUA) nos comprimentos de onda de 470, 663, 647, 653 e 665 nm para

carotenoides, clorofila *a*, clorofila *b*, feofitina *a* e feofitina *b*, respectivamente (Lichtenthaler, 1987). Os conteúdos de pigmentos foram expressos em mg g<sup>-1</sup> de clorofila total e feofitina total com base na massa fresca.

**Fluorescência inicial (Fo) e máxima (Fm) da clorofila e eficiência quântica do fotossistema II (Fv/Fm):** foram aferidas na última folha completamente desenvolvida de perfilho escolhido aleatoriamente na parcela, utilizando um fluorômetro portátil (Os30P + Opti-Sciences) (Lichtenthaler et al., 2005). A avaliação foi realizada entre 8h e 10h am no mês de setembro de 2023. Um período de adaptação da porção da folha ao escuro de 30 minutos foi estabelecido, e então foram submetidas ao pulso de luz vermelha do aparelho.

**Temperatura foliar e trocas gasosas:** utilizou-se um analisador LI-1600 (Steady State Porometer) para aferir a temperatura foliar (°C), a transpiração (*E*, mmol m<sup>2</sup> s<sup>-1</sup> de H<sub>2</sub>O) e a condutância estomática (*G*<sub>s</sub>, mmol m<sup>2</sup> s<sup>-1</sup>) no terço médio da folha mais recentemente expandida de dois perfilhos escolhido aleatoriamente em cada parcela, entre 9 e 11h a.m. em condição natural do experimento.

#### **Análises microbiológicas e carbono orgânico do solo**

Amostras de solo rizosférico e não-rizosférico foram retiradas na camada de 0-10 cm do solo em cada parcela, no mês de outubro de 2023, ao final do período experimental. Uma touceira foi arrancada em cada parcela utilizando um enxadão (*hoe*), o solo fracamente aderido foi retirado, e coletou-se em uma bandeja plástica o solo rizosférico aderido às raízes. O solo não rizosférico foi coletado no local de origem da touceira. As amostras foram acondicionadas em sacos de papel e levadas ao laboratório, passadas em peneira (malha 2 mm), acondicionadas em sacos plásticos e conservadas a 4 °C até análise (Silva et al., 2007a).

**Carbono da biomassa microbiana (SMB-C, mg kg<sup>-1</sup>):** foi determinado seguindo o método da fumigação seguida de extração solução de sulfato de potássio e oxidação do carbono com dicromato de potássio, e titulação com sulfato ferroso amoniacal. O cálculo foi realizado com a equação:  $SMB-C = FC \times kc^{-1}$ , em que, SMB-C corresponde a miligramas de C microbiano por quilograma de solo; FC é fluxo obtido da diferença entre a quantidade de C da amostra fumigada e da não fumigada, e *kc* é o fator de correção (0,33) (Silva et al., 2007b).

**Respiração basal do solo (SBR, mg kg<sup>-1</sup> h<sup>-1</sup>):** foram utilizadas duas subamostras de solo, uma para determinação da umidade do solo, e outra para a respiração basal do solo (Jenkinson & Powlson, 1976; Silva et al., 2007a). Amostras de aproximadamente 50 g de solo úmido foram colocadas em frascos de vidro de 100 mL, e 10 mL da solução de NaOH 1 mol L<sup>-1</sup> foram colocados em frasco semelhante. Ambos foram colocados em recipientes de vidro de 2L que foram hermeticamente fechados e mantidos sob temperatura ambiente entre 25 e 28 °C no escuro por sete dias. Após a incubação, foram adicionados 2 mL de solução de cloreto de bário (10% m/v) no frasco contendo NaOH para precipitação do CO<sub>2</sub>, seguido de titulação com solução de ácido clorídrico padronizada (0,5 mol L<sup>-1</sup>) na presença de indicador fenolftaleína 1%.

Calculou-se a SBR pela equação:  $SBR = (((V_b - V_a) \times M \times 6 \times 1000) / P_s) / T$ , em que: RBS = mg de C-CO<sub>2</sub> liberado pela respiração microbiana por quilograma de solo por hora; V<sub>b</sub> (mL) = volume de ácido clorídrico gasto na titulação da solução controle (branco); V<sub>a</sub> (mL) = volume gasto na titulação da amostra; M = molaridade exata da solução de HCl; P<sub>s</sub> (g) = massa de solo seco e T= tempo de incubação da amostra em horas.

**Quociente metabólico (qCO<sub>2</sub>, mg g<sup>-1</sup> h<sup>-1</sup>):** com os dados obtidos das análises de C da biomassa microbiana e respiração basal do solo, calculou-se o qCO<sub>2</sub> como indicador da eficiência de uso do substrato pela microbiota, utilizando a equação:  $qCO_2 = SBR / (SMB-C \times 10^{-3})$ , na qual qCO<sub>2</sub> é o quociente metabólico em miligrama de C-CO<sub>2</sub> por grama de SMB-C por hora, SBR é a respiração basal do solo e SMB-C é o C da biomassa microbiana do solo (Anderson & Domsch, 1993; Silva et al., 2007b).

**Populações de grupos microbianos:** foi avaliada a densidade da população dos grupos microbianos bactérias esporulantes, leveduras, actinomicetos e microrganismos solubilizadores de fósforo no solo rizosférico e no solo não-rizosférico. Para a contagem das populações, 10g de solo foram pesadas e adicionadas a frascos tipo erlenmeyers contendo 95 mL de tampão pirofosfato de sódio (0,1%) estéril. Os frascos foram incubados por 30 minutos em agitação de 200 rpm a 28 °C para separação física dos agregados de solo.

A partir da solução obtida foram preparadas diluições seriadas, transferindo-se 1 mL para tubos de ensaio contendo 9 mL de tampão pirofosfato 0,1% sucessivamente, até formar a diluição que permitiu a contagem de 30 a 300 colônias por placa, quando plaqueado 0,1 mL em meio seletivo, utilizando-se sendo o meio nutriente-ágar para bactérias esporulantes, meio levedura-ágar-malte com antibióticos para leveduras, meio amido-caseína- ágar para actinomicetos (Wollum, 1982) e meio GES - glicose-extrato de solo e sais inorgânicos para solubilizadores de fosfato (Sylvester-Bradley et al., 1982). As placas foram incubadas em estufa BOD a  $28\text{ }^{\circ}\text{C} \pm 2^{\circ}\text{C}$ , em posição invertida e em duplicata, procedendo a contagem de unidades formadoras de colônia (UFC) após 72 horas para bactérias esporulantes, e após 120 a 168 horas para leveduras, actinomicetos e solubilizadores de fosfato (Vieira & Nahas, 2005).

**Carbono orgânico total do solo ( $\text{g kg}^{-1}$ ):** foi determinado empregando 1 g de amostra (solo não rizosférico) e oxidação por via úmida com mistura de dicromato de potássio e ácido sulfúrico. A solução de dicromato foi adicionada em excesso, de modo que na reação, parte do Cr foi reduzido, e outra parte permaneceu na forma oxidada. A sobra do reagente na forma oxidada foi medida por meio de titulação com solução de sulfato ferroso amoniacal e, a partir da quantidade adicionada e da sobra da reação, foi obtida a quantidade de Cr que oxidou carbono. Essa quantidade permitiu determinar, por cálculo, o teor de C do solo, em  $\text{g kg}^{-1}$ . O procedimento utilizado está baseado no método de Walkley & Black, descrito em (Raij et al., 2001).

### **3.4 Análises estatísticas**

Os dados foram avaliados por meio dos testes de normalidade, homogeneidade e independência dos resíduos do conjunto de dados (Bartlett, 1937; Jarque & Bera, 1980). Após, realizou-se a análise de variância, e as médias consideradas diferentes pelo teste F ( $p < 0,05$ ) foram comparadas pelo teste Tukey ( $p < 0,05$ ). O software estatístico utilizado foi o SPEED Stat versão 3.2 (Carvalho et al., 2020).

## 4. RESULTADOS E DISCUSSÃO

Os resultados referentes ao presente projeto de pesquisa desenvolvido, foram abordados e discutidos em dois artigos científicos, de maneira a explorar com maior profundidade cada aspecto abordado no projeto.

Os artigos intitulados “*Elemental homeostasis and soil microbiota underpin forage grass resilience to drought and productivity gains under subsurface drip irrigation*” e “*Physiological and morphological traits drive differential drought tolerance and irrigation response in tropical forage grasses under field conditions*” estão sob avaliação nos periódicos Scientific Reports e Grass and Forage Science, respectivamente.

Portanto, os resultados do projeto estão contemplados nos artigos conforme descrito a seguir, cujo texto encontra-se sob as normas e formatações exigidas pelos periódicos aos quais foram submetidos.

### 4.1 ARTIGO 1: ELEMENTAL HOMEOSTASIS AND SOIL MICROBIOTA UNDERPIN FORAGE GRASS RESILIENCE TO DROUGHT AND PRODUCTIVITY GAINS UNDER SUBSURFACE DRIP IRRIGATION

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**Abstract:** Perennial forage systems in tropical regions are predominantly rainfed, yet increasing drought frequency due to climate change is driving irrigation adoption. Selecting species with stable elemental composition and beneficial plant-soil interactions is key to sustaining productivity. Was evaluated the drought tolerance of *Zuri* guineagrass and *Mavuno* grass under tropical field conditions, focusing on their responses to subsurface drip irrigation, elemental stoichiometry (C:N:P), and soil microbiota. Under dry conditions, both species showed yield reductions, but *Mavuno* maintained elemental homeostasis and enhanced microbial activity, indicating greater drought resilience. Irrigation improved C:N, C:P, and N:P ratios and increased the abundance of key microbial groups, including sporulating bacteria, yeasts, and actinomycetes. These changes contributed to higher soil carbon, especially in *Mavuno* pastures. Forage yield increased by ~10 Mg ha<sup>-1</sup> of dry matter with irrigation. Notably, *Zuri* under irrigation contributed more to soil microbial biomass and total soil carbon, suggesting potential for long-term sustainability. Our results highlight species-specific drought responses and the role of irrigation in modifying plant-soil interactions and nutrient stoichiometry. Understanding these dynamics is essential to enhance the resilience and productivity of tropical forage systems under future climate scenarios.

**Keywords:** irrigated pasture, *Megathyrsus* spp., plant nutrition, soil microbiota, subsurface drip irrigation, *Urochloa* spp., water deficit

## 1. Introduction

Tropical forage grasses from different species are widely cultivated in pastures to enhance livestock production efficiency [1]. However, water deficits caused by annual agricultural drought in tropical regions with dry winters result in seasonal forage production, concentrated during the rainy season [2, 3]. In addition to limiting forage yield, drought alters soil microbial communities [4], with plant species influencing these interactions, as observed for forage legumes [5, 6]. While grass cultivation generally enhances microbial abundance in soil [7], the effects of tropical forage grass species on soil microbiota under drought remain underexplored, and it is unclear how these microbial communities contribute to plant drought tolerance under field conditions.

Water deficits negatively affect plants by reducing leaf water potential and transpiration [8], and by impairing nutrient uptake and disrupting stoichiometric balance among carbon (C), nitrogen (N), and phosphorus (P) in leaf tissues [9]. Although C:N:P homeostasis is essential for efficient nutrient assimilation and dry matter production, it is frequently disturbed by abiotic stress [10, 11], which can impair plant metabolism and reduce the availability of organic substrates for soil microorganisms [12]. Despite its relevance, most studies assessing pasture performance under different soil water regimes do not consider elemental stoichiometric homeostasis, which is key to understanding biomass responses. In parallel, soil microbial activity and biomass are valuable indicators of environmental stress and management practices in pasture systems [13], both of which influence plant nutrient dynamics.

To mitigate the adverse effects of drought on forage production, irrigation systems combined with maintenance fertilization have been widely adopted [14]. Among these, subsurface drip irrigation has been recommended as a more efficient system [15], minimizing water loss through evaporation and runoff compared to sprinkler systems, albeit at higher initial cost [16, 17]. Therefore, it is important to evaluate the performance of newer cultivars, such as *Megathyrsus maximus* cv.

Zuri and *Urochloa brizantha* × *U. ruziziensis* cv. Mavuno, under this irrigation strategy.

As with other agronomic practices, irrigation can influence the activity and composition of soil microbial groups [18, 19]. Since soil organic matter (SOM) accumulation is largely driven by root inputs and microbial biomass [20], well-managed irrigation may also enhance soil C stocks, although microbial respiration may initially increase [21]. The integration of species adapted to both dryland and irrigated systems represents a sustainable alternative, yet further research is required to elucidate the mechanisms governing plant-microbe interactions, their impact on nutrient homeostasis, and subsequent effects on forage yield.

Based on these considerations, this study tested the following hypotheses: (i) the tolerance of different grass species to dryland cultivation is influenced by C:N:P homeostasis and soil microbiota; (ii) grass species induce differences in microbial biomass, activity, and composition in soil under dryland and irrigated conditions; and (iii) subsurface drip irrigation mitigates drought stress on forage grasses and soil microbiota. Thus, the objective of this study was to understand the drought tolerance of forage grasses during the dry season, their responsiveness to irrigation, and their impact on soil microbiota in dryland and irrigated pastures. The findings aim to support more accurate grass species selection based on water availability, while highlighting the potential of drip irrigation for improving the sustainability of pasture systems.

If these hypotheses are validated, the interplay between soil microbiota and plant nutritional homeostasis will be better understood, enabling the identification of forage species better suited to diverse pasture management systems at minimal cost. This would ultimately support the sustainability of grass-based pasturelands across tropical regions.

## **2. Material and methods**

### **2.1 Experimental Conditions**

A field experiment was conducted at the experimental site of São Paulo State University (UNESP), in Jaboticabal, Brazil (21°14'54" S, 48°17'06" W; 560 m elevation). The region has a tropical wet climate with a dry winter (Aw, Köppen classification). The study was carried out from April to October 2023, encompassing

the dry season. Meteorological data, including evapotranspiration, air temperature, global solar radiation, and rainfall, were recorded at the university's weather station (Fig. 1a, b).

The soil was classified as Oxisol [22]. It was sampled for initial chemical analysis [23], yielding the following results for the 0-20 and 20-40 cm soil layers, respectively: pH = 5.8 and 6.0, organic matter (SOM) = 22 and 22 g dm<sup>-3</sup>, phosphorus (P) = 78 and 51 mg dm<sup>-3</sup>, potassium (K) = 5.5 and 3.6, calcium (Ca) = 46 and 44, magnesium (Mg) = 15 and 15 mmolc dm<sup>-3</sup>, sulfur (S) = 3 and 4, boron (B) = 0.32 and 0.25, copper (Cu) = 3.4 and 3.5, iron (Fe) = 6 and 5, manganese (Mn) = 25.5 and 18.6, zinc (Zn) = 1.6 and 1.0 mg dm<sup>-3</sup>, H+Al = 21 and 18 mmolc dm<sup>-3</sup>, cation exchange capacity (CEC) = 88 and 81 mmolc dm<sup>-3</sup>, base saturation ( $V = \text{Ca} + \text{Mg} + \text{K} / \text{CEC}$ ) = 76 and 77%.

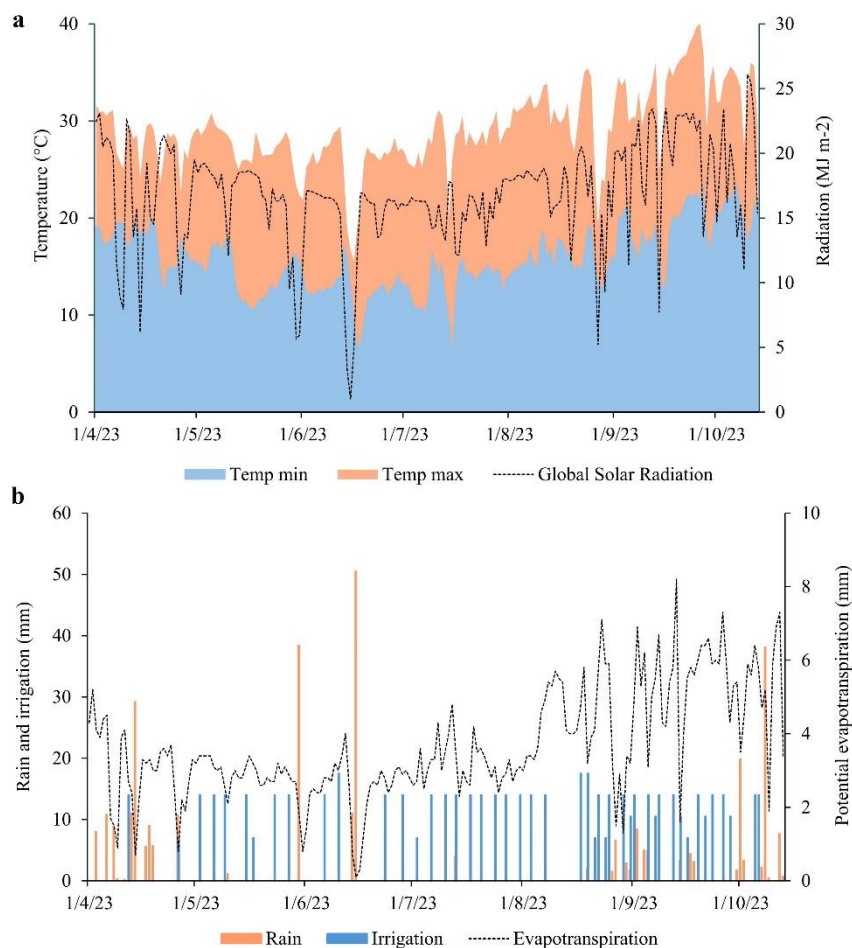


Figure 1. Meteorological data. a) Meteorological data on air temperature, solar radiation and b) precipitation, irrigation, and evapotranspiration during the experimental period.

## 2.2 Experimental design and management

The experiment followed a  $2 \times 2$  factorial design comprising two tropical forage grass species *U. brizantha*  $\times$  *U. ruziziensis* cv. Mavuno and *M. maximus* cv. Zuri, grown under two water management conditions: dryland (rainfed) and irrigated. Treatments were arranged in a randomized block design with four replicates. In the irrigated treatments, a subsurface drip irrigation system was installed in April 2022 following soil preparation by harrowing. Drip lines were buried at a depth of 20 cm [15] and spaced 80 cm apart. Emitters had a nominal discharge of  $1.45 \text{ L h}^{-1}$  at 100 kPa and were positioned at 30 cm intervals. Preliminary testing confirmed uniform water distribution [24].

Experimental plots measured  $3 \times 4.8 \text{ m}$ . Forage grasses were sown manually at a rate of  $10 \text{ kg ha}^{-1}$  of pure viable seed, distributed evenly across the soil surface. The forage grass seeds were donated by the commercial supplier WolfSeeds. After seedling emergence, a uniformity cut was performed to stimulate tillering using a brush cutter (Husqvarna 31R/330-2), and the removed biomass was discarded. Sprinkler irrigation was applied during the establishment phase to ensure uniform germination and pasture development.

In April 2023, once the pasture was fully established, plots were standardized with a maintenance cut, leaving a residual height of 15 cm for Mavuno and 30 cm for Zuri [25, 26]. Water treatments were then implemented: either rainfed (no irrigation) or irrigated with subsurface drip. For irrigated plots, irrigation depth and frequency were calculated to fully replenish crop evapotranspiration, with a maximum allowable soil water deficit of 22 mm. This was based on the soil's water holding capacity (120 mm in the 0-40 cm layer), as determined from the soil water retention curve [27].

Given that initial soil fertility was adequate for grass production, only maintenance fertilization was conducted after each cut. Nutrient replacement rates for N, P, K, and S were based on the quantities exported by the forage biomass under each treatment [28, 29]. Fertilizer sources included urea, potassium chloride, ammonium sulfate, and purified monoammonium phosphate (for fertigation), while simple superphosphate was used under rainfed conditions. Fertilizers were applied through fertigation in irrigated plots and broadcast manually in rainfed plots.

## **2.3 Variables analyzed**

### **2.3.1 Forage sampling and data collection**

Data were collected from April to October 2023. Pasture cuts and biomass sampling were performed when grasses under optimal water supply (irrigated plots) reached the recommended pre-grazing heights: 85 cm for Zuri guineagrass and 35 cm for Mavuno grass [25, 26]. Plant height (cm) was recorded immediately prior to cutting at five points per plot using a graduated pasture ruler. Based on this criterion, five harvests were conducted in irrigated plots, whereas only one harvest was possible under rainfed conditions due to limited growth.

To assess dry matter (DM) yield, forage was harvested between 7 and 9 a.m. using a sickle, leaving a post-harvest residue height of 35 cm for Zuri and 15 cm for Mavuno. Forage was collected within metal quadrats of 2.25 m<sup>2</sup> for Zuri and 1.0 m<sup>2</sup> for Mavuno, placed within each plot. Fresh biomass was weighed immediately, and a subsample (~100 g) was oven-dried at 65 ± 5 °C for 72 hours in a forced-air oven to determine DM yield (Mg ha<sup>-1</sup>). After each cut, tiller density (tillers m<sup>-2</sup>) was estimated by counting both live and dead tillers within a 635 cm<sup>2</sup> frame [30]. Forage mass density (kg ha<sup>-1</sup> cm<sup>-1</sup> DM) was then calculated by dividing the DM yield by pasture height.

### **2.3.2 Soil moisture**

Volumetric soil water content ( $\theta$ , cm<sup>3</sup> cm<sup>-3</sup>) was measured at the peak of the dry season in September 2023 using Time Domain Reflectometry (TDR) with a portable soil moisture sensor (HydroSense II, 20 cm rods). Measurements were taken at three locations per plot. Sensor accuracy was confirmed by calibration against gravimetric soil moisture at the same sampling points, yielding an R<sup>2</sup> of 0.84 ( $y = 1.0294x - 3.2562$ ) and a standard deviation of 1% [31].

### **2.3.3 Leaf water potential and relative water content**

Leaf water potential ( $\Psi_w$ , MPa) was measured between 3 and 5 a.m. in September using a Scholander-type pressure chamber (Model 3000F01, Soil Moisture Equipment). Measurements were taken from the middle third of the most recently expanded leaf (ligule fully emerged) by gradually increasing chamber pressure until sap exudation from the leaf midrib was observed [32].

Relative water content (RWC, %) was determined from ten leaf discs (129 mm<sup>2</sup> each) collected from the same leaf. Fresh mass (FM) was recorded immediately, followed by 6 hours of rehydration in deionized water to obtain turgid mass (TM). Discs were then oven-dried at 80 °C for 24 hours to determine dry mass (DM). RWC was calculated as:  $RWC = [(FM - DM) / (TM - DM)] \times 100$  [33].

#### **2.3.4 Elemental stoichiometry in leaf of forage grasses**

To evaluate the C:N, C:P, N:P, and C:Si stoichiometric ratios in forage biomass, concentrations of carbon (C), nitrogen (N), phosphorus (P), and silicon (Si) in dry matter (DM) were first determined. For irrigated treatments, samples were pooled and homogenized from all collection dates. For rainfed treatments, a subsample was taken from the single cut, as described in Section 2.3.1.

Nitrogen content was determined by the Kjeldahl method with titration following sulfuric acid digestion [34]. Carbon content was assessed via oxidation with potassium dichromate in sulfuric acid and titration with ammonium ferrous sulfate [35]. Phosphorus was determined after nitric-perchloric digestion, followed by a colorimetric reaction with a metavanadate-molybdate reagent, and absorbance was measured at 420 nm using a spectrophotometer [36]. For Si, alkaline digestion was carried out with hydrogen peroxide at 120 °C, followed by a colorimetric reaction with ammonium molybdate and absorbance reading at 410 nm [37]. Elemental concentrations in DM were expressed in g kg<sup>-1</sup>, and the respective stoichiometric ratios were then calculated.

#### **2.3.5 Microbiological and soil organic carbon analyses**

Soil samples were collected from the 0–10 cm layer in each plot in October 2023, at the end of the experimental period. A clump of soil and roots was excavated using a hoe, and loosely attached soil was gently removed. Rhizosphere soil was defined as that remaining adhered to the root surface and was carefully collected in a plastic tray. Non-rhizosphere soil was sampled from the original clump location. All samples were transferred to paper bags for transport, then sieved through a 2 mm mesh, placed into plastic bags, and stored at 4 °C until analysis [38].

**Microbial biomass carbon (SMB-C)** Microbial biomass carbon (SMB-C) was quantified using the fumigation–extraction method with 0.5 mol L<sup>-1</sup> potassium sulfate (K<sub>2</sub>SO<sub>4</sub>) as the extractant. Extracted organic carbon was oxidized with

potassium dichromate ( $K_2Cr_2O_7$ ) in acidic medium and quantified by titration with ammonium ferrous sulfate. SMB-C was calculated using the equation:  $SMB-C = FC \times kc^{-1}$ , where SMB-C is microbial C in  $mg\ kg^{-1}$  soil; FC is the flux from the difference between the fumigated and non-fumigated samples, and kc is the correction factor (0.33) [39].

**Soil basal respiration (SBR)** was measured using two soil subsamples: one for gravimetric moisture determination and another for  $CO_2$  evolution [40, 38]. Approximately 50 g of moist soil was placed into a 100 mL glass jar, which was enclosed in a 2 L hermetically sealed glass container along with a second jar containing 10 mL of  $1\ mol\ L^{-1}$  NaOH solution. Incubation was carried out in the dark at ambient temperature ( $25-28\ ^\circ C$ ) for seven days. After incubation, 2 mL of 10% (w/v) barium chloride ( $BaCl_2$ ) solution was added to the NaOH to precipitate  $CO_2$ . The remaining NaOH was titrated with standardized  $0.5\ mol\ L^{-1}$  HCl using 1% phenolphthalein as an indicator. SBR was calculated using the equation:  $SBR\ (mg\ kg^{-1}\ h^{-1}) = (((V_b - V_a) \times M \times 6 \times 1000) / P_s) / T$ , where: SBR = mg C- $CO_2$  released by microbial respiration per kg of soil per hour;  $V_b$  (mL) = volume of acid used in the titration of the control (blank) solution;  $V_a$  (mL) = volume used in the titration of the sample; M = exact molarity of the HCl solution;  $P_s$  (g) = mass of dry soil, and T = incubation time of the sample in hours.

**Metabolic quotient ( $qCO_2$ )** was calculated using the equation:  $qCO_2\ (mg\ g^{-1}\ h^{-1}) = SBR / (SMB-C \times 10^{-3})$ , where  $qCO_2$  is the metabolic quotient in mg of C- $CO_2$  per gram of SMB-C per hour, SBR is the basal soil respiration, and SMB-C is the microbial biomass carbon [41, 39].

**Microbial population:** Microbial populations were quantified for sporulating bacteria, yeasts, actinomycetes, and phosphorus-solubilizing microorganisms in both rhizosphere and non-rhizosphere soils. For microbial enumeration, 10 g of soil were added to 95 mL Erlenmeyer flasks containing sterile 0.1% sodium pyrophosphate buffer. The flasks were incubated for 30 minutes at 200 rpm and  $28\ ^\circ C$  to facilitate disaggregation of soil particles. Serial dilutions were prepared by transferring 1 mL of the suspension to test tubes containing 9 mL of the same buffer, continuing until suitable dilutions were obtained for plate counts (30–300 CFU per plate). Inoculated plates were incubated at  $28 \pm 2\ ^\circ C$ . Colony-forming units (CFU)

were recorded after 72 hours for sporulating bacteria, and between 120 and 168 hours for yeasts, actinomycetes, and phosphorus-solubilizing microorganisms [42].

**Total Organic Carbon in Soil:** It was determined by using 1 g of sample (non-rhizosphere soil) and oxidizing it through wet digestion with a potassium dichromate and sulfuric acid mixture. The dichromate solution was added in excess, so that during the reaction, part of the Cr was reduced, while another part remained in its oxidized form. The remaining reagent in the oxidized form was measured by titration with an ammonium ferrous sulfate solution. From the amount added and the excess reagent after the reaction, the amount of Cr that oxidized carbon was determined. This allowed for the calculation of the soil C content ( $\text{g kg}^{-1}$ ). The procedure followed is based on the Walkley and Black method, as described by [23].

## 2.4 Statistical Analysis

An analysis of data normality assumptions, homogeneity of variances, and independence of residuals was performed [43, 44], followed by analysis of variance (F-test,  $p < 0.05$ ). For significant F, means were compared using Tukey test ( $p < 0.05$ ) with SPEED Stat software version 3.4 [45]. A Pearson correlation network among the variables was established, and principal component analysis was conducted based on the covariance matrix, overlapped by hierarchical clustering analysis using Euclidean distance and a cluster analysis by the single linkage method [46]. All analyses were performed using R Studio version 4.3.3.

## 3. Results

### 3.1 Soil and plant water content, and elemental stoichiometry

The results showed an interaction effect between water conditions and forage species ( $p < 0.05$ ) for leaf water potential (Fig. 2 a-d) and for the C:P and N:P stoichiometric ratios in the biomass of the grazed stratum (Fig. 1 e-h). Irrigated cultivation, in comparison to dryland conditions, increased the volumetric water content (VWC), which was reflected in higher relative water content (RWC) and increased leaf  $\Psi_w$  for both Mavuno (87%) and Zuri (92%). Under dryland conditions, Zuri grass exhibited lower VWC and leaf  $\Psi_w$  compared to Mavuno, whereas no differences were observed between the species under irrigated

conditions for these variables. When compared within each water condition, the species did not differ in terms of leaf relative water content (Fig. 2 a-d).

In the comparison between species, Zuri grass showed a higher organic carbon content than Mavuno under dryland (+10%) and irrigated (+6%) conditions. Irrigation promoted an increase in soil organic carbon content in the Mavuno grass, compared to dryland conditions (Fig. 2 b). Under dryland conditions, Mavuno grass exhibited higher C:P, N:P, and C:Si ratios compared to Zuri grass, while under irrigation, no differences were found between species for elemental stoichiometry (Fig. 2 e-h).

Volumetric soil water content (VWC) (a), soil organic carbon content (b), relative water content in the leaf (RWC) (c), leaf water potential ( $\Psi_w$ ) (d), and stoichiometric ratios C:N, C:P, N:P, and C:Si (e, f, g, h) in the grazing stratum biomass of Mavuno grass and Zuri grass under dryland or irrigated conditions.

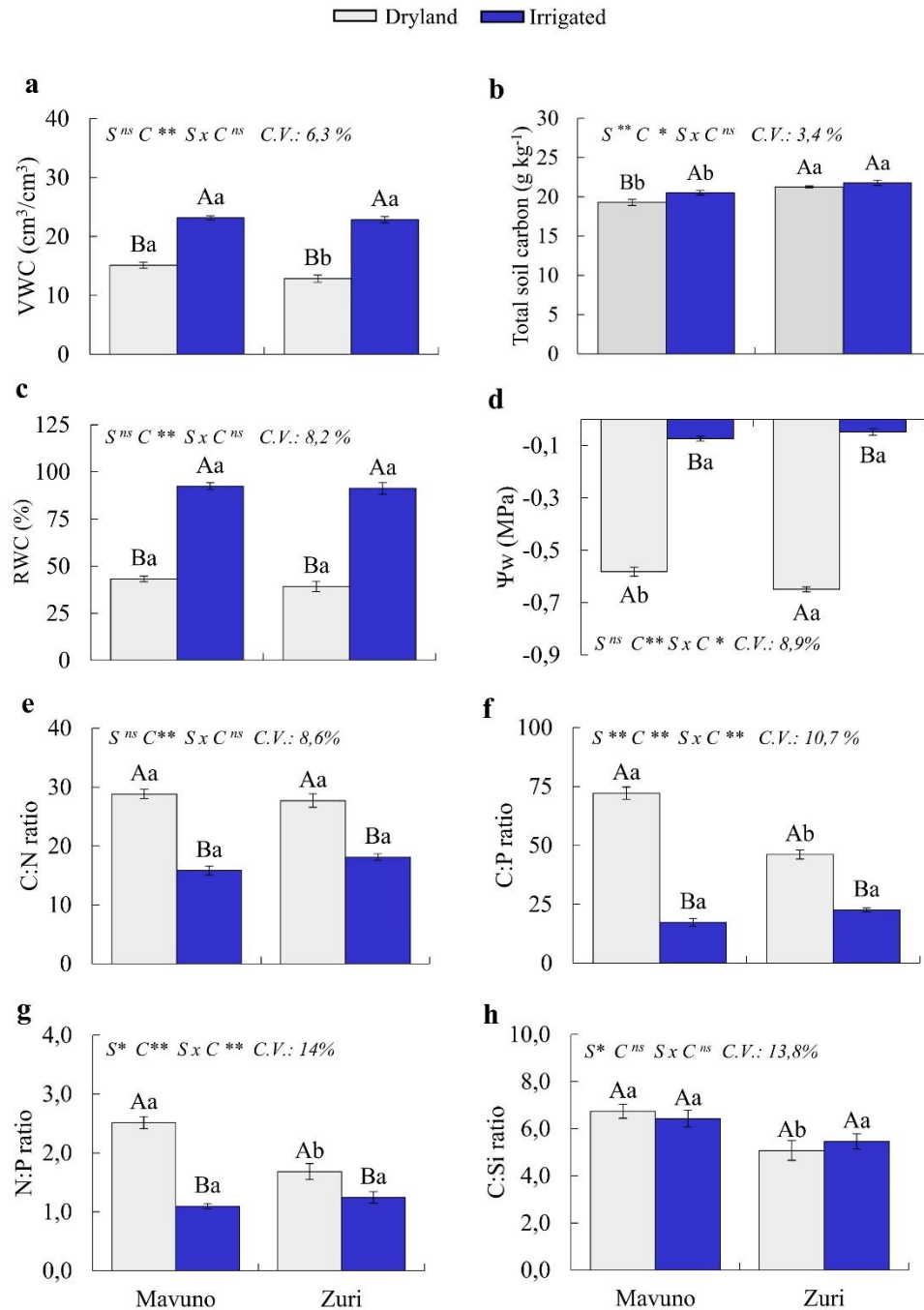


Figure 2. Volumetric soil water content (VWC) (a), total soil organic carbon content (b), relative water content in the leaf (RWC) (c), leaf water potential ( $\Psi_w$ ) (d), and stoichiometric ratios C:N, C:P, N:P, and C:Si (e, f, g, h) in the grazing stratum biomass of Mavuno grass and Zuri grass under dryland or irrigated conditions. Uppercase letters compare water conditions for each species, and lowercase letters compare species under each water condition according to the Tukey test ( $p < 0.05$ ),  $n = 4$ .

### 3.2 Biomass and microbial activity in the soil

Interaction effects were observed between water regimes and forage species ( $p < 0.05$ ) for the variables SMBC, SBR, and  $qCO_2$  in both rhizosphere and non-rhizosphere soils, except for basal respiration in rhizosphere soil (Fig. 3 a-f), where no interaction between the factors was found. When comparing water regimes, irrigation increased SMBC in Mavuno grass cultivation (49% in rhizosphere soil) and Zuri grass cultivation (124% in rhizosphere soil and 21% in non-rhizosphere soil) (Fig. 3 a, d). Irrigated conditions, compared to dryland, increased SBR in rhizosphere soil under both forage species (Fig. 3 b, e), and reduced  $qCO_2$  in the soil microbiota of Zuri grass pasture, both in the rhizosphere (-65%) and non-rhizosphere (-52%) zones (Fig. 3 c, f).

Differences between species under dryland conditions were observed, such as a higher SBR in Mavuno grass soil compared to Zuri in both the rhizosphere and non-rhizosphere zones (Fig. 3 b, e). In irrigated pasture, Zuri grass soil exhibited higher SMBC in both the rhizosphere (36%) and non-rhizosphere (27%) zones, as well as lower SBR (-16%) and  $qCO_2$  (-52%) in the rhizosphere compared to Mavuno grass under the same conditions (Fig. 3 a-f).

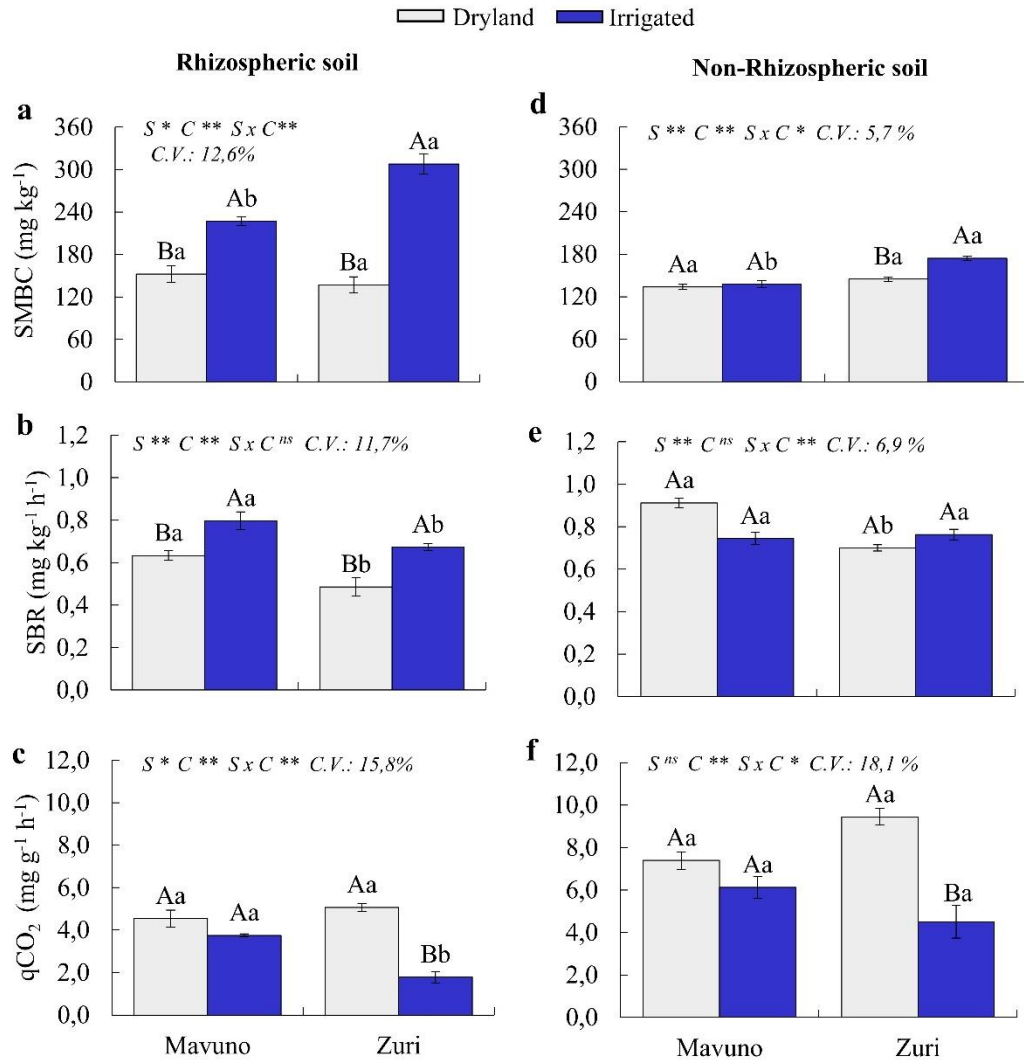


Figure 3. Soil microbial biomass carbon (SMBC), soil basal respiration (SBR), and microbial metabolic quotient (qCO<sub>2</sub>) in rhizosphere soil (a, b, c) and non-rhizosphere soil (d, e, f) cultivated with Mavuno grass and Zuri grass under dryland or irrigated conditions. Upper-case letters compare water regimes within each species, while lower-case letters compare species within each water regime, according to Tukey test (p<0.05), n=4.

### 3.3 Microbial community in soil

The interaction between water regime and forage species was significant (p<0.05) for most microbial groups analyzed, including total bacteria (SBac) and phosphate-solubilizing microorganisms (P-solub) in both rhizosphere and non-rhizosphere soil, as well as actinomycetes in non-rhizosphere soil (Fig. 4a-h).

Compared to dryland conditions, irrigation increased SBac counts in Zuri grass rhizosphere soil (+424%) and in Mavuno grass non-rhizosphere soil (+30%), while reducing SBac in Zuri grass non-rhizosphere soil (-51%) (Fig. 4a, e). Irrigation

also increased yeast counts in non-rhizosphere soil cultivated with Mavuno grass (+35%) and Zuri grass (+41%), with no effect in rhizosphere soil (Fig. 4b, f). Actinomycete counts increased by 71% in irrigated Mavuno grass compared to dryland conditions in non-rhizosphere soil (Fig. 4c, g). Similarly, irrigation enhanced P-solub counts in rhizosphere soil cultivated with Mavuno grass compared to dryland conditions (Fig. 4d, h).

Regarding species effects, yeast counts did not differ between forage species regardless of water regime. Under dryland conditions, SBac counts were higher in Mavuno grass rhizosphere soil (+60%) compared to Zuri grass, whereas in non-rhizosphere soil, SBac counts were lower in Mavuno than in Zuri (-21%). In non-rhizosphere soil, actinomycete counts were 54% higher in Zuri grass than in Mavuno grass under dryland conditions (Fig. 4a-g). In dryland conditions, P-solub counts were higher in Zuri grass rhizosphere soil (+102%) but lower in non-rhizosphere soil (-15%) compared to Mavuno grass. Under irrigation, SBac counts were higher in Zuri grass rhizosphere soil (+125%) but lower in non-rhizosphere soil (-52%) compared to Mavuno grass (Fig. 4a, e). In rhizosphere soil, P-solub counts were higher in Mavuno grass than in Zuri a grass (Fig. 4d, h).

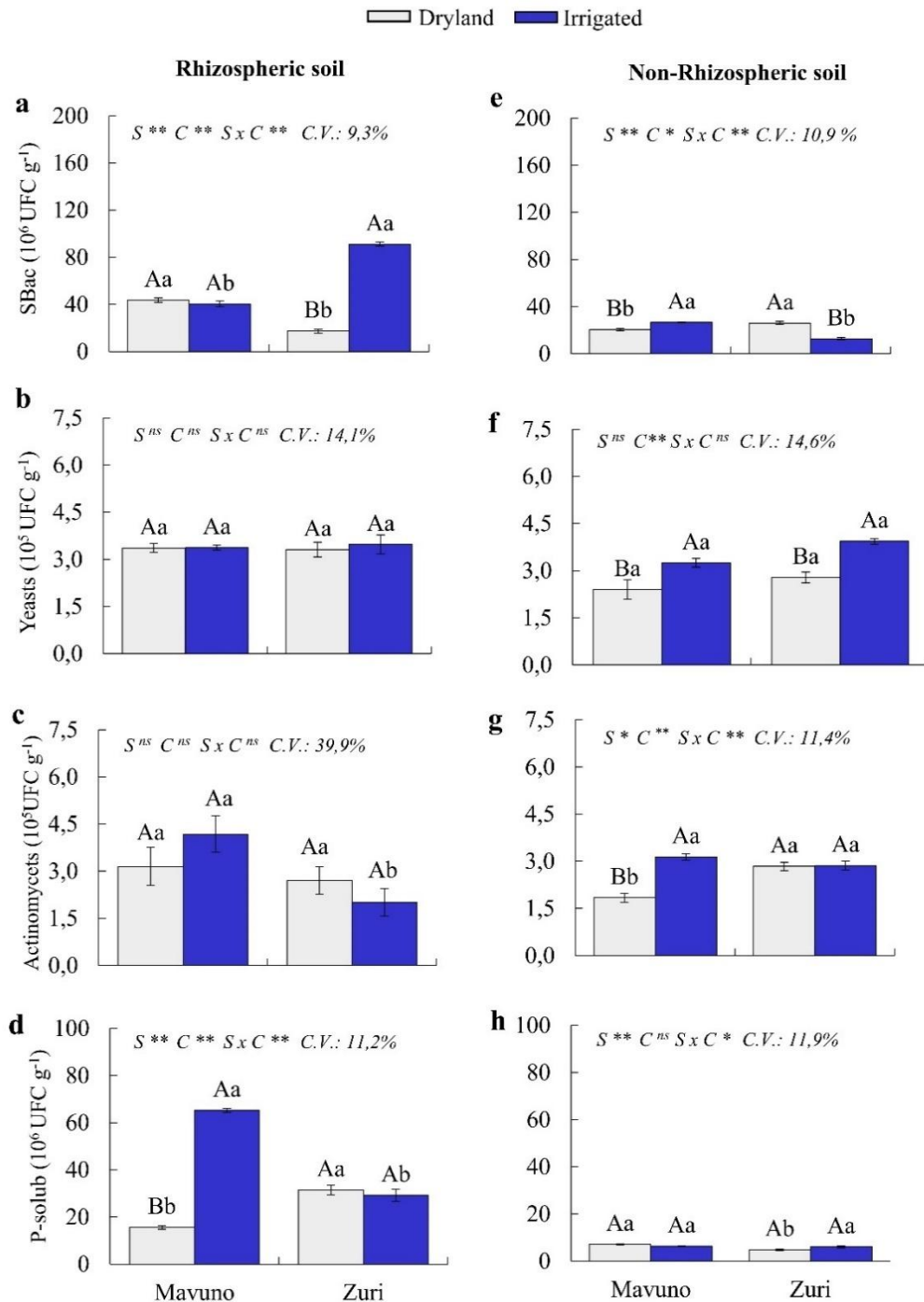


Figure 4. Counts of sporulating bacteria (SBac), yeasts, actinomycetes, and phosphate-solubilizing microorganisms (P-solub) in rhizosphere soil (a, b, c, d) and non-rhizosphere soil (e, f, g, h) cultivated with Mavuno grass and Zuri grass under dryland or irrigated conditions. Upper case letters compare water conditions within each species, and lower case letters compare species within each water condition using Tukey test ( $p < 0.05$ ),  $n = 4$ .

### 3.4 Effects of water condition and forage species on biomass production

Dry matter (DM) production was influenced independently by water condition and forage species, whereas canopy height, pasture density, and tiller number exhibited significant interactions between the analyzed factors ( $p < 0.05$ ) (Fig. 5a-d).

Irrigated cultivation of Mavuno grass, compared to dryland conditions, increased canopy height by 35% and tiller number by 147%, while simultaneously reducing pasture density by 23%. Under irrigation, Mavuno grass produced 11.7 t ha<sup>-1</sup> of DM, a gain of 10.3 t ha<sup>-1</sup> compared to dryland conditions (Fig. 5a-d). For Zuri grass, irrigation increased canopy height by 79% and tiller number by 94% compared to dryland conditions, without affecting pasture density. Irrigated Zuri grass produced 10.1 t ha<sup>-1</sup> of DM, representing an increase of 9.7 t ha<sup>-1</sup> compared to dryland conditions (Fig. 5a-d).

Under dryland conditions, Mavuno grass exhibited a higher canopy density and shorter height than Zuri grass, with no differences in tiller number between species. The dryland DM production was 1.4 and 0.4 t ha<sup>-1</sup> for Mavuno and Zuri grasses, respectively. Under irrigation, Mavuno and Zuri grasses produced 11.7 and 10.1 t ha<sup>-1</sup> of DM, respectively, with Mavuno grass showing greater density and tiller number (Fig. 5b, c).

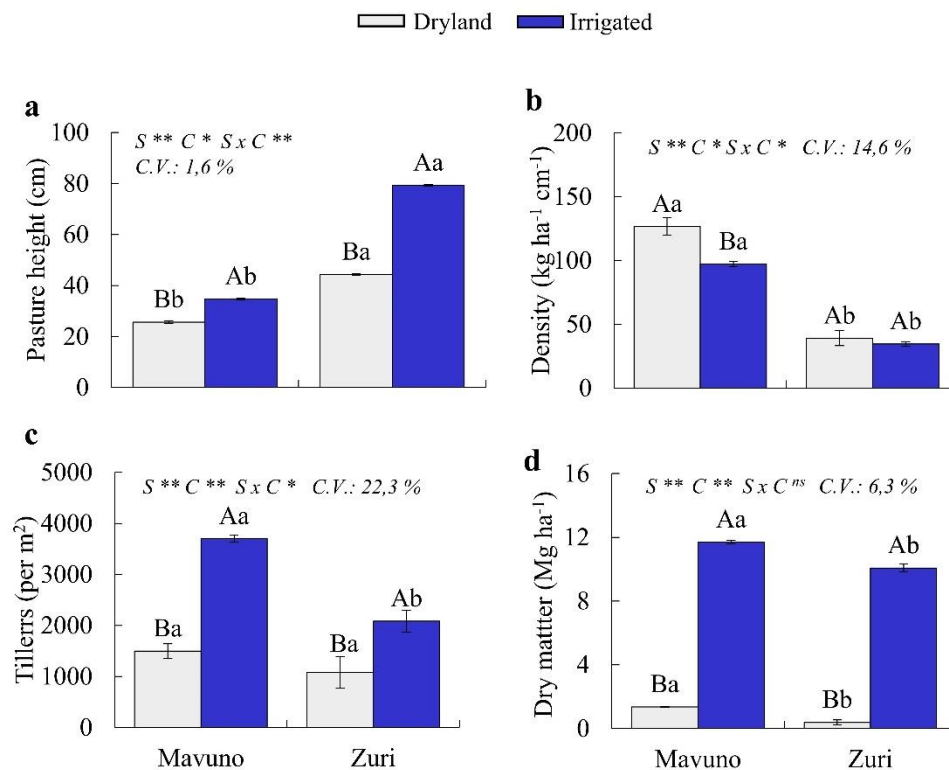


Figure 5. Canopy height (a), pasture density (b), tiller number per square meter (c), and forage dry matter production (d) of Mavuno grass and Zuri grass under dryland or irrigated conditions. Uppercase letters compare water conditions within each species, and lowercase letters compare species within each water condition using Tukey test ( $p < 0.05$ ),  $n = 4$ .

### 3.5 Correlation and Principal Component Analyses

Pearson correlation (Fig. 6a, c) revealed positive relationships between soil water content and leaf water potential ( $r = 0.85^{**}$ ) as well as dry mass ( $r = 0.93^{**}$ ). In the rhizosphere, P-solubilizing microorganisms were associated with the number of tillers ( $r = 0.66^{**}$ ) and dry mass ( $r = 0.62^{**}$ ), whereas this association was not observed in non-rhizosphere soil. In non-rhizosphere soil, a high C:N ratio was linked to lower microbial metabolic efficiency ( $r = 0.52^{**}$ ). Negative correlations were observed between soil water content and the C:N ratio ( $r = -0.90^{**}$ ), as well as between the C:N ratio and dry mass ( $r = -0.94^{**}$ ). The C:P and N:P ratios also showed a negative relationship with leaf water potential and dry mass. An increase in  $qCO_2$  reduced microbial biomass carbon both in the rhizosphere and in non-rhizosphere soil.

Principal component analysis (PCA) (Fig. 6b, d) explained 67.5% of the variance in rhizosphere soil and 61% in non-rhizosphere soil. The main variables contributing to this variation were microbial biomass carbon, dry mass production, P-solubilizing microorganisms, and the C:N ratio. Cluster analysis highlighted that spore-forming bacteria had a significant influence, particularly in treatments with irrigated Mavuno grass and rainfed Zuri grass. The variables spore-forming bacteria and the C:N ratio primarily contributed to the variance observed in the rainfed Zuri grass treatment but also influenced the rainfed Mavuno and irrigated Zuri treatments. This was indicated by the overlap between groups, suggesting the relevance of these variables across all treatments.

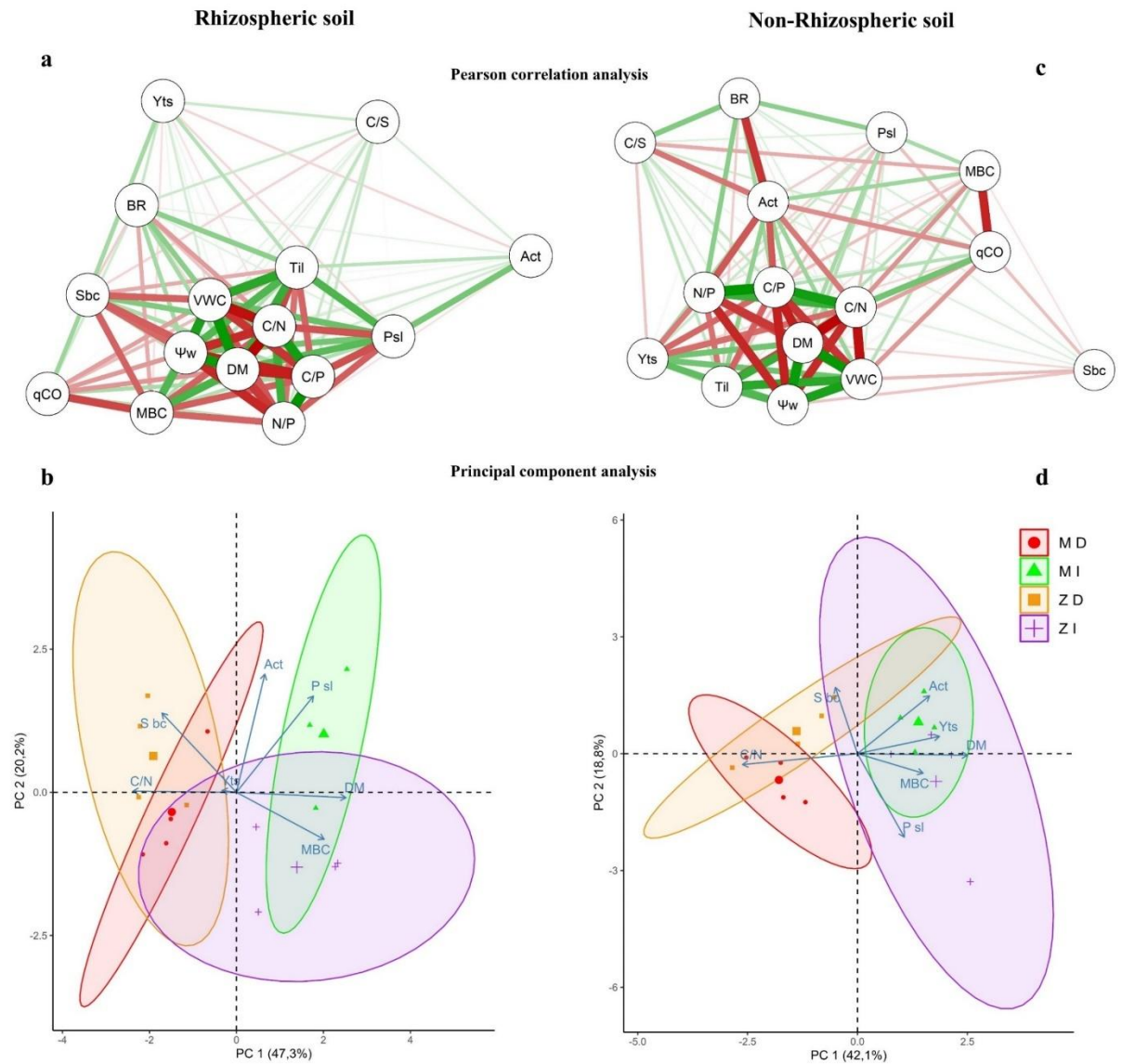


Figure 6. Pearson correlation network (a) and principal component analysis combined with cluster analysis using standardized data (b) among response variables studied in the field cultivation of Mavuno and Zuri grasses under rainfed and irrigated conditions. BR, soil basal respiration; qCO<sub>2</sub>, metabolic quotient; C/N, C/P, C/Si, N/P stoichiometry ratios in the biomass of the grazing stratum; VWC, volumetric soil water content;  $\Psi_w$ , leaf water potential; Til, number of tillers; DM, dry matter production; Sbc, sporulating bacteria; Act, actinomycetes; Psl, P-solubilizing; Yts, yeasts; MBC, microbial biomass carbon.

## 4. Discussion

### 4.1 Forage grass species and water availability influence the activity and composition of pasture soil microbiota

Forage grass cultivation predominates in pastures across tropical regions; however, field-based studies exploring the interaction between forage species and soil microbiota under dryland and irrigated conditions remain scarce. This study demonstrated that under dryland conditions, forage species distinctly influenced soil microbial parameters, as evidenced by the higher basal soil respiration observed under Mavuno grass compared to Zuri grass, in both rhizosphere and non-rhizosphere soils. The stimulatory effect of Mavuno grass on microbial activity is likely linked to its capacity to maintain higher leaf water potential, which promotes enhanced metabolism and greater exudation of labile carbon compounds by plants [47, 48]. In contrast, Zuri grass resulted in lower microbial respiration, consistent with the higher organic carbon content observed in soils cultivated with this species under dryland conditions.

The analysis of dryland effects on pasture soil microbiota further revealed associations between sporulating bacteria, phosphorus-solubilizing microorganisms, yeasts, and actinomycetes in soils under both forage species. These microbial groups may facilitate grass tolerance to water deficit through phytohormone production (e.g., auxins and gibberellins) [49, 50] or by enhancing osmotic adjustment via amino acid accumulation [51]. Notably, phosphorus-solubilizing microorganisms in rhizosphere soil were positively correlated with tiller density ( $r = 0.66^{**}$ ), highlighting their functional role in plant productivity under limited water availability.

Under irrigated conditions, the influence of forage species on the soil microbiota remained evident, consistent with previous findings in grass-legume systems [18, 52]. Mavuno grass promoted the abundance of phosphorus-solubilizing microorganisms, which increased microbial metabolic activity and elevated the metabolic quotient under favorable moisture conditions [53]. Conversely, in the rhizosphere of Zuri grass, higher abundance of sporulating bacteria was observed, organisms that enhance microbial persistence and resilience. These compositional shifts in the microbial community were associated with higher total organic carbon in soils cultivated with Zuri grass under irrigation. Thus, the second hypothesis was

confirmed: forage species influence both the composition and functional activity of pasture soil microbiota under contrasting water regimes.

Importantly, irrigation exerted species-specific effects on microbial parameters. For instance, it reduced the microbial metabolic quotient ( $qCO_2$ ) by 50% in soils under Zuri grass, while simultaneously increasing microbial biomass carbon (SMB-C) in both forage systems relative to dryland conditions. This outcome reflects the role of irrigation in optimizing plant physiological processes and increasing root exudation [54]. In contrast, under dryland conditions, nutrient acquisition is restricted, and both root turnover and litter inputs are diminished [55, 56], limiting microbial substrate availability.

The increase in basal respiration under irrigation is indicative of a proliferation of copiotrophic microorganisms, which respond positively to resource-enriched environments [53]. Nonetheless, this did not constrain the accumulation of microbial biomass C or total soil organic carbon in Mavuno based systems. Principal component analysis (PCA) showed that SMB-C and dry matter production were among the key contributors to variance in irrigated treatments for both forage species and soil compartments, reinforcing the role of irrigation in enhancing microbial biomass and aboveground productivity in pasture systems.

The positive response of sporulating bacteria and yeasts under irrigation, and the selective stimulation of actinomycetes in Mavuno grass soils, indicate that the irrigation strategy employed in this study was appropriate, avoiding excessive moisture that could impair microbial communities [21]. Furthermore, hierarchical clustering combined with PCA revealed that sporulating bacteria in rhizosphere soils significantly contributed to the variance observed in irrigated Mavuno grass treatments. Therefore, irrigation management of tropical forage systems promotes soil carbon retention by both increasing microbial biomass C (in Zuri and Mavuno grass soils) and enhancing microbial metabolic efficiency, particularly in *M. maximus* cv. Zuri dominated systems.

These findings confirm the third hypothesis, indicating that irrigation mitigates drought-induced losses in soil microbiota and enhances the carbon efficiency of pasture systems in tropical environments. The contrasting microbial responses to Mavuno and Zuri grasses highlight the importance of integrating forage species selection and plant physiological traits with irrigation management to optimize soil microbial function and carbon sequestration. By promoting microbial

biomass and metabolic efficiency, particularly under irrigated conditions, these strategies contribute to improved soil health, resilience to climate variability, and the long-term sustainability of pasture-based livestock systems.

#### **4.2 Differences in water relations and elemental homeostasis distinguish forage grasses and their productive potential under dryland or irrigated conditions**

Forage grass cultivars are adaptable to systems with varying technological inputs, making it essential to understand the physiological and nutritional traits that underpin their tolerance to dryland conditions and responsiveness to irrigation. This study explored the contrasting performance of Mavuno and Zuri grasses in pastures managed with subsurface drip irrigation, highlighting key differences in their water status parameters and elemental homeostasis.

Under dryland conditions, both forage grasses exhibited a common drought-avoidance strategy characterized by the coordination between leaf morphology and leaf rolling (Fig. 7a, b), a mechanism that enhances water use efficiency by reducing transpiration [57]. However, this response entails stomatal closure, restricting CO<sub>2</sub> diffusion into the mesophyll, decreasing stomatal conductance, and limiting photosynthetic carbon assimilation, ultimately impairing growth transpiration [58]. As a result, both grasses showed reductions in plant height and dry matter (DM) yield, although Mavuno grass outperformed Zuri under dryland conditions, producing 3.5 times more DM. This superior performance was associated with smaller reductions in leaf water potential ( $\Psi_w$ ) and higher volumetric soil water content, likely reflecting more efficient drought signaling pathways transpiration [59].

At the nutritional level, the study revealed shifts in C:N:P stoichiometry under water-limited conditions, with increases in C:N, C:P, and N:P ratios in both species, likely due to reduced nutrient diffusion and uptake transpiration [55]. These ratios were negatively correlated with DM production, indicating that nutrient imbalance constrains biomass accumulation. Notably, Mavuno grass maintained higher carbon accumulation per unit of phosphorus under stress, reflecting greater nutrient-use efficiency, a key trait for sustaining productivity during drought or nutrient-limited conditions transpiration [9, 60, 11]. Additionally, Mavuno grass higher tiller density may compensate for reductions in tiller number under water stress,

whereas species with more upright growth forms, such as Zuri (*M. maximus* cv. Zuri), generally exhibit lower tiller density and depend more on canopy height for biomass accumulation [61, 62].

Under irrigated conditions, both species showed balanced  $\Psi_w$  values, confirming the efficiency of subsurface drip irrigation in maintaining optimal soil moisture during the dry season. This water availability supported full recovery from drought symptoms (Fig. 7c, d), leading to increased plant height and tillering, as well as the resumption of cell division and expansion due to improved nutrient uptake [63]. The restoration of C:N:P homeostasis under irrigation further supported the third hypothesis, suggesting that irrigation mitigates the stoichiometric imbalances induced by water deficit and promotes physiological recovery.

Forage production under irrigation increased by approximately  $10 \text{ t ha}^{-1}$  for both species, compared to  $1.3 \text{ t ha}^{-1}$  and  $0.4 \text{ t ha}^{-1}$  under dryland conditions for Mavuno and Zuri grasses, respectively. This substantial yield increase underscores the potential of irrigation to intensify pasture use, enhancing livestock productivity per hectare and reducing pressure on native ecosystems.

Taken together, the tolerance of forage species to dryland conditions and their responsiveness to irrigation depend directly on how grasses maintain or adjust the balance of C, N, and P in leaf tissues in response to water availability. Mavuno and Zuri grasses modulate soil microbial biomass, composition, and activity, which in turn influences their capacity to sustain elemental homeostasis, growth, and productivity under both dryland and irrigated conditions.

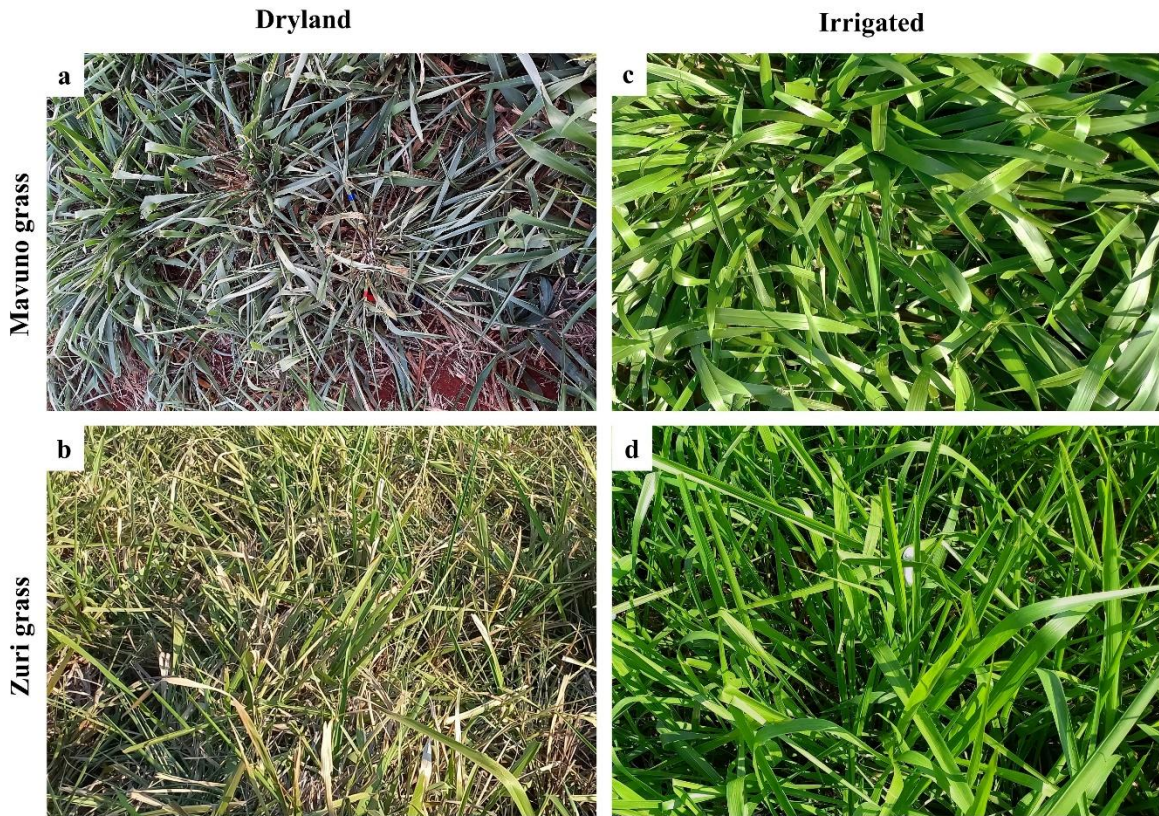


Figure 7. Visual response of forage grasses to dryland and irrigated conditions. Visual appearance of Mavuno and Zuri grasses pastures **(a, b)** illustrating leaf rolling and senescence under dryland conditions; **(c, d)** normal leaf morphology under irrigated conditions in Jaboticabal, São Paulo, Brazil.

## 5. Conclusions

The interaction between forage grass species and soil microbiota is significantly influenced by water availability, which directly impacts plant productivity, elemental homeostasis, and soil microbial dynamics. The Mavuno species demonstrated higher drought tolerance, exhibiting increased productivity under rainfed conditions, attributed to better water and nutritional balance. In contrast, Zuri grass promoted organic carbon accumulation in the soil under irrigated conditions.

Irrigation proved to be a critical factor in enhancing the metabolic efficiency of the forage grasses, as well as biomass production and microbial activity in the soil. Irrigated conditions led to a 10 Mg ha<sup>-1</sup> increase in dry matter production during the dry season for both Mavuno and Zuri grasses.

The maintenance of the C:N:P balance in plants is essential for sustaining growth and productivity across varying water availability scenarios. These findings provide valuable insights for pasture management, particularly in tropical regions, by optimizing both productivity and soil sustainability. The results are particularly relevant for pasture management in both rainfed and irrigated agricultural systems. This study underscores the importance of irrigation in enhancing productivity and improving soil quality and suggests that the C:N:P ratio may serve as an indicator of soil health and plant nutritional efficiency.

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## 4.2 ARTIGO 2: PHYSIOLOGICAL AND MORPHOLOGICAL TRAITS DRIVE DIFFERENTIAL DROUGHT TOLERANCE AND IRRIGATION RESPONSE IN TROPICAL FORAGE GRASSES UNDER FIELD CONDITIONS

(Grass and Forage Science - Submission ID d809c614-b09f-4d46-8cc0-095fd03d9a0b)

**Abstract:** Water deficit during the dry season in tropical regions limits pasture development, requiring the use of drought-tolerant forage species or the adoption of irrigation. However, the physiological and morphological mechanisms that confer drought tolerance and irrigation responses in tropical grasses remain poorly understood under field conditions. This study evaluated the physiological strategies of *Urochloa brizantha* × *U. ruziziensis* (Mavuno) and *Megathyrsus maximus* (Zuri) grown under rainfed and subsurface drip irrigation in a field experiment. Under drought conditions, Mavuno exhibited greater tolerance, evidenced by reduced soil water loss, higher membrane integrity, increased biomass accumulation, and elevated levels of phenolic compounds. In contrast, Zuri was more sensitive, exhibiting greater losses in photosynthetic pigments, membrane stability, and photosystem II efficiency. Under irrigation, both cultivars demonstrated improved performance, with increased amino acid content, reduced leaf toughness, and higher forage yield. The results indicate that Mavuno is recommended for regions prone to dry periods, while subsurface drip irrigation significantly enhances productivity and physiological traits of these forages. This study contributes to the development of efficient and sustainable pasture systems adapted to water limitations expected under climate change scenarios.

**Keywords:** drought stress; leaf gas exchange; photosynthetic efficiency; subsurface drip irrigation; tropical pasture

### 1. Introduction

Climate change has increased the frequency, duration, and intensity of drought events on a global scale, due to changes in precipitation patterns that challenge forage production and the sustainability of livestock production systems in various tropical regions (Sloat et al. 2018; Santos et al. 2024). In areas where livestock production relies on pastures, water deficits directly impair the growth and productivity of forage grasses, as water balance is essential for basic physiological processes such as nutrient uptake and the photosynthetic assimilation of carbon (C) (Melo et al. 2025; Pereira-Flores et al. 2023).

The drought period discussed here refers to the time of year when soil moisture declines due to low rainfall and high evapotranspiration rates, resulting in a water deficit. During this agricultural drought, the amount of water available in the soil becomes insufficient to meet the crop's requirements, forcing forage plants to undergo physiological and biochemical adjustments, such as the accumulation of osmoprotectant and antioxidant compounds, to maintain cellular water homeostasis and mitigate oxidative stress (Rocha et al. 2022). In this context of insufficient rainfall and/or irregular seasonal distribution, the cultivation of drought-adapted grasses becomes essential, along with the implementation of targeted pasture management strategies to support forage production stability (Tulu et al. 2023).

Tropical forage grasses have significantly boosted forage production in Brazil, particularly through the genetic improvement of *Urochloa* spp. (syn. *Brachiaria*) and species of the genus *Megathyrsus* (syn. *Panicum*). The scientific community has made considerable efforts to elucidate the drought tolerance of various grasses under controlled conditions, highlighting the adverse effects of water stress on gas exchange, membrane integrity, photosynthetic pigments, and, consequently, forage yield (Mastalerczuk and Borawska-Jarmulowicz, 2021; Zuffo et al. 2022; Oliveira et al. 2022). However, such studies under field conditions remain scarce (Olivera-Viciedo et al. 2021), despite their importance in guiding the selection of forage species and appropriate pasture management strategies.

Alongside the search for drought-tolerant species, there is growing interest in practices that enhance the resilience and/or tolerance of grasses to drought, aiming to intensify livestock production systems sustainably. One such strategy is the use of subsurface drip irrigation (SDI) in pastures (Rocha et al. 2025). This technique can markedly improve forage productivity during dry periods (Pereira-Flores et al. 2023), offering high water use efficiency by delivering water directly to the root zone and minimizing evaporative losses (Cahn and Hutmacher 2024). Although the limited cost-effectiveness of SDI has been a potential barrier to its adoption in temperate forage systems (Finger et al. 2015), its use may be viable for tropical grasses, which exhibit high biomass accumulation potential (Rocha et al. 2024). Nonetheless, scientific evidence from field studies on the physiological and productive responses of *Megathyrsus* and *Urochloa* cultivars to subsurface drip irrigation remains limited.

In this context, two hypotheses were investigated: (i) the dry season affects tropical forage grass species differently, depending on the species-specific physiological and biochemical mechanisms activated in response to water deficit; and (ii) subsurface drip irrigation can mitigate the effects of drought by promoting physiological and productive gains in Mavuno and Zuri grasses.

Therefore, the objectives of this study were to compare the physiological, biochemical, and productive responses of *M. maximus* cv. Zuri and *U. brizantha* × *U. ruziziensis* cv. Mavuno under rainfed conditions, identifying the mechanisms associated with drought tolerance in tropical forage grasses, and to evaluate their responsiveness to subsurface drip irrigation. The outcomes of this research are expected to support the selection of more drought-tolerant grass species and to quantify the potential benefits of irrigation for intensively managed systems, aiming to achieve more uniform forage production and improved pasture-based livestock performance.

## **2. Materials and Methods**

### **2.1 Location and edaphoclimatic conditions of the experimental site**

A field experiment was conducted at the experimental farm of São Paulo State University located in the municipality of Jaboticabal, Brazil, at coordinates 21°14'54" S and 48°17'06" W, at an altitude of 560 m. The region is characterized by a tropical savanna climate, Köppen classification type Aw, with rainfall concentrated in the summer and a dry winter. The experimental period extended from April to October 2023, coinciding with the dry season. Meteorological data were obtained from a weather station installed on the university near the experimental site. The recorded parameters included rainfall volume, potential evapotranspiration, air temperature, and global solar radiation (Figure 1a, b). Over the course of the experimental period, cumulative evapotranspiration and rainfall were 595 mm and 184 mm, respectively.

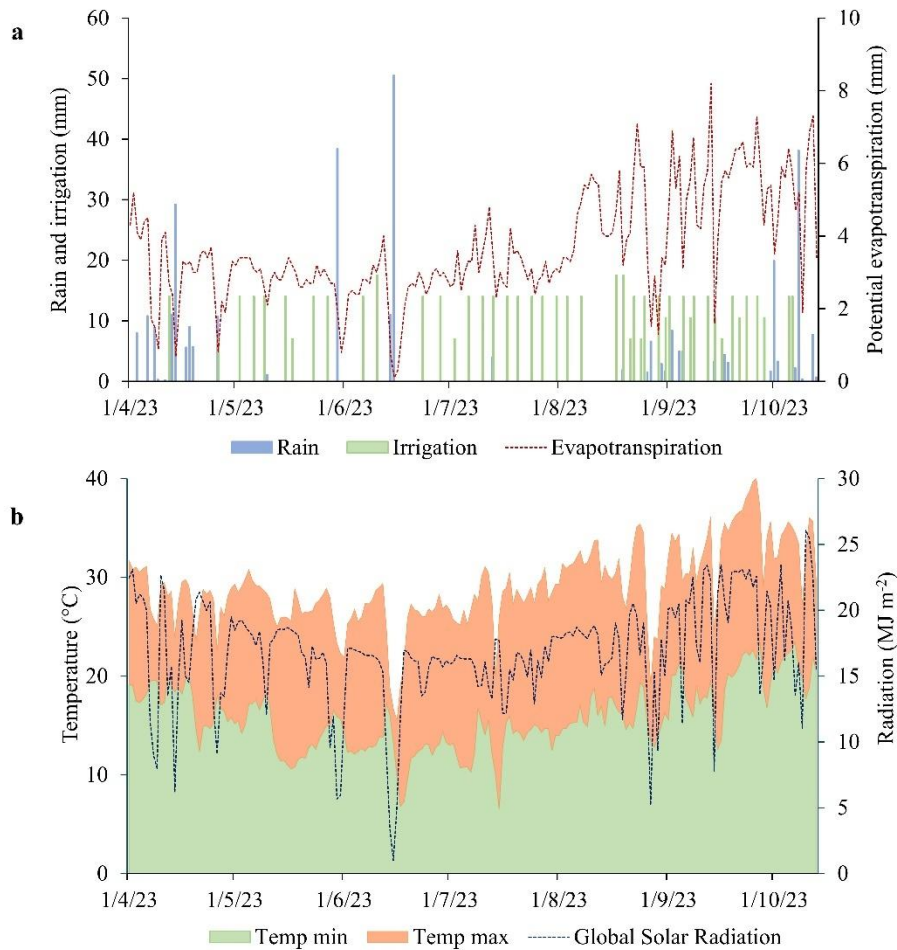


Figure 1. Observed data during the experimental period in Jaboticabal, Brazil: (a) precipitation (mm), irrigation (mm), and potential evapotranspiration (mm); (b) air temperature (°C) and global solar radiation (MJ m<sup>-2</sup> day<sup>-1</sup>).

Soil samples were collected from the experimental area for initial chemical characterization (Raij et al. 2001). The results for the 0-20 cm and 20-40 cm soil layers, respectively, were as follows: pH = 5.8 and 6.0; organic matter (OM) = 22 g dm<sup>-3</sup>; phosphorus (P) = 78 and 51 mg dm<sup>-3</sup>; potassium (K) = 5.5 and 3.6; calcium (Ca) = 46 and 44; magnesium (Mg) = 15 mmolc dm<sup>-3</sup>; sulfur (S) = 3 and 4; boron (B) = 0.32 and 0.25; copper (Cu) = 3.4 and 3.5; iron (Fe) = 6 and 5; manganese (Mn) = 25.5 and 18.6; zinc (Zn) = 1.6 and 1.0 mg dm<sup>-3</sup>; H<sup>+</sup>+Al<sup>3+</sup> = 21 and 18 mmolc dm<sup>-3</sup>; cation exchange capacity (CEC) = 88 and 81 mmolc dm<sup>-3</sup>; and base saturation ( $V = \text{Ca}^{2+} + \text{Mg}^{2+} + \text{K}^{+} / \text{CEC}$ ) = 76% and 77%. The soil is classified as Oxisol (Soil Survey Staff 2022).

## 2.2 Treatments and experimental establishment

The experiment followed a  $2 \times 2$  factorial design, considering two tropical forage grass species: Mavuno (*Urochloa brizantha*  $\times$  *U. ruziziensis*) and Zuri (*Megathyrsus maximus*) and two water regimes (rainfed non-irrigated and irrigated). The experimental design was a randomized complete block design with four replicates. The experiment was established in April 2022, following soil preparation by harrowing. In the irrigated plots, a subsurface drip irrigation system was installed. Drip lines were buried at a depth of 20 cm, with 80 cm spacing between crop rows (Rocha et al. 2024). Emitters had a flow rate of  $1.45 \text{ L h}^{-1}$ , operated at 100 kPa, and were spaced 30 cm apart, ensuring uniform water distribution according to Bernardo et al. (2009).

Each plot measured  $3 \times 4.8 \text{ m}$  and was manually sown with pure live seeds at a seeding rate equivalent to  $10 \text{ kg ha}^{-1}$ . After germination and initial growth, a cut was performed to stimulate tillering: to a height of 10 cm for Mavuno and 20 cm for Zuri, using a handheld brush cutter (Husqvarna 31R/330-2). During the establishment phase, sprinkler irrigation was used to ensure uniform plant development. In April 2023, after full pasture establishment, a standardization cut was performed, leaving a post-cut height of 15 cm for Mavuno and 30 cm for Zuri (Barbosa et al. 2021; Sbrissia et al. 2020).

## 2.3 Treatment application and pasture fertilization

Following the standardization cut, the different water regimes were applied: plots sown with either Mavuno or Zuri grass were maintained under rainfed conditions (non-irrigated) or subjected to subsurface drip irrigation. The soil water holding capacity in the 0-40 cm layer was previously determined from field capacity and permanent wilting point values, obtained from soil water retention curves using undisturbed samples (Klute 1986), and was equal to 50 mm. Irrigation scheduling (volume and frequency) was designed to fully replace evapotranspiration losses, applying a management allowable depletion factor of 0.6. This resulted in a total irrigation depth of 577 mm over the experimental period (Figure 2). Under rainfed conditions, soil moisture remained below the critical threshold of 20 mm throughout the experiment, except during sporadic rainfall events in June.

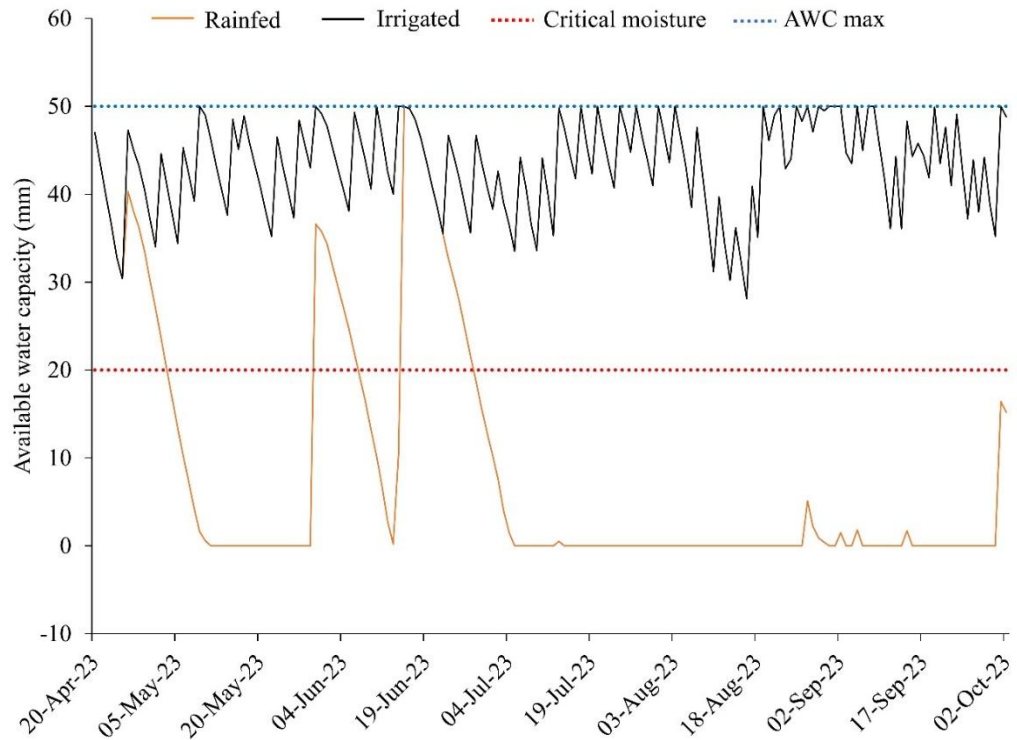


Figure 2. Soil available water data during the experimental period from April to October 2023 at the experimental site in Jaboticabal, Brazil. The rainfed condition is represented by the yellow line, and the irrigated condition by the black line. Dashed horizontal lines indicate the soil maximum water holding capacity (blue line) and the critical soil moisture threshold established for irrigation management (red line).

Initial soil fertility was considered adequate for the cultivation of the grasses, requiring only maintenance fertilization after each pasture growth cycle to replace nitrogen (N), P, K, and S exported in the dry matter (DM) of each treatment (Dubeux Jr. et al. 2007; Quaggio et al. 2022). The fertilizers used included urea, potassium chloride, ammonium sulfate, and purified monoammonium phosphate for fertigation, whereas phosphorus in the rainfed system was applied as single superphosphate. Fertilization in the irrigated plots was carried out via fertigation, while fertilizers were manually broadcast in the rainfed areas.

## 2.4 Measurements and analyses

Pasture height, tiller density, and DM production were monitored throughout the experimental period. Pasture was harvested whenever irrigated plots reached the recommended pre-grazing heights of 85 cm for Zuri and 35 cm for Mavuno (Barbosa et al. 2021; Silva et al. 2020). One cut was performed under rainfed

conditions and five cuts under irrigation. Prior to each harvest, plant height was recorded at five random points per plot using a graduated sward ruler.

Sampling was carried out between 07 and 10 h, using a 2.25 m<sup>2</sup> frame in Zuri plots and 1.0 m<sup>2</sup> frame in Mavuno plots. Forage within the frame was clipped with sickle to a residual height of 35 cm for Zuri and 15 cm for Mavuno. Fresh mass (FM) was determined, and a 100 g subsample was taken for drying at 65 ± 5 °C for 72 h to determine DM content. Forage production (Mg ha<sup>-1</sup> DM) was calculated using DM content and sward height, and forage mass density (kg ha<sup>-1</sup> cm<sup>-1</sup> DM) was derived accordingly. After each harvest, live and dead tillers were counted within a fixed 635 cm<sup>2</sup> frame per plot (Bahmani et al. 2003). Net live tiller density (tillers per m<sup>-2</sup>) was estimated by subtracting dead from total tillers.

In September 2023, at the peak of the dry season, the following water and physiological variables were measured:

**Soil volumetric water content ( $\theta$ , cm<sup>3</sup> cm<sup>-3</sup>):** estimated using Time Domain Reflectometry (TDR) with a portable HydroSense II moisture sensor equipped with 20 cm rods, showing satisfactory accuracy ( $R^2 = 0.84$ ; standard deviation = 1%) (Gonzalez-Porras et al. 2024). Measurements were taken at three points per plot.

**Leaf water potential ( $\Psi_w$ , MPa):** determined using a Scholander pressure chamber (model 3000F01, Soil Moisture Equipment) between 3 and 5 a.m. Three tillers were randomly selected per plot, and the youngest fully expanded leaf (with exposed ligule) was excised. The middle portion of the leaf was used, and pressure gradually applied until sap exuded from the main vein, following Turner (1981).

**Relative leaf water content (RWC, %):** Ten leaf discs (26 mm<sup>2</sup>) were collected from the youngest fully expanded leaf at 6 a.m. Discs were weighed to obtain FM, then floated in deionized water for 6 h and reweighed for turgid mass (TM). Discs were oven-dried at 80 °C for 24 h to determine DM. RWC was calculated using the formula:  $[(FM-DM)/(MT-MS)] \times 100$  (Barrs and Weatherley 1962).

**Leaf firmness index (N):** Leaf firmness was measured with a digital penetrometer equipped with an 8 mm tip, capable of applying 5 to 200 N ± 1 N (Impac, Model IP-90DI, São Paulo, Brazil), following the method of Calbo et al. (2010). Six

measurements were taken on the midsection of the youngest fully expanded leaf from randomly selected tillers.

**Electrolyte leakage index:** Ten leaf discs (26 mm<sup>2</sup>) from the youngest fully expanded leaf were collected at 6 a.m. Discs were immediately transported in a cooled container to the laboratory and immersed in deionized water for 2 h. Initial electrical conductivity (EC<sub>1</sub>) was measured with a portable conductivity meter (TSD-3). Samples were autoclaved at 121 °C for 20 min, cooled, and final conductivity (EC<sub>2</sub>) measured. Electrolyte leakage was calculated as:  $(EC_1 / EC_2) \times 100$  (Dionisio-Sese and Tobita 1998).

**Total phenolic compounds:** Fresh samples (0.1 g) of the youngest fully expanded leaf were extracted in methanol for 3 h. A colorimetric reaction was performed using Folin-Ciocalteu reagent (2 N) for 3 minutes, followed by 20% sodium carbonate. After 2 h, absorbance was measured at 765 nm (B442 spectrophotometer, Micronal). Total phenolics were quantified using a gallic acid standard curve and expressed as gallic acid equivalents per 100 g fresh weight (GAE 100 g<sup>-1</sup>) (Singleton and Rossi 1965).

**Soluble carbohydrates and total amino acids:** One gram of dry plant material (from the grazing layer) was extracted with 10 mL of methanol–chloroform–water (60:25:15%, v/v) in Falcon tubes, stored at 4 °C for 48 h. Four milliliters of the supernatant were mixed with 1 mL chloroform and 1.5 mL deionized water, then refrigerated for 24 h. Soluble carbohydrates were determined by reacting 0.5 mL extract with 0.5 mL 5% phenol and 2 mL concentrated sulfuric acid, and reading absorbance at 490 nm (DuBois et al. 1956). Amino acids were analyzed by mixing 1 mL extract with 500 µL 0.2 M sodium citrate, 200 µL 5% ninhydrin in monomethyl ether of ethylene glycol, and 1 mL 0.0002 M KCN. The mixture was heated at 100 °C for 20 minutes, cooled for 10 minutes under running water, and 1 mL 60% ethanol added before reading absorbance at 570 nm (Yemm et al. 1955).

**Total chlorophyll, total pheophytin, carotenoids, and flavonoids:** Discs weighing 0.025 to 0.030 g were randomly collected from the middle third of the youngest fully expanded leaf of each tiller. Samples were placed into Eppendorf tubes with 1.5 mL of 80% acetone. Tubes were stored in darkness until complete

pigment extraction (approximately 48 h). Absorbance readings were taken using a spectrophotometer (DU640, Beckman, USA) at wavelengths of 470, 663, 647, 653, and 665 nm for carotenoids, chlorophyll *a*, chlorophyll *b*, pheophytin *a*, and pheophytin *b*, respectively (Lichtenthaler 1987). Pigment contents were expressed in mg g<sup>-1</sup> of fresh mass.

**Chlorophyll fluorescence parameters (Fo, Fm) and quantum efficiency of photosystem II (Fv/Fm):** Measurements were taken on the youngest fully expanded leaf from a randomly selected tiller in each plot using a portable fluorometer (Os30P<sup>+</sup>, Opti-Sciences) (Lichtenthaler et al., 2005). Assessments were conducted between 8 and 10 a.m. in September 2023 after a 30-minute dark adaptation period, followed by a red-light pulse.

**Leaf temperature and gas exchange:** Leaf temperature (°C), transpiration rate (*E*, mmol m<sup>-2</sup> s<sup>-1</sup> H<sub>2</sub>O), and stomatal conductance (*G*<sub>s</sub>, mmol m<sup>-2</sup> s<sup>-1</sup>) were measured with a LI-1600 steady-state porometer on the middle third of the most recently expanded leaf from two randomly selected tillers per plot, between 9 and 11 a.m., under natural environmental conditions.

## 2.5 Statistical analysis

The data were assessed for normality, homogeneity, and independence of residuals using standard diagnostic tests (Bartlett 1937; Jarque and Bera 1980). Subsequently, analysis of variance was performed, and means that were significantly different according to the F-test (*p* < 0.05) were compared using Tukey test at the 5% significance level. Statistical analyses were conducted using SPEED Stat software, version 3.2 (Carvalho et al. 2020).

## 3. RESULTS

### 3.1 Plant water status and leaf toughness

A significant interaction (*p* < 0.05) was observed between the irrigation regime (rainfed vs. irrigated) and forage species, affecting both soil and leaf water parameters, as well as the leaf toughness index (Figure 3a-d). Cultivation under rainfed conditions, compared with irrigated conditions, reduced soil water content by 34% and 44% and relative leaf water content by 53% and 57% for Mavuno and Zuri grasses, respectively. In addition, leaf water potential decreased, whereas the

leaf toughness index increased in both species under rainfed conditions. Among the forages grown without irrigation, Zuri grass exhibited lower soil water content (–15%) and lower leaf water potential (–10%), but a higher leaf toughness index (+24%) compared to Mavuno grass (Figure 3a, c). Irrigation increased all soil and plant water status variables for both species, while it reduced the leaf toughness index (Figure 3a-d). Under these conditions, no significant differences were observed between species for the variables analyzed.

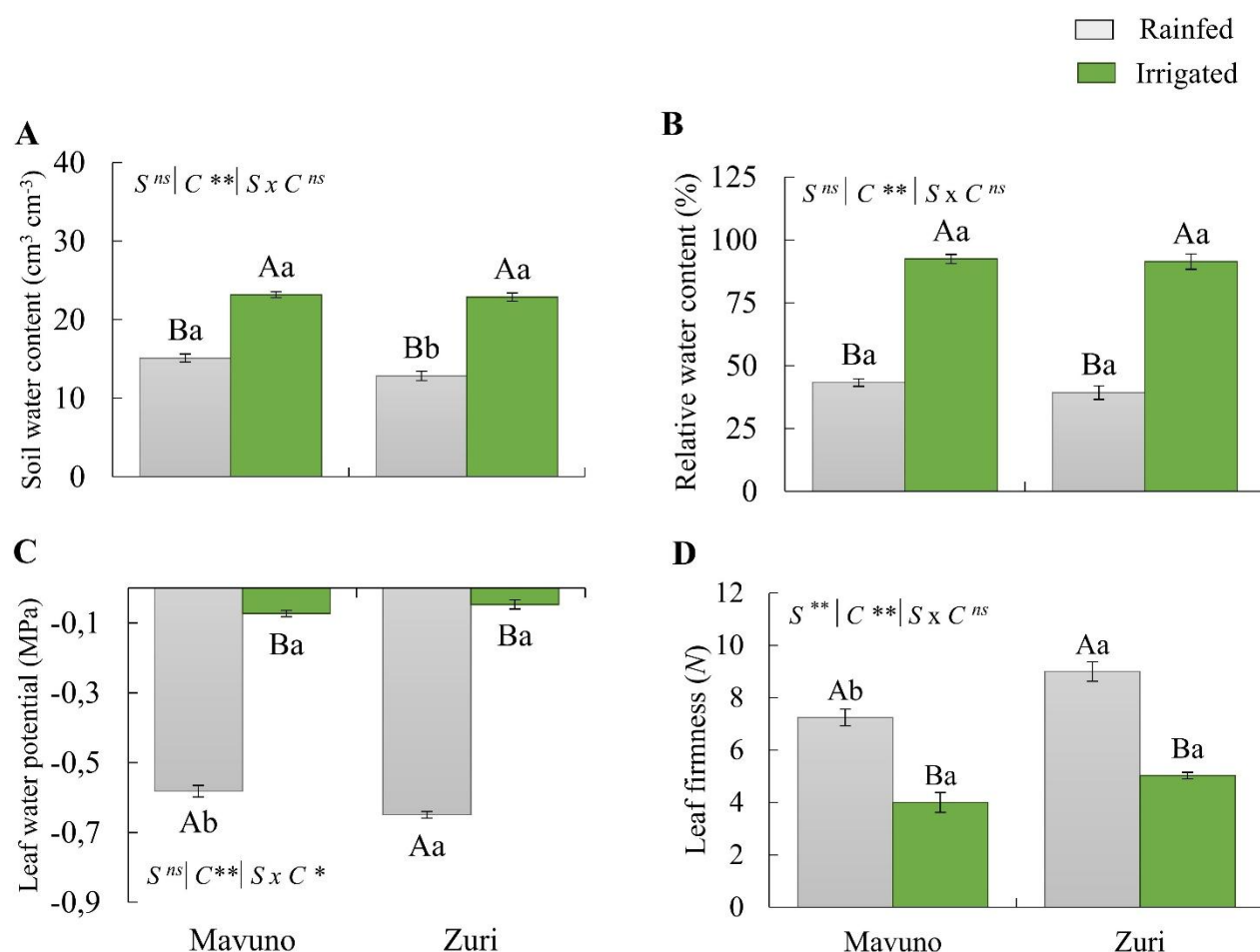


Figure 3. Volumetric soil water content (A), relative leaf water content (B), leaf water potential (C), and leaf toughness index (D) in forage biomass of Mavuno and Zuri grasses under rainfed or irrigated conditions. Uppercase letters indicate comparisons between water regimes within each species; lowercase letters indicate comparisons between species within each water regime.

### 3.2 Stress related variables in grasses

The water regime (rainfed vs. irrigated) influenced all parameters related to water stress, while forage species significantly affected ( $p < 0.05$ ) electrolyte leakage and soluble carbohydrate content (Figure 4a-f). A significant interaction ( $p < 0.05$ ) between water regime and species was observed for electrolyte leakage, soluble carbohydrates, and carotenoid content (Figure 4a, c, f).

Under rainfed conditions compared with irrigation, Zuri grass showed greater electrolyte leakage (+57%), and both species exhibited increased phenolic compound content (+25%) (Figure 4a, b). In addition, soluble carbohydrate content increased in Mavuno (+9.8%) and Zuri (+24%) grasses, whereas total free amino acid levels decreased by approximately 60% in both species (Figure 4c, d). The flavonoid index was also higher under rainfed conditions, with increases of 83% in Mavuno and 128% in Zuri grasses (Figure 4e).

Irrigation reduced electrolyte leakage in Zuri (−36%) and decreased phenolic compounds, soluble carbohydrates, and flavonoids in both species (Figure 4a-c, e). In contrast, total amino acids increased by approximately 160% under irrigation in both grasses, along with carotenoid content, particularly in Mavuno grass (Figure 4d, f). Under rainfed conditions, Zuri grass showed higher electrolyte leakage than Mavuno (+66%). In contrast, under irrigation, Mavuno grass exhibited higher soluble carbohydrate content compared with Zuri (+14%) (Figure 4a-f).

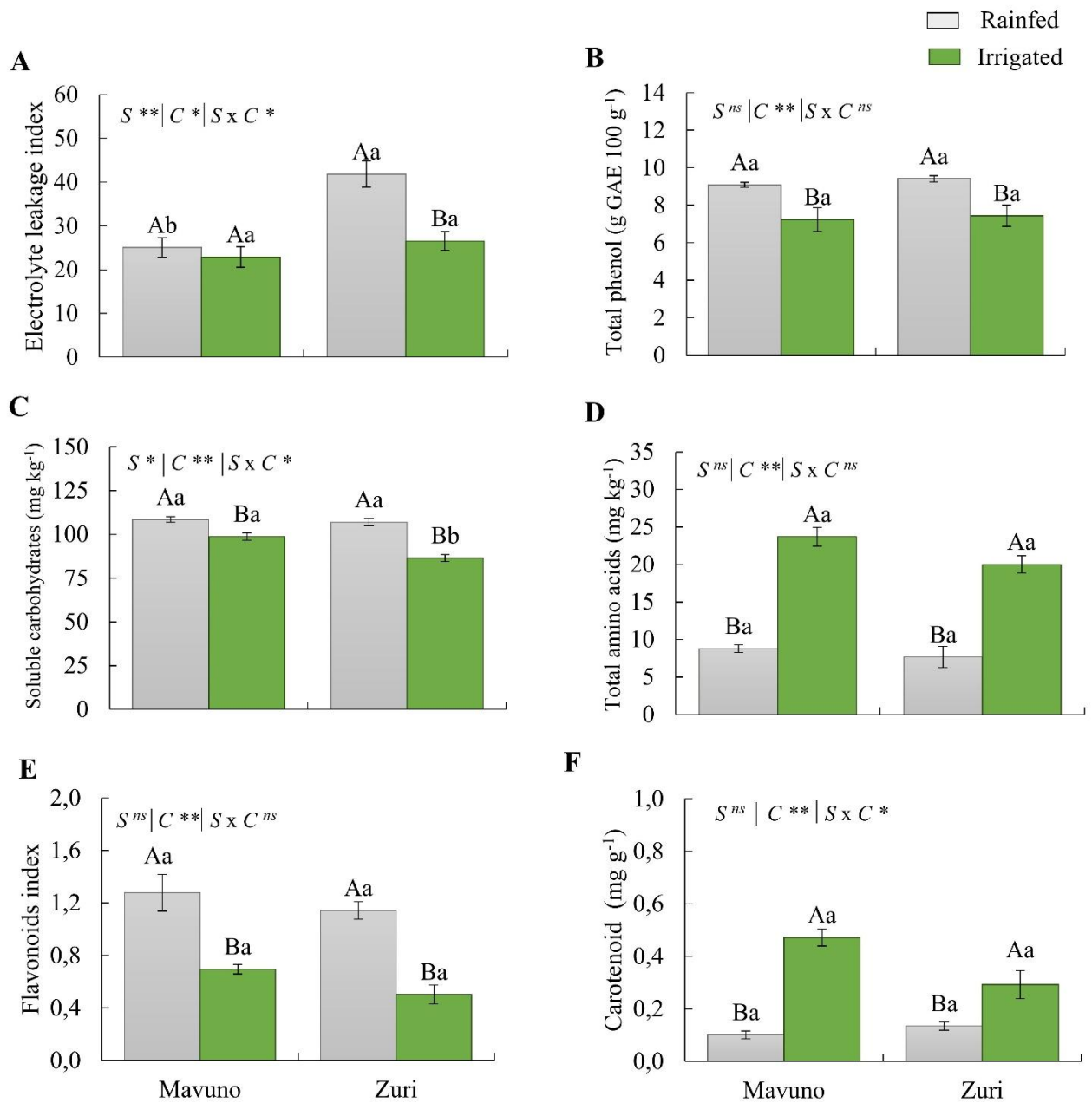


Figure 4. Electrolyte leakage index (A), total phenols (B), soluble carbohydrates (C), total amino acids (D), flavonoid index (E), and total carotenoids (F) in the forage biomass of Mavuno and Zuri grasses grown under rainfed and irrigated conditions. Uppercase letters indicate comparisons between water regimes within each species; lowercase letters indicate comparisons between species within each water regime.

### **3.3 Gas exchange, pigments, and photosynthetic efficiency**

Gas exchange and photosynthesis variables were significantly affected by water availability ( $p < 0.05$ ), with differences observed between cultivars and in the interaction between these factors for total chlorophyll and total pheophytin contents (Figure 5a-h).

Rainfed conditions increased leaf temperature in both species (from 34 to 36 °C) and reduced transpiration in Mavuno (–20%) and Zuri (–50%) compared with irrigated conditions (Figure 5a, b). Stomatal conductance was also reduced by approximately 60% in both grasses under rainfed conditions (Figure 5e). Total chlorophyll and total pheophytin contents decreased in Mavuno and Zuri (–85% and –75% for both pigments, respectively) (Figure 5c, d).

Initial fluorescence increased in Zuri under rainfed conditions (+7.8%), while maximum fluorescence was reduced under rainfed conditions in Mavuno (–46%) and Zuri (–28%) (Figure 5f, g). The quantum efficiency of PSII was impaired by water limitation, with a 27% reduction in Mavuno and 15% in Zuri (Figure 5h). Under irrigation, total chlorophyll and pheophytin contents were higher in Mavuno compared with Zuri (+18% and +33%, respectively) (Figure 5c, d).

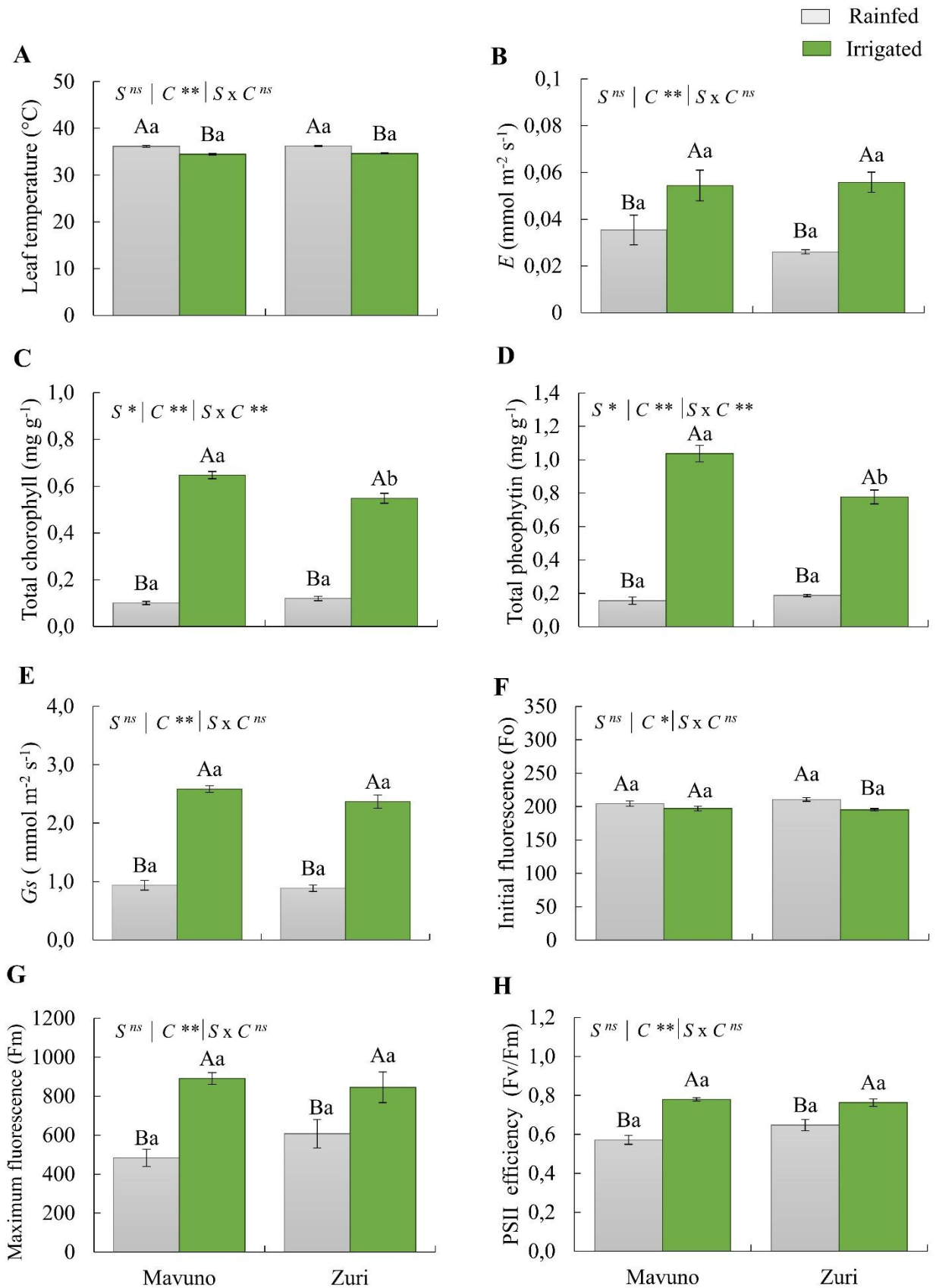


Figure 5. Leaf temperature (A), transpiration rate (B), total chlorophyll content (C), total pheophytin (D), stomatal conductance ( $G_s$ ) (E), initial fluorescence ( $F_o$ ) (F), maximum fluorescence ( $F_m$ ) (G), and quantum efficiency of photosystem II ( $F_v/F_m$ )

(H) in the plants of Mavuno and Zuri grasses under rainfed or irrigated conditions. Uppercase letters indicate comparisons between water regimes within each species; lower-case letters indicate comparisons between species within each water regime.

### **3.4 Morphological traits and forage yield**

Water regime and forage species had significant effects ( $p < 0.05$ ) on forage density, tiller number, canopy height, and dry matter (DM) yield (Figure 6a-d). A significant interaction ( $p < 0.05$ ) between these factors was also observed for forage density, tiller number, and canopy height.

Under rainfed conditions, compared with irrigation, both grasses showed reductions in tiller number (–59% for Mavuno; –48% for Zuri), canopy height (–26% and –44%, respectively), and DM yield (–88% and –64%) (Figure 6b-d). In addition, Mavuno exhibited greater forage mass density under rainfed conditions than under irrigation (Figure 6a).

Compared with rainfed conditions, irrigation decreased forage density in Mavuno (Figure 6a) and increased tiller number in both species, with a more pronounced effect in Mavuno (Figure 6b). Irrigation also increased canopy height, especially in Zuri (Figure 6c), and enhanced DM yield, with increments of 10.3 Mg ha<sup>-1</sup> in Mavuno and 9.7 Mg ha<sup>-1</sup> in Zuri (Figure 6d).

When comparing species, Mavuno had greater forage mass density than Zuri under both rainfed and irrigated conditions (Figure 6a) and produced a higher number of tillers under irrigation (+77%). Mavuno also yielded more DM than Zuri under both rainfed (by 0.97 Mg ha<sup>-1</sup>) and irrigated conditions (+1.6 Mg ha<sup>-1</sup>).

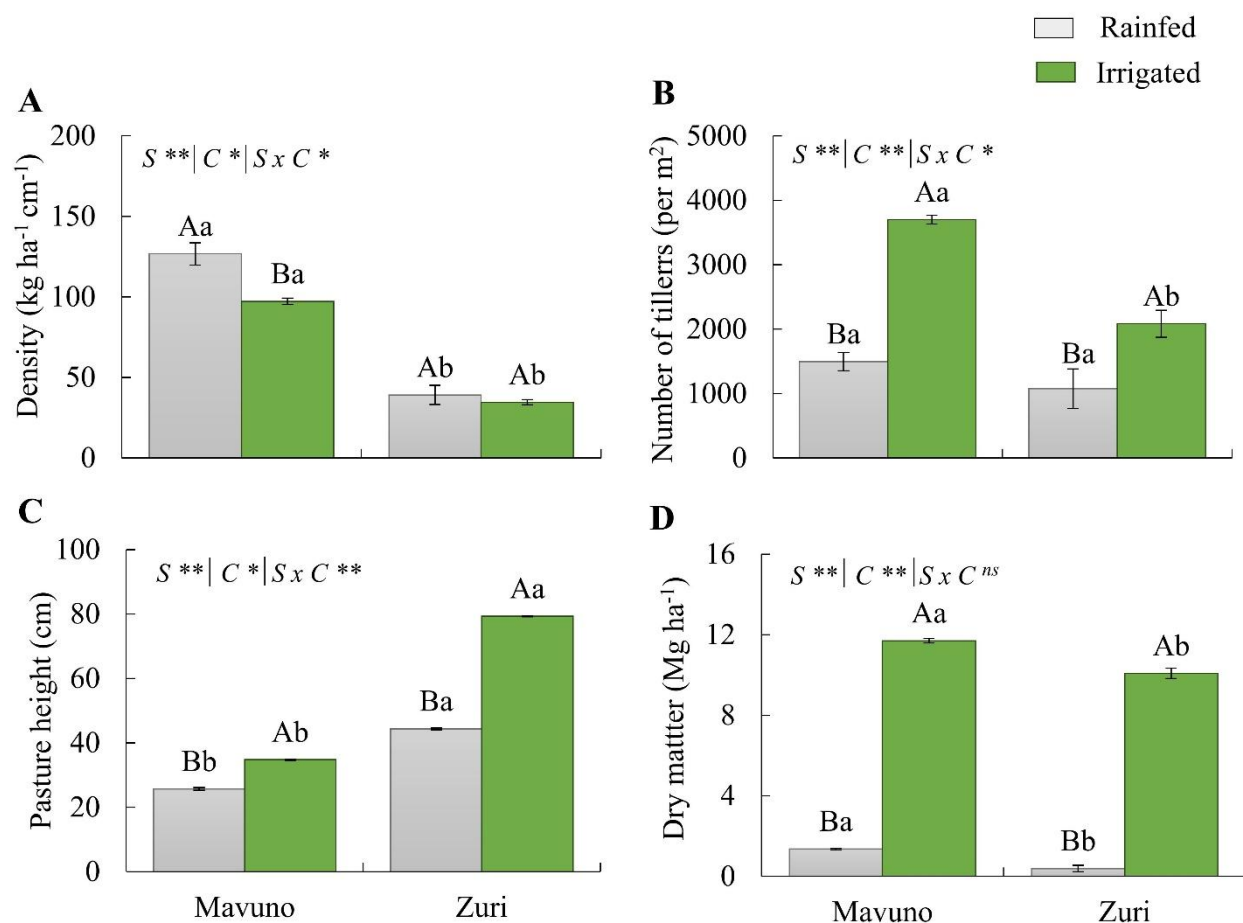


Figure 6. Forage mass density (A), tiller number per square meter (B), canopy height (C), and dry matter yield (D) of Mavuno and Zuri grasses grown under rainfed or irrigated conditions. Uppercase letters indicate comparisons between water regimes within each species; lower-case letters indicate comparisons between species within each water regime.

## 4. Discussion

### 4.1 Water status and antioxidant compound production confer greater drought tolerance to Mavuno grass under field conditions

The increasing frequency and severity of droughts, driven by climate change, have resulted in physiological impairments in forage plants and reduced forage production in tropical regions, where rainfed pastures predominate. One strategy to mitigate the effects of drought is the use of more drought-tolerant forage species. However, the physiological responses of recently released cultivars, such as *M. maximus* cv. Zuri and *U. brizantha* × *U. ruziziensis* cv. Mavuno, to water deficit under field conditions remain poorly understood. This study, conducted under real agricultural drought conditions, elucidated the main biological strategies underlying the differential drought tolerance of these tropical forage grasses. Gas exchange, photosynthetic efficiency, and forage production were all reduced under water deficit, but to varying degrees between the two species.

The water balance of both grasses was negatively affected under rainfed conditions, as the progressive reduction in soil water content resulted in lower leaf water potential. This response reflects efforts to maintain plant water uptake. It is associated with increased soluble carbohydrate content in the grasses, which contributes to osmotic adjustment (Ghosh et al. 2021), a key mechanism for drought tolerance in the species studied, particularly in *M. maximus*.

We found that both forage species grown under rainfed conditions exhibited increased total phenolic and flavonoid contents, which possess antioxidant activity in plants under stress by scavenging reactive oxygen species (ROS), such as hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) (Fini et al. 2011; Rocha et al. 2022). This strategy likely contributed to maintaining cell membrane integrity and functionality, enabling Mavuno grass to sustain similar electrolyte leakage levels under both water regimes (rainfed and irrigated), as previously observed for forage grasses in temperate climates (Shariatipour et al. 2022). However, this mechanism was insufficient to mitigate cellular damage and leakage in *M. maximus* cv. Zuri.

The observed differences in electrolyte leakage under drought, with *M. maximus* exhibiting greater sensitivity, suggest differential stability of cell membranes between the forage species. This may be related, for instance, to membrane composition, since a higher proportion of unsaturated fatty acids in the

cell membrane enhances foliar dehydration tolerance in grasses by increasing membrane fluidity (Xu et al. 2011; Zhang et al. 2017). This trait could be decisive for drought tolerance against oxidative damage (Shi et al. 2012) and may explain the lower cellular leakage observed in Mavuno compared to Zuri grass in this study.

The loss of leaf water balance under rainfed conditions also caused a 2 °C increase in leaf temperature in both grasses, due to reduced transpiration, particularly in Zuri. Generally, decreased transpiration induces stomatal closure under water deficit to maintain water balance, resulting in elevated leaf temperature (Liu et al. 2011). Reduced transpiration was confirmed by lower stomatal conductance in both Mavuno and Zuri under rainfed conditions compared to irrigation, indicating sensitivity of both grasses in maintaining leaf gas exchange under water deficit, which restricts CO<sub>2</sub> assimilation for photosynthesis (Mganga et al. 2023).

In addition to impairments in gas exchange caused by water deficit, reductions in photosynthetic pigment content, resulting from decreased synthesis or pigment degradation under stress, directly affect photosynthesis in grasses (Rocha et al. 2022). In this study, the grasses experienced severe losses (exceeding 70%) in total chlorophyll and pheophytin contents under water deficit, compromising photosynthetic efficiency. Chlorophyll absorbs light in reaction centers, while pheophytin acts as the primary electron acceptor following chlorophyll excitation (Klimov 2003). This likely contributed to the increased initial fluorescence in Zuri and decreased maximum fluorescence in both grasses (particularly Mavuno) under drought, not ruling out the possibility of structural and functional damage to photosystem II (D1 protein) or thylakoids (Henriques 2009). Consequently, PSII efficiency decreased by 15% and 27% in Zuri and Mavuno, respectively, indicating greater sensitivity of Mavuno regarding photosynthetic efficiency due to reduced maximum fluorescence and pigment content.

Another important aspect of drought impact on grasses is nutrient uptake impairment, since reduced transpiration flow compromises ion-root contact and, consequently, nutrient absorption necessary for metabolic and biosynthetic plant processes (Melo et al. 2025; Prado 2021). This can result in alterations in elemental homeostasis among C, N, and P in grasses such as sugarcane and *M. maximus* (Oliveira Filho et al. 2021; Olivera-Viciedo et al. 2024), thereby compromising physiological processes and biomass production (Costa et al. 2024).

Regarding morphological and productive traits, it was notable that Mavuno grass produced one tonne more dry matter than Zuri grass under rainfed conditions, confirming its greater drought tolerance despite a similar 50% reduction in tillering for both grasses. Under controlled conditions, a study demonstrated that *U. brizantha* can be one of the most drought-tolerant species within the genus *Urochloa* due to higher root production and photosynthetic efficiency (Mattos et al. 2005), which was confirmed in relation to photosynthesis in this field study for the Mavuno hybrid.

Among morphological factors, canopy height and forage mass density had a decisive influence on forage yield, with the tussock-forming Zuri grass more negatively affected in these traits, and consequently in biomass accumulation. Indeed, drought-induced reduction in leaf turgor pressure inhibits cell differentiation and expansion, decreasing leaf elongation rate in grasses and slowing growth, which can be exacerbated by nutrient deficiencies (Coussement et al. 2021; Melo et al. 2023) and reduced photosynthetic efficiency.

Therefore, this study demonstrates that agricultural drought affects the physiological and productive performance of the tropical grasses *M. maximus* cv. Zuri and *U. brizantha* × *U. ruziziensis* cv. Mavuno, with distinct responses between them, supporting the first hypothesis tested. We observed that Mavuno exhibited greater drought tolerance due to lower soil water loss, higher cell membrane integrity, and greater biomass production, associated with increased phenolic compound accumulation. In contrast, Zuri was more sensitive to stress, showing greater losses in membrane integrity, photosynthetic pigments, and photosystem II efficiency, differing from results reported for other *M. maximus* cultivars under imposed drought in controlled environments (Zuffo et al. 2022).

This research proposes cultivating Mavuno grass as a sustainable strategy to mitigate the adverse effects of seasonal forage production variability caused by drought in tropical regions, aligning with sustainable development goals. This practice entails no additional cost as it involves forage species selection, requiring only seed acquisition already considered in pasture establishment. It is worth noting that Mavuno is a hybrid with productive genetics and tolerance to pasture spittlebug, potentially contributing to tropical pasture-based production systems facing challenges imposed by climate change.

## **4.2 Responsiveness of Mavuno and Zuri grasses to subsurface drip irrigation is linked to amino acid synthesis and tillering capacity**

In intensive pasture production systems, irrigation is commonly used, significantly contributing to forage productivity and the sustainability of livestock activities (Pereira-Flores et al. 2023), especially in regions experiencing severe water scarcity. The use of more efficient irrigation systems, such as drip irrigation, is necessary for the rational use of freshwater, a finite natural resource. Accordingly, this study evaluated the response potential of tropical forage grasses to subsurface drip irrigation and demonstrated under field conditions that this practice can mitigate the physiological and productive impairments of two forage species. This is possible because subsurface drip irrigation maintained adequate soil moisture during the dry season, increasing relative water content and leaf water potential in the grasses, thereby reducing the risk of oxidative damage compared to non-irrigated plants.

Under irrigated conditions, Mavuno exhibited a marked increase in tillering and forage density, which underpinned its superior productivity relative to Zuri, further enhancing the cost-effectiveness of this technology. Additionally, Mavuno showed higher soluble carbohydrate content, beneficial for grass regrowth following grazing (Fulkerson and Donaghy, 2001), and contributes to forage quality, as soluble carbohydrates provide a rapid energy source in the rumen important for efficient utilization of forage protein (Villalba et al. 2021).

In addition to the increase in soluble carbohydrate content, other benefits to forage quality were identified in this study. Assessment of leaf firmness showed that irrigated cultivation, compared to rainfed conditions, promoted the production of more tender leaves by the grasses that is, leaves with a lower firmness index, which enhances forage intake and nutritive value (Silva et al. 2019). Moreover, forage grown under irrigation exhibited higher amino acid content, likely favored by improved water availability and nutrient uptake necessary for amino acid synthesis (Melo et al. 2025). Amino acids are precursors of proteins and secondary metabolites.

The benefits of irrigation on plant metabolism were further confirmed by the optimized synthesis of photosynthetic pigments, chlorophyll and pheophytin, as well as increased carotenoid content in both grass species. These accessory pigments absorb light that drives photosynthesis (Zulfiqar et al. 2021). A difference observed between species under irrigation was the higher chlorophyll and pheophytin

contents in Mavuno compared to Zuri, however, this did not translate into greater photosynthetic efficiency. Nevertheless, improvements in gas exchange under irrigation were sufficient to mitigate drought-induced damage.

With optimized water balance and photosynthetic metabolism under irrigation, tropical grasses expressed their high productive potential even during the dry season, achieving forage yields close to 12 Mg ha<sup>-1</sup> in a short period (April to October). This productive response is directly related to the marked increase in tillering promoted by adequate water availability, a trait responsive in both grasses. The superior dry matter production of Mavuno compared to Zuri is attributed to its higher tiller number and greater forage mass density, morphological traits typical of *Urochloa* spp. (Ochola et al. 2024), while *M. maximus* stands out for greater canopy height.

This researcher clearly demonstrated the strong responsiveness of the evaluated tropical grasses, particularly Mavuno to subsurface drip irrigation, with physiological and morphological improvements that indicate the potential of this irrigation system for use in tropical pastures. We showed that both Mavuno and Zuri grasses are suitable for intensive pasture-based production systems. However, Mavuno stands out for its higher productivity. Nevertheless, forage species selection should take into account not only biomass yield but also other important factors, such as forage quality, management familiarity, and the species adaptability to the edaphoclimatic conditions of each farming system.

Future studies are expected to advance toward identifying genes in both species, at the transcriptomic level, that are associated with the biochemical, physiological, and growth mechanisms of grasses cultivated under rainfed and irrigated conditions. Such genetic knowledge will support the development and performance prediction of new forage grass cultivars under varying water availability conditions, contributing to more precise recommendations for drought resilience and improved irrigation efficiency in pasture systems.

## Conclusions

Water deficit during the dry season impairs the physiological and productive performance of *M. maximus* cv. Zuri and *U. brizantha* × *U. ruziziensis* cv. Mavuno, with Mavuno showing greater tolerance to drought stress. Subsurface drip irrigation

proved effective in improving water status and morphophysiological traits in both forage species, although Mavuno exhibited superior productivity under irrigation.

This research offers insights into how different forage species respond to drought under field-grown, rainfed conditions and how they perform under irrigation. The findings are aligned with the Sustainable Development Goals by contributing to the strategic use of available plant genetic resources and irrigation to support more stable forage production and greater efficiency in pasture-based livestock systems.

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## 6. PARTICIPAÇÃO EM BANCAS DE CONCLUSÃO

6.1 Participação em banca de João Vitor Silva e Silva. Inoculação com *Azospirillum brasilense* no híbrido *Brachiaria brizantha* x *Brachiaria ruziziensis* atenua o aquecimento prolongado, aumentando o armazenamento de carbono no solo e a eficiência na absorção e no uso de nutrientes. 2025. Exame de qualificação (Mestrando em Pós-graduação em Ciência do Solo- FCAV/UNESP Jaboticabal).

6.2 Participação em banca de Patrícia Messias Ferreira. Sinergia silício e potássio mitiga os danos causados pelo déficit hídrico ao estimular o mecanismo de defesa

antioxidante em plantas de feijão e milho. 2025. Defesa de Tese (Doutoranda em Pós-graduação em Produção Vegetal – FCAV/UNESP Jaboticabal).

## **7. ORIENTAÇÕES**

Não houve orientações.

## **8. DISCIPLINAS MINISTRADAS COM A RESPECTIVA CARGA HORÁRIA E/OU A REALIZAÇÃO DE ATIVIDADES DE EXTENSÃO**

Não se aplica.

## **9. COMENTÁRIOS DO SUPERVISOR**

A pós-doutoranda executou a pesquisa com sucesso atendendo as atividades de pesquisa programadas e sugiro aprovação do presente relatório.