RAFAEL CARVALHO AMARAL

CUTTING MANAGEMENT AND INOCULANTS ALTER THE FERMENTATIVE PROFILE AND QUALITY OF ELEPHANT GRASS SILAGE CULTIVAR BRS CAPIAÇU

LAVRAS-MG
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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Microbiologia Agrícola para a obtenção do título de Doutor.

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RAFAEL CARVALHO AMARAL

MANEJO DE CORTE E INOCULANTES ALTERAM O PERFIL FERMENTATIVO E A QUALIDADE DA SILAGEM DE CAPIM ELEFANTE CULTIVAR BRS CAPIAÇU

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APROVADA em 20 de janeiro de 2023.

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LAVRAS – MG
2023

Aos meus pais Arriel e Imelda e meu irmão Douglas

que sempre me apoiaram e deram

forças nessa jornada,

Dedico.

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RESUMO

O capim elefante BRS Capiaçu se destaca pelo alto rendimento e qualidade da forragem, sendo uma ótima opção para a produção de silagens, porém fermentações indesejadas são comuns nessa forragem que podem ser evitadas pelo ajuste da concentração de MS ou a utilização de inoculantes. O objetivo deste estudo foi avaliar o efeito de novos inoculantes em silagens de capim elefante cultivar BRS Capiaçu com diferentes concentrações de MS e diferentes tempos de estocagem. O capim elefante foi ensilado com duas concentrações de MS denominadas: MS baixa (90 dias de rebrota) e MS alta (110 dias de rebrota) e inoculados com *Lactiplantibacillus plantarum* – CCMA 1394 (LP), *Lentilactobacillus hilgardii* – CCMA 0170 (LH), e sua combinação (LP + LH). Essas cepas foram selecionadas por reduzirem perdas de MS e aumentarem a estabilidade aeróbia de silagem de capim elefante. Foram avaliados o perfil fermentativo, a população de bactérias do ácido lático (BAL), leveduras, fungos filamentosos, enterobactérias, bactérias aeróbias formadoras de esporos (BAFES) e microrganismos mesófilos, a distribuição de partículas, a estabilidade aeróbia e a análise metataxonômica das silagens após 62 e 104 dias de estocagem. A forrageira colhida com a MS baixa apresentou concentração de MS de 167,7 $g.kg^{-1}$ matéria fresca (MF) e a forrageira colhida com a MS alta apresentou a concentração de MS de 242,6 $g.kg^{-1}$ da MF. A forrageira com a MS alta apresentou maiores concentrações de ácido acético e fibra em detergente neutro (FDN), menores concentrações de carboidratos solúveis em água (CSA) e maior população dos microrganismos avaliados. Todos os inoculantes reduziram a população de microrganismos indesejáveis nas silagens, embora não tenham reduzido o pH da silagem em relação ao controle. Os inoculantes LH e LP + LH se destacaram por reduzir as perdas de MS das silagens com a MS baixa (32,3 e 21,7 $g.kg^{-1}$ MS, respectivamente), reduzir a concentração de etanol com 104 dias de ensilagem (6,01 e 3,36 g kg^{-1} MS, respectivamente), reduzir a população de leveduras com 62 dias de estocagem (2,44 e 2,12 log $UFC.g^{-1}$, respectivamente) e aumentar a estabilidade aeróbia na silagem com a MS alta com 62 dias de ensilagem (45,75 e 45,50 h, respectivamente). As silagens com a MS baixa tiveram as menores perdas de MS (média de 53,0 $g.kg^{-1}$ vs 70,9 $g.kg^{-1}$ MS da silagem com a MS alta), maior produção de ácido lático (média de 64,06 g kg^{-1} MS vs 28,76 $g.kg^{-1}$ MS da silagem com a MS alta) e menores valores de pH (4,55 vs 4,73 da silagem com a MS alta). O tempo de estocagem afetou o perfil fermentativo, população microbiana e distribuição de partículas das silagens. O perfil da comunidade de procariotos se diferenciou nas silagens com diferentes concentrações de MS. A cepa CCMA 0170 (LH) é promissora para a utilização como inoculante em silgens de capim elefante BRS Capiaçu. Devido ao melhor perfil fermentativo apresentado, recomenda-se ensilar a forragem com a MS baixa.

Palavras-chave: Capim elefante. Silagem. Inoculante. Matéria seca. Tempo de estocagem.

ABSTRACT

BRS Capiaçu elephant grass stands out for its high yield and forage quality, being a great option to produce silages, but undesirable fermentations are common in this forage that can be avoided by adjusting the DM concentration or the use of inoculants. The aim of this study was to evaluate the effect of new inoculants on silages of elephant grass cultivar BRS Capiaçu with different DM concentrations and different storage times. Elephant grass was ensiled with two different DM concentrations called: low DM (90 days of regrowth) and high DM (110 days of regrowth) and inoculated with the strains CCMA 1394 – *Lactiplantibacillus plantarum* (LP), CCMA 0170 – *Lentilactobacillus hilgardii* (LH), and their combination (LP + LH). These strains were selected for reducing DM losses and increasing the aerobic stability of elephant grass silage. Fermentative profile, lactic acid bacteria (LAB) count, yeast, filamentous fungi, enterobacteria, aerobic spore-forming bacteria (EASFB) and mesophilic microorganisms, particle distribution, aerobic stability and metataxonomic analysis of silages with 62 and 104 days of storage were evaluated. The forage harvested with low DM had a DM concentration of 167.7 $g.kg^{-1}$ of fresh matter (FM) and the forage harvested with high DM had a DM concentration of 242.6 $g.kg^{-1}$ of FM. The forage with high DM showed higher concentrations of acetic acid and neutral detergent fiber (NDF), lower concentrations of water-soluble carbohydrates (WAC) and higher population of microorganisms evaluated. All inoculants reduced the population of undesirable microorganisms in the silages, although they didn't reduce the pH of the silage in relation to the control. The LH and LP + LH inoculants stood out for reducing the DM losses of silages with low DM (32.3 and 21.7 g kg^{-1} DM, respectively), reducing the ethanol concentration after 104 days of ensiling (6 .01 and 3.36 g kg^{-1} DM, respectively), reduce the yeast population with 62 days of storage (2.44 and 2.12 log $CFU.g^{-1}$, respectively) and increase the aerobic stability in the silage with high DM after 62 days of ensiling (45.75 and 45.50 h, respectively). Silages with low DM had the lowest DM losses (mean of 53.0 $g.kg^{-1}$ vs 70.9 g kg^{-1} DM of silage with high DM), higher production of lactic acid (mean of 64.06 $g.kg^{-1}$ DM vs 28.76 $g.kg^{-1}$ DM of silage with high DM) and lower pH values (4.55 vs 4.73 of silage with high DM). Storage time affected the fermentative profile, microbial population and particle distribution of silages. The prokaryotic community profile differed in silages with different DM concentrations. The CCMA 0170 strain is promising for use as an inoculant in silages of elephant grass BRS Capiaçu. Due to the better fermentative profile presented, it is recommended to ensile forage with low DM.

Keywords: Elephant grass. Silage. Inoculant. Dry matter. Storage time.

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO

Os capins tropicais possuem um grande potencial para a utilização como silagem em regiões de climas quentes devido ao seu alto rendimento, sendo que a maior parte desse rendimento ocorre durante a estação chuvosa, tornando a ensilagem uma ferramenta fundamental para otimizar seu uso durante o ano (DANIEL et al., 2019). No processo de ensilagem ocorre a fermentação de açúcares pelas bactérias do ácido láctico (BAL), que em ambiente anaeróbio crescem rapidamente e se tornam, na maioria das vezes, os microrganismos dominantes na cultura ensilada (MUCK 2010).

Quando colhidos para a ensilagem com adequado valor nutritivo, os capins tropicais possuem alto teor de umidade e baixa concentração de carboidratos solúveis em água (CSA) (BERNARDES et al., 2018). Dessa forma, durante o processo de ensilagem, pode haver liberação de efluentes e fermentações indesejáveis como as realizadas por bactérias do gênero *Clostridium* (GEBREHANNA et al., 2014).

Para evitar perdas durante o processo fermentativo da silagem, estratégias devem ser tomadas. O emurchecimento e a adição de alimentos secos absorventes são algumas alternativas comumente utilizadas. No entanto essas técnicas têm sido questionadas. No emurchecimento, os capins tropicais e com colmos grossos são difíceis de serem murchados, além disso, tal técnica torna as plantas susceptíveis ao aumento das perdas de campo. Já a utilização de alimentos absorventes, além de poder haver problemas de logística, é dependente de regiões onde há a disponibilidade desses produtos e que sejam economicamente viáveis. Com isso, a utilização de aditivos se torna a opção mais adotada pelo produtor, em especial os inoculantes contendo BAL (DANIEL et al., 2019). Os inoculantes são a classe de aditivos mais comuns e são compostos principalmente por BAL, cuja finalidade é garantir um processo fermentativo adequado no silo (MUCK, 2010). Apesar da diversidade de estudos envolvendo essas bactérias, o potencial desses microrganismos ainda pode ser explorado, cepas de uma mesma espécie podem apresentar diferentes resultados dependendo das características do substrato, tornando a escolha do inoculante um fator de extrema importância (CARVALHO et al., 2021).

Dentre os capins tropicais, o capim elefante (*Pennisetum purpureum* Schum.) é uma forrageira com excelente potencial de produção de matéria seca (MS), facilidade de propagação, cultivo e colheita (RUSDY, 2016), características que o torna no capim perene mais utilizado na produção de silagens (CÂNDIDO et al., 2007). Em 2016, o programa de melhoramento de capim-elefante da Embrapa Gado de Leite lançou a cultivar BRS Capiaçu que se destaca em relação a outras cultivares pelo alto rendimento (produção de MS anual de 49,75 $t.ha^{-1}$) e

qualidade da forragem (teor de proteína bruta de 9,10 %), facilidade para a colheita mecânica e resistência ao tombamento. (PEREIRA et al., 2017).

Recomenda-se a colheita para a ensilagem da cultivar BRS Capiaçu quando a planta estiver entre 90 e 110 dias de idade de rebrota, com o objetivo de se obter a melhor relação entre produção de biomassa, valor nutritivo e concentração de MS, associado ao o uso de aditivos para melhorar o processo fermentativo (PEREIRA et al., 2016). No entanto, até o momento, não existem dados que demonstrem o efeito da MS sobre a fermentação dessa cultura.

2 REFERENCIAL TEÓRICO

2.1 Importância da conservação de forragens em países de clima tropical

Em muitos países, onde há uma estação que restringe o crescimento de plantas forrageiras, como o inverno e épocas de baixa precipitação pluviométrica, a produção de forragens é sazonal, como consequência, sua disponibilidade não ocorre de forma uniforme durante todo o ano, resultando em alguns períodos com excedente e outros com déficit (PAHLOW et al., 2003; DUNIÈRE et al., 2013). Segundo Daniel et al. (2019), na América do Sul, a maioria das atividades de agricultura e produção animal está localizada em regiões de climas tropical de savana e subtropical úmido, seguindo a classificação climática de Köppen-Geiger. Esses climas possuem como característica duas estações bem definidas, denominadas: estação chuvosa, época em que há excedente de forragem de boa qualidade e estação seca, época em que as forragens são escassas e de baixa qualidade.

Na estação seca, a forragem disponível para os ruminantes apresenta baixa qualidade nutricional, podendo causar queda no desempenho desses animais. Diante dessa situação fazendas de gado de corte e gado de leite que não fizeram um bom planejamento forrageiro, podem passar por um déficit de forragem no período de quatro a seis meses no ano. De modo a contornar esse problema, as práticas de conservação de forragens se tornam uma estratégia atrativa, pois possibilitam aumentar a taxa de lotação e o desempenho dos animais durante a estação seca (DANIEL et al., 2019).

Outro ponto importante é que atualmente o número de fazendas de gado de leite que substituem o sistema de pastejo por confinamento, como free stall e compost barn estão aumentando, assim como as fazendas de gado de corte que tem adotado a terminação de bovinos

em confinamento. Tais práticas necessitam da disponibilidade de forragem conservada durante o ano todo (DANIEL et al., 2019).

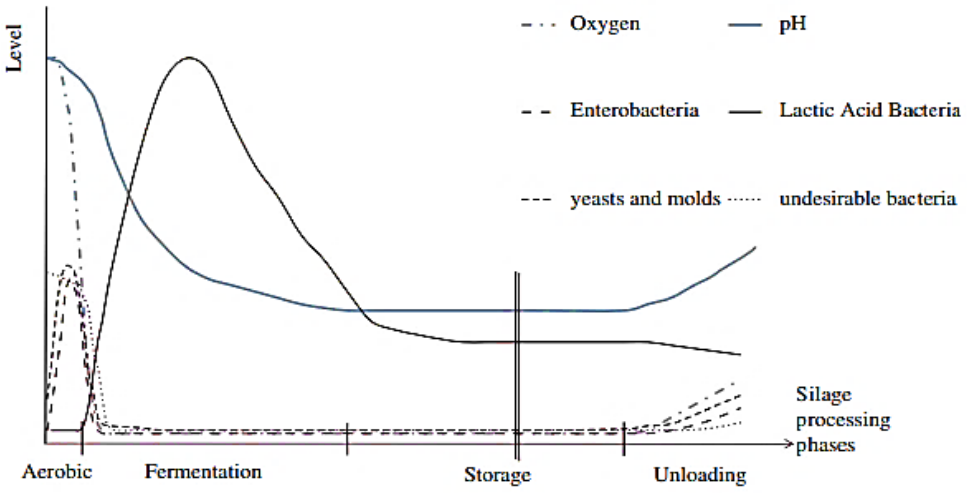
A preservação da forragem pode ser realizada através da produção de feno ou silagem. Na produção de feno, a forragem é desidratada de modo que iniba a atividade enzimática da planta e a ação de microrganismos deteriorantes (MUCK e SHINNERS, 2001). No entanto, em ambientes tropicais, existem algumas barreiras que dificultam o emprego dessa técnica, como a alta umidade e chuvas frequentes. Desta forma, o emprego da técnica de ensilagem se torna mais adequado nos países que apresentam clima quente e úmido (ADESOGAN, 2009; BERNARDES e DO RÊGO, 2014). A técnica de ensilagem pode ser definida como o método de conservação de forragens úmidas através de dois processos fundamentais: a criação de um ambiente anaeróbio e a fermentação de carboidratos solúveis em ácido lático e outros produtos por bactérias do ácido lático (BAL). O ácido lático é principal responsável pela queda do pH, inibindo a atuação de microrganismos deteriorantes (MUCK, 2010).

Segundo Wilkinson e Rinne (2018), a silagem desempenha dois importantes papéis quanto à nutrição na pecuária: [1] como fonte de nutrientes digestíveis conservados em dietas para gados de alta produção, mantendo uma ideal função ruminal e reduzindo riscos de doenças como acidose ruminal e deslocamento do abomaso e [2] como alimento suplementar quando o crescimento das pastagens não é suficiente para atender a demanda animal, situação característica de épocas de seca e inverno.

2.2 Processo de ensilagem

O processo de ensilagem envolve as etapas desde a colheita no estágio ideal de maturação da planta, passando pelo transporte da planta, compactação e vedação em silos para a exclusão do ar e armazenamento até a posterior abertura desses silos para a alimentação dos animais (DUNIÈRE et al., 2013). Tal processo pode ser dividido em 4 fases: [1] fase aeróbia, momento em que a forragem é depositada no silo imediatamente após a colheita, [2] fase de fermentação, [3] fase de armazenamento ou de estabilidade, e [4] fase de descarga ou alimentação - fase em que o silo é aberto e o material ensilado é exposto ao ar (WILKINSON e DAVIES, 2013) (Figura 1). Essas quatro etapas são aquelas as quais podem ocorrer eventos bioquímicos e microbiológicos (WEINBERG e ASHBELL, 2003).

Figura 1: Fases do processo de ensilagem



Fonte: Dunière et al., 2013 adaptado de Pitt e Sniffen (1985)

Na primeira fase ocorre a denominada “respiração residual”, que consiste na atividade enzimática das células vegetais intactas, consumindo o oxigênio que se encontra aprisionado na silagem e metabolizando os carboidratos (SHAO et al., 2005). Também ocorre o desenvolvimento da microbiota aeróbia epifítica como enterobactérias, fungos filamentosos e leveduras até que haja o consumo total do oxigênio e acidificação suficiente para interromper o metabolismo desses microrganismos (DUNIÈRE et al., 2013).

Heron et al. (1993), identificaram as espécies de enterobactérias dominantes da planta fresca de azevém (*Lolium multiflorum*) e as espécies dominantes logo após a ensilagem. As espécies que frequentemente dominaram a planta fresca antes da ensilagem foram identificadas como *Erwinia herbicola* e *Rahnella aquitilis*, as quais foram rapidamente substituídas pelas espécies *Hafnia alvei*, *Escherichia coli* e *Serratia fonticola* após a ensilagem do azevém. AMARAL et al. (2020) identificaram as espécies *Klebsiella variicola*, *Enterobacter asburiae* e *Staphylococcus xylosus* na forragem de capim elefante cultivar Napier. Essa fase normalmente leva apenas algumas horas, pois corresponde ao tempo em que o oxigênio presente entre as partículas da planta é reduzido (OUDE ELFERINK et al., 2000).

Em seguida tem-se a fase da fermentação, que inicia quando o ambiente se torna anaeróbio. Nessa etapa, tem-se uma diversidade de grupos de microrganismos aeróbios facultativos ou anaeróbios que competem pelos nutrientes. Quando a silagem é bem manipulada, as BAL dominam o processo fermentativo, tornando o pH da silagem baixo (entre 3,8 a 5, dependendo da cultura a ser ensilada), essa condição favorece na preservação da silagem (DUNIÈRE et al., 2013).

Os principais gêneros de BAL presentes na silagem são *Lactobacillus, Pediococcus, Lactococcus, Enterococcus, Streptococcus* e *Leuconostoc* (PAHLOW et al., 2003). No entanto, caso haja contaminação via solo, prolongamento da fase aeróbia ou lenta acidificação da silagem, as comunidades microbianas presentes na silagem durante essa fase serão dominadas por microrganismos indesejáveis como leveduras, fungos filamentosos, *Clostridium* sp. e *Listeria* sp. (DRIEHUIS e OUDE ELFERINK, 2000). A fase de fermentação pode durar de vários dias a várias semanas, esse tempo depende das características da forragem a ser ensilada e das condições em que foi ensilada (OUDE ELFERINK et al., 2000).

KHOTA et al. (2016) identificaram as populações de BAL naturais nas silagens de capim guiné com 20,18% de MS e capim elefante cv. Napier com 17,88% de MS após 30 dias de fermentação com base nas características morfológicas, bioquímicas e na análise da sequência do gene 16S rRNA, as espécies encontradas foram: *Lactiplantibacillus plantarum*, *Lacticaseibacillus casei*, *Lactobacillus acidipiscis*, *Leuconostoc pseudomesenteroides*, *Leuconostoc garlicum, Weissella confusa e Lactococcus lactis.*

AMARAL et al. (2020) isolaram e identificaram BAL na silagem de capim elefante cv. Napier com 10, 30 e 45 dias de estocagem. Com 10 dias foram identificadas as espécies *Latilactobacillus sakei*, *Pediococcus pentosaceus* e *Lactiplantibacillus plantarum*. Após 30 dias, *L. plantarum* dominou o processo de fermentação e permaneceu na silagem até os 45 dias. A espécie *Levilactobacillus brevis* foi identificado apenas aos 45 dias.

A terceira fase corresponde ao período de estabilidade, que se caracteriza por ocorrer pequenas mudanças, desde que as condições de anaerobiose e baixo pH sejam mantidas. Com o tempo ocorre a queda da viabilidade das BAL e outros microrganismos, com exceção dos formadores de endósporos e leveduras tolerantes à acidez que permanecem em estado quase inativo (VISSERS et al., 2006; STORM et al., 2010). As BAL especializadas pertencentes a espécie *Lentilactobacillus buchneri*, também são capazes de continuar ativas em baixo número (DRIEHUIS et al., 1999; OUDE ELFERINK et al., 2000). A fase de armazenamento pode durar meses (DUNIÈRE et al., 2013).

Por fim, tem-se a fase de descarregamento para a alimentação dos animais, momento em que o silo é aberto, e o ar penetra na silagem, proporcionando o crescimento de microrganismos aeróbios indesejáveis inicialmente presentes na silagem (DRIEHUIS e OUDE ELFERINK, 2000). Segundo OUDE ELFERING et al. (2000), essa fase pode ser dividida em duas etapas, na primeira etapa ocorre o início da deterioração devido à degradação dos ácidos orgânicos pelas leveduras, e ocasionalmente pelas bactérias do ácido acético, causando o aumento do pH, já a segunda etapa está associada ao aumento da temperatura e à atividade de

deterioração de outros microrganismos aeróbios ou aeróbios facultativos como fungos filamentosos e enterobactérias.

SOUZA et al. (dados não publicados) avaliando as características das silagens de capim elefante cv. BRS Capiaçu com dois teores de matéria seca (MS baixa e alta) associada à deterioração aeróbia observaram que após 6 dias de exposição ao ar, na silagem com a MS baixa o pH que antes era de 4,44 atingiu o valor de 7,33, nessa silagem também houve o aumento na contagem de leveduras. Na silagem com a MS alta houve maior contagem de leveduras, maior produção dos ácidos succínico, acético e propiônico. Nas duas silagens, a concentração do ácido lático aos 6 dias de exposição ao ar chegou a níveis não detectáveis.

2.3 Principais metabólitos e microrganismos envolvidos na ensilagem

O ácido lático produzido pelas BAL geralmente é o ácido com a maior concentração em silagens. É o principal responsável pela queda do pH, pois apresenta um pKa de 3,86, que é cerca de 10 a 12 vezes mais forte que outros ácidos comumente encontrados na silagem, como o ácido acético que apresenta o pKa de 4,75 e o ácido propiônico que apresenta o pKa de 4,87. A concentração do ácido lático em silagens geralmente é em torno de 2 a 4% da MS total, podendo ser consideravelmente maior em silagens com MS abaixo de 30%. Quando o alimento é fornecido ao animal, o ácido lático é convertido em ácido propiônico no rúmen (KUNG JR et al., 2018).

As BAL podem ser classificadas de acordo com os produtos da fermentação dos açúcares em três grupos: homofermentativas obrigatórias, heterofermentativas facultativas e heterofermentativas obrigatórias. As bactérias homofermentativas são capazes de produzir 2 mols de ácido lático a partir de um mol de glicose e as heterofermentativas são capazes de produzir 1 mol de ácido lático, 1 mol de dióxido de carbono (CO_2) e 1 mol de etanol ou ácido acético a partir de 1 mol da glicose (MUCK, 2010).

As espécies homofermentativas obrigatórias chegam a produzir mais de 90% de ácido lático do total de metabólitos produzidos a partir de hexoses (DUNIÈRE et al, 2013) pela via de Embden-Meyerhof-Parnas (EMP), porém são incapazes de metabolizar pentoses, pois não possuem a enzima fosfoquetolase. As espécies heterofermentativas facultativas utilizam a mesma via que as homofermentativas obrigatórias para metabolizar hexoses e também são capazes de metabolizar as pentoses, pois possuem as enzimas aldolase e fosfoquetolase. As espécies heterofermentativas obrigatórias são capazes de fermentar pentoses e hexoses pela mesma via metabólica (fosfoquetolase) (DRIEHUIS e OUDE ELFERINK, 2000). A maioria

das BAL são pertencentes à esses dois últimos grupos (MUCK, 2010). Na tabela 1 é mostrado com mais detalhes as reações típicas de fermentação por BAL.

Tabela 1: Reações típicas de fermentação realizadas por BAL.

Grupo	Fermentação
Homofermentativas obrigatórias	1 açúcar 6–C → 2 ácido lático
Heterofermentativas facultativas	1 açúcar 6–C → 2 ácido lático
	1 açúcar 5–C → 1 ácido lático + 1ácido acético
Heterofermentativas obrigatórias	1 açúcar 6–C → 1 ácido lático + 1 ácido acético + CO_2
	1 açúcar 6–C → 1 ácido lático + 1 etanol + CO_2
	1 açúcar 5–C → 1 ácido lático + 1 ácido acético
	1 ácido lático → 1 ácido acético + CO_2

Fonte: adaptado de Muck (2010).

Depois do ácido lático, o ácido acético é o ácido encontrado em maior quantidade na silagem, entre 1 a 3% da MS. Em concentrações moderadas, a presença desse ácido na silagem é desejável devido às suas características antifúngicas, são capazes de inibir leveduras, resultando em maior estabilidade aeróbia quando a silagem é exposta ao ar. Assim como no ácido lático, a concentração do ácido acético é inversamente proporcional ao teor de MS. Quando fornecido aos ruminantes, pode ser utilizado como fonte de energia ou ser incorporado no leite ou gordura corporal (KUNG JR et al., 2018). Os ácidos lático e acético, em conjunto com o pH baixo, são inibidores de vários microrganismos aeróbios (MUCK, 2010).

Quando há a presença do ácido butírico na silagem pode ser um indicativo de fermentação indesejável por clostrídios, pois algumas espécies são capazes de converter açúcares e o ácido lático em ácido butírico. Geralmente a presença desse ácido é associada com valores de pH altos, concentrações baixas do ácido lático e concentrações acima da média de ácido acético, nitrogênio amoniacal (N-NH3) e proteína solúvel (KUNG JR et al., 2018) e pode gerar alguns riscos à saúde do ruminante de forma direta pelo consumo da silagem mal fermentada ou de forma indireta através da redução do consumo energético dos componentes dietéticos da silagem (DRIEHUIS et al., 2018). Por possuir fortes características antifúngicas, quando o ácido butírico está presente na silagem, tende a torná-la mais estável à deterioração aeróbia (DANNER et al., 2003; KUNG JR et al., 2018).

Em silagem bem preservadas, o ácido propiônico é encontrado em concentrações baixas, (menor que 0,1% da MS). A partir da conversão da glicose por propionibactérias tem-se como produtos os ácidos propiônico, butírico e lático, no entanto, embora ocasionalmente encontradas em silagens, populações epifíticas dessas bactérias são improváveis de crescer na maioria das silagens (KUNG JR et al., 2018). Altas concentrações do ácido propiônico na silagem (entre 0,5a 1,3%) estão relacionadas com a fermentação de clostrídios, em particular a espécie *C. propionicum* ou pelo uso de aditivos com a finalidade de aumentar a estabilidade aeróbia (KUNG JR et al., 2018). Existem poucas informações na literatura a respeito da utilização de propionibactérias em inoculantes (HIGGINBOTHAM et al., 1998; ROWGHANI et al., 2008), nesses estudos as silagens apresentaram tendência a se aquecerem mais lentamente. Quando consumido pelos animais, esse ácido é absorvido no rúmen e convertido em glicose no fígado (KUNG JR et al., 2018).

Dentre os álcoois e ésteres, o etanol é o álcool mais encontrado em silagens, podendo ser produzido por uma variedade de microrganismos, como leveduras, enterobactérias e BAL heteroláticas. Altas concentrações de etanol em silagens estão associadas a altas populações de leveduras e perdas de MS. A presença de um grande número de leveduras na silagem pode torná-la mais suscetível à deterioração aeróbia, pois algumas leveduras são capazes de assimilar o ácido lático na presença do oxigênio (KUNG JR et al., 2018). Quando o etanol é ingerido pelo ruminante, ele é convertido em ácido acético ou absorvido pela parede do rúmen (BRUNING e YOKOYAMA, 1988), podendo ser convertido em gordura do leite ou disponível para o metabolismo do corpo ou crescimento, e em situações em que a concentração desse álcool é alta, pode ocorrer alterações no sabor do leite (KUNG JR et al., 2018).

Segundo Kung Jr et al. (2018), em silagens de milho e leguminosas a concentração de etanol geralmente é baixa, variando de 0,5 a 1,5% da MS, contrário a silagens de cana-de-açúcar que apresentam muitas leveduras epifíticas que convertem a sacarose em etanol, podendo chegar a concentrações de até 15% da MS (DANIEL et al., 2013). Alguns estudos relacionados ao uso de inoculantes para silagens de cana-de-açúcar (ÀVILA et al., 2010; ÀVILA et al., 2014; CARVALHO et al., 2014), em particular os compostos por cepas de BAL das espécies *L. brevis* e *L. hilgardii* têm se mostrado eficientes na redução da concentração de etanol.

A presença do 1,2-Propanodiol em silagens é geralmente resultado do metabolismo do ácido lático por populações de BAL da espécie *L. buchneri*. Em silagens inoculadas com *L. buchneri* com o intuito de melhorar a estabilidade aeróbia, os níveis detectáveis de 1,2-Propanodiol podem chegar de 0,25 a 1,5 % da MS, no entanto, algumas vezes esse metabólito é erroneamente usado como parâmetro para avaliar se inoculantes contendo *L. buchneri* foram

eficientes em dominar o processo fermentativo, uma vez que uma variedade de microrganismos é capaz de produzi-lo. Quando consumido pelos ruminantes, o 1,2-Propanodiol pode ser convertido em glicose no fígado ou convertido em ácido propiônico no rúmen (KUNG JR et al., 2018).

2.4 Capins tropicais: importância e desafios na produção de silagens

Assim como o milho e o sorgo, os capins tropicais possuem um grande potencial para a utilização como silagem em regiões de climas quentes devido ao seu alto rendimento, sendo que a maior parte desse rendimento ocorre durante a estação chuvosa, tornando a ensilagem uma ferramenta fundamental para melhorar seu uso durante o ano (DANIEL et al., 2019).

Quando colhidos no momento em que apresentam adequado valor nutritivo, os capins tropicais possuem alto teor de umidade (NUSSIO, 2005) e baixa concentração de carboidratos solúveis em água (CSA) (BERNARDES et al., 2018), dessa forma, durante o processo de ensilagem pode haver liberação de efluente e fermentações indesejáveis como as realizadas por bactérias do gênero *Clostridium* (GEBREHANNA et al., 2014).

2.4.1 Geração de efluentes

A produção de efluentes é resultante da ruptura celular e extravasamento de conteúdo citoplasmático durante o período em que a planta forrageira é armazenada, levando a perdas significativas na qualidade da silagem (SCHMIDT et al., 2011). Além da perda de matéria seca (MS), o efluente pode reduzir a quantidade de nutrientes presentes na silagem (GEBREHANNA et al., 2014).

O impacto ambiental que pode ser gerado também tem sido outro motivo de preocupação, visto que o efluente proveniente da silagem é rico em demanda bioquímica de oxigênio (DBO), nitrogênio, fósforo e possui baixo pH (GALANOS et al., 1995), sendo assim, se liberado sem que haja o tratamento correto, pode afetar negativamente a qualidade das águas superficial e subterrânea (OMAFRA, 2004 apud GEBREHANNA et al., 2014). Quando atinge cursos de água próximo ao silo, os componentes solúveis são metabolizados por microrganismos que consomem o oxigênio disponível na água, dessa forma, pode haver a escassez para outros organismos aquáticos (SCHMIDT et al., 2011).

O efluente apresenta pH ácido, o que o torna corrosivo devido às altas concentrações de ácidos orgânicos que o compõem, podendo assim danificar estruturas de concreto e ferro

(OMAFRA, 2004 apud GEBREHANNA et al., 2014), e também matar as vegetações contaminadas (SCHMIDT et al., 2011).

Segundo Schmidt et al. (2011), a DBO é um método de mensuração de potencial poluidor. Esse método avalia a concentração de matéria orgânica em águas residuárias e efluentes de esgotos domésticos e industriais, que se baseia no consumo de oxidante necessário para a completa oxidação dos componentes solúveis. Os autores ressaltam que a legislação ambiental brasileira determina valores de DBO de até 60 mg/L para esgotos e dejetos lançados em cursos de água e rios, no entanto, esse valor é bem inferior à DBO que pode ser observada em efluentes de silagens. Segundo Gebrehanna et al. (2014), a DBO dos efluentes gerados pela silagem pode exceder o valor de 49.000 mg/L.

2.4.2 Presença de *Clostridium spp.*

Clostridium spp. são bactérias gram-positivas formadoras de esporo, a maioria é anaeróbia obrigatória. São capazes de crescer e se prosperar em silagens com baixo teor de açúcar, sendo seu crescimento comum quando a planta apresenta as seguintes características: umidade superior a 70%, pH superior a 4,6, temperatura superior a 30 °C, alta capacidade tamponante (DRIEHUIS et al., 2018; QUEIROZ et al., 2018) e alta atividade de água (0,952 a 0,971) (DRIEHUIS et al., 2018). Sendo assim, as plantas que apresentam altas concentrações de umidade na colheita ou ensilagem e que possuem alta capacidade tamponante, atrasando o declínio do pH durante o processo fermentativo, podem favorecer o crescimento de clostrídios (DRIEHUIS et al., 2018), é o que ocorre com silagens de capins e alfafa, que diferentemente do milho não atingem facilmente o pH inferior a 4,0 (MUCK, 2010). A presença dessas bactérias na silagem ocorre principalmente pela contaminação do solo ou pela aplicação de adubo orgânico contaminado (QUEIROZ et al., 2018), sendo que geralmente seus efeitos sobre a qualidade da silagem ocorre algum tempo após as BAL cessarem o crescimento ativo (MUCK, 2010).

As espécies de *Clostridium* mais importantes presentes na silagem podem ser classificadas em três grupos de acordo com os principais compostos que metabolizam: [1] clostrídios proteolíticos que fermentam principalmente aminoácidos, nesse grupo estão inclusos tanto os clostrídios que possuem capacidade limitada para fermentar carboidratos (*C. sporogenes* e *C. bifermentans*) quanto os que são capazes de fermentar uma variedade de carboidratos (*C. sphenoides*), [2] grupo *Clostridium butyricum* (também inclui *C. beijerincki* e *C. acetobutylicum*) que fermentam carboidratos e [3] *Clostridium tyrobutiricum* que além de

fermentar alguns açúcares, é capaz de fermentar ácidos orgânicos, com destaque ao ácido lático. Como resultado do catabolismo de aminoácidos, há uma diversidade de produtos gerados, sendo os principais as amônias, aminas e o dióxido de carbono. Já os grupos de *C. butiricum* e *C. tyrobutyricum* produzem o ácido butírico, ácido acético, hidrogênio e o dióxido de carbono (DRIEHUIS et al., 2003; MUCK, 2010). Clostrídios proteolíticos incapazes de fermentar carboidratos não são normalmente encontrados na silagem, apesar de *C. botulinum* pertencer a esse grupo, a sua presença em silagens é atípica (DRIEHUIS et al., 2003).

Muck (2010) cita alguns prejuízos na qualidade da silagem que as fermentações indesejáveis por clostrídios podem causar. Em primeiro, com o aumento da atividade de clostrídios na silagem (concentração de ácido butírico superior a 5 $g.kg^{-1}$ de MS) pode haver a redução do consumo pelos animais, sendo que a causa dessa redução ainda é incerta, já que o ácido butírico é um ácido comum no rúmen. Apesar das aminas estarem associadas a queda de ingestão por pequenos ruminantes, em bovinos ainda é incerto. Em segundo, o consumo de silagens com alta atividade de clostrídios por vacas produtoras de leite em transição pode torná-las suscetíveis à cetose devido à alta quantidade de ácido butírico consumido. E em terceiro, a fermentação do ácido lático em ácido butírico pode gerar uma perda de até 51% de MS e 18% de perda de energia bruta.

Além dos efeitos na qualidade da silagem, os esporos presentes no alimento fornecido aos animais podem sobreviver ao processo digestivo e através do esterco entram em contato com o úbere e contaminam o leite (VISSERS et al., 2007). A presença de clostrídios no leite pode causar sabores desagradáveis e o denominado “estufamento tardio” em queijos duros e semiduros, no qual há a formação excessiva de gases, seguida de defeitos na textura e sabor desagradável durante o período de maturação (AURELI e FRANCIOSA, 2003). Além disso, a contagem superior de 1000 esporos de bactérias do ácido butírico (BAB)/L de leite no tanque das fazendas tem consequência econômica negativa para o produtor em países que adotam o critério de contagem inferior a esse valor para o pagamento do leite (VISSERS et al., 2007).

Driehuis et al. (2016) avaliaram a presença de esporos de bactérias produtoras do ácido butírico em amostras de solo, silagem de milho, silagem de capim, fezes de vacas leiteiras e leite de tanque em fazendas leiteiras na Holanda. Foi possível detectar 3 principais espécies encontradas em todas as amostras, sendo duas dessas, pertencentes ao gênero *Clostridium* (*C. tyrobutyricum* e *C. beijerinckii*) e outra pertencente ao gênero *Paenibacillus* (*P. polymyxa*). Segundo os autores, as espécies foram encontradas em proporções semelhantes nas amostras, indicando que as três populações de esporos compartilham as mesmas fontes e a mesma rota de

contaminação do leite. As duas espécies de *Clostridium* encontradas são comumente relatadas em queijos com estufamento tardio e são capazes de produzir o ácido butírico.

Calamari et al., (2018) avaliaram a presença de população de esporos anaeróbios e anaeróbios facultativos em amostras de solo, silagem de milho, ração mista total (TMR), fezes e leite em 49 fazendas leiteiras. Dentre as três espécies que foram detectadas em todas as amostras, uma delas é pertencente ao gênero *Clostridium* (C. *tyrobutyricum*). A contagem de esporos da TMR foi influenciada pela quantidade de silagem de milho deteriorada presente na TMR. A maioria das fazendas que eliminaram a fração de silagem com presença de bolores foram capazes de manter a contagem de TMR abaixo de 4,0 log esporos/g. Quando o número superou 4,5log esporos/g na TMR, as fezes apresentaram contaminação superior a 3,0 log esporos/g. A maioria das fazendas que apresentaram contaminação de fezes acima de 5,0 log esporos/g tiveram uma contaminação de esporos no leite superior a 1.000 esporos/L. Esses resultados evidenciam que não somente boas práticas de ordenha são suficientes para assegurar baixos níveis de contaminação no leite, e que é fundamental um manejo correto de silagem e eliminação das frações deterioradas, já que possuem um grande potencial em contaminar o leite com esporos.

As espécies mais comumente encontradas na silagem incluem *C. tyrobutyricum*, *C. beijerinckii*, *C. butyricum* e *C. sporogenes*, no entanto, essas bactérias não são patogênicas (DRIEHUIS, 2013). Apesar de existir algumas espécies patogênicas como *C. perfringens*, *C. difficile*, *C. tetani* e *C. botulinum* (DOYLE et al., 2015), segundo Driehuis et al. (2018), apenas *C. botulinum* é ocasionalmente associado à silagem.

C. botulinum é o agente causador do botulismo, uma intoxicação causada pela ingestão de alimentos contaminados com toxinas de origem microbiana mais potentes conhecidas. Após a ingestão pelos animais, essas toxinas resistem à degradação proteolítica no trato gastrointestinal e são absorvidos pela mucosa intestinal, atuando nas junções neuromusculares (SILVA et al., 2016), as proteínas SNARE, responsáveis pela liberação de neurotransmissores excitatórios nas junções neuromusculares são clivadas (AOKI et al., 2010), e dessa forma, ocorre a redução da liberação de acetilcolina nas junções neuromusculares, levando à incapacidade de ocorrer a contração muscular (LOBATO et al., 2013).

Apesar de não ocorrer com muita frequência, nos últimos 13 anos alguns surtos de botulismo foram registrados em fazendas que utilizavam silagens de capim, e a presença de *C. botulinum* estava associada a valores de pH muito altos, entre 5,3 e 6,4 (DRIEHUIS et al., 2018).

Na Finlândia, em 2006, 9 de 90 animais em uma fazenda de gado de leite morreram após serem alimentados com silagem de capim mal preservada e contaminada com carcaça de animais. O gene da neurotoxina botulínica do tipo C foi detectado em uma novilha por reação em cadeia da polimerase (PCR) e a neurotoxina foi detectada pelo bioensaio em camundongos (MYLLYKOSK et al., 2009).

Em 2010, na Áustria 6 vacas Simental de um rebanho de 29 animais foram afetadas e mostraram os sinais típicos de botulismo, como decúbito e dificuldades de deglutição, segundo relatos, os animais teriam sido alimentados com silagem de capim mal preservada e contaminada com carcaça de gatos descartadas no pasto. O surto foi diagnosticado clinicamente pela confirmação das neurotoxinas C e D de *C. botulinum* por bioensaio em camundongos (KUMMEL et al., 2012).

Em 2015, na França, 80 de 110 vacas Holstein morreram após um surto de botulismo bovino do tipo D e C em uma fazenda com criação de aves e bovinos leiteiros. As investigações epidemiológicas e laboratoriais indicaram que o surto foi causado provavelmente pela alimentação do gado com silagem de capim insuficientemente acidificada e que a fonte dos esporos apesar de incerta, pode ter sido uma pilha de cama de frango armazenada na pastagem de capim antes da colheita (RELUN et al., 2017).

2.4.3 Estratégias para melhorar o processo fermentativo

O emurchecimento ou a aplicação de aditivos são frequentemente necessários para reduzir o risco de fermentação butírica e produção de efluentes em silagens de capins tropicais (NUSSIO, 2005). Segundo Daniel et al. (2019), apesar da prática de emurchecimento ser uma estratégia viável para diminuir o teor de umidade em forragens que apresentam caules curtos e finos, tem-se vários tipos de capins tropicais altos e com caules grossos que são difíceis de serem murchados, como é o caso do capim elefante (*Pennisetum purpureum*). Ainda de acordo com os autores, outro importante ponto negativo do emurchecimento é o fato de que tal prática pode aumentar as perdas de campo.

A adição de alimentos secos na silagem pode reduzir a lixiviação pela absorção e adicionar uma melhor digestibilidade da matéria seca (MS). Como exemplos tem-se a polpa cítrica, casca de soja, farelo de trigo, grão de milho, entre outros. No entanto, em regiões onde tais produtos são escassos, ou que apresentam altos valores, essa técnica tem sido questionada, tornando os produtores dependentes de aditivos, cuja maioria utilizada é baseada em

inoculantes compostos por BAL. No entanto, a utilização de inoculantes tem apresentado resultados inconsistentes (DANIEL et al., 2019).

Todavia, essa imprecisão dos resultados nos estudos envolvendo inoculantes pode ocorrer devido à falta de compatibilidade entre o microrganismo e a planta forrageira utilizada, uma vez que o teor de carboidratos e a população epifítica podem variar entre as diversas plantas ensiladas (MUCK, 2008; ÁVILA et al., 2009), além disso, pode haver diferenças entre as cepas pertencentes à mesma espécie de bactéria inoculadas, resultando em silagens com diferentes características químicas e microbiológicas (ÁVILA et al., 2010), segundo Ávila et al. (2014), essa compatibilidade pode ser avaliada pela capacidade dos microrganismos de usar os carboidratos disponíveis e produzir metabólitos de interesse. Recentemente tem aumentado o número de pesquisas relacionadas à seleção de inoculantes estimulados pela obtenção de cepas promissoras através de testes em laboratório (SAARISALO et al., 2007; SANTOS ET AL., 2013; ÁVILA et al., 2014; CARVALHO et al., 2014; AMARAL et al. 2020).

2.5 Utilização de inoculantes

Os inoculantes são a classe de aditivos mais comuns e são compostos principalmente por BAL, cuja finalidade é suplementar as BAL epifíticas presentes em determinada cultura e auxiliá-las a garantir um processo fermentativo consistente no silo (MUCK, 2010). As BAL devem estar presentes no inoculante em número suficiente para dominar de forma eficiente o processo de fermentação, sendo a dose ideal aplicada entre 10^5 a 10^6 unidades formadoras de colônia (UFC) por grama de forragem fresca (KUNG JR et al., 2003).

Os inoculantes homofermentativos são os inoculantes bacterianos mais antigos e mais comumente utilizados na produção de silagens por décadas (MUCK et al., 2018). Porém, atualmente a maioria das bactérias que antes pertenciam a esse grupo são agrupadas taxonomicamente como heterofermentativas facultativas (PAHLOW et al., 2003), pois possuem a enzima fosfoquetolase, a qual permite que essas bactérias fermentem pentoses, produzindo principalmente os ácidos lático e acético. Como principais exemplos de espécies heterofermentativas facultativas tradicionalmente utilizadas como inoculantes incluem *Lactiplantibacillus plantarum*, *Lacticaseibacillus casei*, *Enterococcus faecium* e várias espécies de *Pediococcus* (MUCK et al., 2018)

Os efeitos que ocorrem em silagens tratadas com essa classe de inoculante incluem concentrações mais baixas de pH, menores concentrações dos ácidos acético e butírico e N-

NH_3 e altas concentrações de ácido lático com maior recuperação de MS (MUCK e KUNG JR, 1997).

Apesar de inconsistentes, alguns estudos mostram que os inoculantes contendo bactérias homofermentativas obrigatórias ou heterofermentativas facultativas têm como principal problema a redução na estabilidade aeróbia da silagem. Como possível explicação, acredita-se que devido a menor produção do ácido acético, pode haver o aumento de populações de leveduras e dessa forma contribuir para a aceleração do processo de deterioração da silagem, já que esse ácido possui propriedades antifúngicas (MUCK et al., 2018).

Em meados dos anos 90, a classe de inoculantes com bactérias heterofermentativos obrigatórias foram desenvolvidos com o objetivo de proporcionar melhor estabilidade aeróbia à silagem, principalmente às culturas mais suscetíveis à deterioração (KUNG JR, 2009). A principal espécie de BAL heterofermentativa obrigatória estudada é *Lentilactobacillus buchneri*, no entanto, outras espécies como *Levilactobacillus brevis*, *Lentilactobacillus diolivorans*, *Lentilactobacillus hilgardii*, *Lentilactobacillus kefiri* e, recentemente, *Lentilactobacillus parafarraginis* e *Letilactobacillus farraginis* são ocasionalmente avaliadas como inoculantes. Até então, o aumento na estabilidade aeróbia tem sido relacionado a produção do ácido acético, mas também há a hipótese de que efeitos múltiplos ou sinérgicos podem ser responsáveis por melhorias na estabilidade aeróbica além do ácido acético (MUCK et al., 2018), como a produção dos ácidos fenilático e 4-hidroxi-fenil-lático que possuem propriedades antifúngicas e que são produzidos por *L. hilgardii* (VALERIO et al., 2004).

No início dos anos 2000, iniciaram-se estudos com inoculantes que combinam BAL heterofermentativas facultativas e heterofermentativas obrigatórias com o objetivo de alcançar os benefícios de ambas em um único inoculante, ou seja, a BAL heterofermentativa facultativa teria como finalidade controlar o processo fermentativo inicial inibindo microrganismos indesejáveis como enterobactérias e clostrídios reduzindo as atividades de proteólise e perda de MS pelo rápido decréscimo do pH, e a BAL heterofermentativa obrigatória, no caso *L. buchneri* converteria lentamente o ácido lático em ácido acético após o período inicial de fermentação, dessa forma aumentaria a estabilidade aeróbia da silagem e elevaria o pH (MUCK et al., 2018).

Durante os últimos anos, várias cepas de BAL heterofermentativas facultativas ou homofermentativas obrigatórias foram utilizadas em associação com a espécie mais estudada, a bactéria *L. buchneri* (MUCK et al., 2018). Alguns estudos mostram que essa combinação foi eficiente na redução da contagem de fungos e aumento na produção do ácido acético (SHIMIDT e KUNG JR, 2010), maior recuperação da MS e menor concentração de etanol (REICH e

KUNG, 2010) e rápido declínio do pH (SCHMIDT et al., 2009; ADDAH et al. 2012; ARRIOLA et al., 2015).

Zopollatto et al. (2009) fez em um levantamento de 15 artigos publicados em periódicos científicos sobre a utilização de inoculantes em silagens de capins tropicais no Brasil. Foram avaliadas as médias de diferentes parâmetros em resposta a utilização de inoculantes contendo bactérias homofermentativas, heterofermentativas ou a combinação entre as duas. As silagens inoculadas com bactérias homofermentativas apresentaram em média maior queda de pH, maior concentração de carboidratos solúveis em água (CSA), maior taxa recuperação de matéria seca (MS) e uma leve redução nas concentrações de fibra em detergente neutro (FDN) e fibra em detergente ácido (FDA), contudo, não foram capazes de reduzir a produção de efluentes. As bactérias heterofermentativas, em média, apresentaram maior concentração de proteína bruta (PB) e queda na recuperação de MS. Já a combinação entre as bactérias homofermentativas e heterofermentativas apresentou uma taxa de recuperação de MS razoável, altas concentrações de PB e MS, redução na produção de efluentes e queda no teor de FDA, porém o pH teve um leve aumento.

Rabelo et al. (2016) fizeram um levantamento da utilização de inoculantes homofermentativos, heterofermentativos e a combinação entre esses dois em silagens de capins tropicais. Foram observados os dados de 45 estudos, a maioria desses estudos (18) foram realizados com o capim elefante (cultivar Napier e Cameroon). A taxa de aplicação dos inoculantes nos experimentos variou de 5×10^4 a 8×10^{10} UFC/g de forragem fresca. Como resultado, as silagens tratadas com inoculantes contendo bactérias homofermentativas apresentaram uma média geral de aumento na concentração do ácido lático em 29,4%, com uma queda de pH de 4,75 (controle) para 4,47, as perdas de MS e concentração de $N\text{-}NH_3$ diminuíram cerca de 11,4 e 11,7%, respectivamente. Segundo os autores, a queda do $N\text{-}NH_3$ é provavelmente devido a uma rápida queda do pH, evitando a proteólise pela planta e microrganismos indesejáveis, como clostrídios e enterobactérias. Apesar da proposta principal dos inoculantes contendo bactérias homofermentativas não seja provocar o aumento na estabilidade aeróbia, curiosamente, a média geral dos estudos mostrou um aumento na estabilidade aeróbia de 59,5 para 114 horas.

Nos trabalhos avaliados com inoculantes contendo bactérias heterofermentativas, a espécie utilizada foi *L. buchneri*. No geral, os resultados mostram que houve queda na qualidade da silagem pelo aumento do pH, $N\text{-}NH_3$ e FDN e redução do teor de proteína bruta (PB). O número de estudos que utilizaram inoculantes combinados foram baixos e os resultados

inconsistentes, no entanto, o pH e a concentração de N-NH_3 apresentaram tendência em reduzir, e as perdas de MS e teor de PB apresentaram tendência em aumentar.

2.6 Capim elefante BRS Capiaçu: características e uso como silagem

O capim elefante (*Pennisetum purpureum* Schum.) é uma monocotiledônea pertencente à família Poaceae e ao gênero *Pennisetum*, sendo que muitas de suas cultivares foram desenvolvidas para se adequar às condições nas mais diversas regiões, apresentando alto potencial produtivo e adequado valor nutritivo. O alto potencial produtivo de MS, facilidade de propagação, cultivo e colheita são os motivos da sua frequente adoção entre os agricultores (RUSDY, 2016). É o capim perene mais utilizado para produção de silagens no Brasil (CÂNDIDO et al., 2007), capaz de se adaptar às condições climáticas e ao solo de praticamente toda a extensão territorial do Brasil (DERESZ, 1999).

Recentemente, o programa de melhoramento de capim elefante da Embrapa Gado de Leite desenvolveu a cultivar BRS Capiaçu (Figura 2), registrada pelo Ministério da Agricultura Pecuária e Abastecimento (MAPA) em 2015. Como características principais, essa cultivar apresenta alto porte, touceiras de formato ereto, folhas largas e compridas, colmos grossos, elevada densidade de perfilhos basais, florescimento tardio, e se destaca em relação a outras cultivares pelo alto rendimento e qualidade da forragem (Tabela 2), facilidade para a colheita mecânica e resistência ao tombamento (PEREIRA et al., 2017).

Tabela 2: Rendimento e teor de proteína bruta (PB) da cultivar BRS Capiaçu.

Cultivares	Produção total de MS anual ($t.ha^{-1}$)	Produção de MS foliar anual ($t.ha^{-1}$)	Teor de PB (%)
BRS Capiaçu	49,75	21,60	9,10
Mineiro	36,79	16,16	6,94
Cameroon	29,87	14,32	7,17

Fonte: adaptado de Pereira et al., (2017).

A cultivar BRS Capiaçu pode ser empregada tanto para a produção de silagens como para o fornecimento da planta verde picada direto no cocho, e apresenta alta tolerância ao estresse hídrico, sendo uma alternativa ao cultivo do milho em regiões com alto risco de ocorrência de veranicos. Comparada a outras culturas para silagem apresenta menor custo de produção, com produção média de 100 t/ha/corte de massa verde, ou seja, considerando três

cortes anuais, tem-se uma produtividade de 300 t/ha/ano, representando cerca de três vezes a produção de biomassa de culturas como o milho e o sorgo (PEREIRA et al., 2016).

Assim como ocorre em outros capins tropicais, quando a cultivar BRS Capiaçu alcança o melhor valor nutritivo, as concentrações de CSA e MS são baixas, podendo gerar efluentes e ocorrer fermentação indesejável. Dessa forma, torna-se necessária a realização da colheita quando a planta estiver mais madura, com o objetivo de se obter a melhor relação entre produção de biomassa, valor nutritivo e concentração de MS. (PEREIRA et al., 2016). Por ser uma cultivar introduzida recentemente, definir qual o momento ideal de corte em função da concentração de MS é um desafio para produzir silagens de boa qualidade.

Figura 2: Capim elefante cultivar BRS Capiaçu

Fonte: autor.

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SEGUNDA PARTE – ARTIGO

Cutting management and inoculants alter the fermentative profile and quality of elephant grass silage cultivar BRS Capiaçu

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ABSTRACT

The BRS Capiaçu elephant grass stands out for its high yield and forage quality, being an option to produce silages. Still, unwanted fermentations are common in this silage that can be avoided by adjusting the forage dry matter (DM) concentration and using inoculants. This study aimed to evaluate the effect of new inoculants previously selected for elephant grass cultivar BRS Capiaçu silages harvested with different DM contents at different storage times. Fermentative profile, microbial population, particle distribution, digestibility, meta taxonomy of prokaryotes, and aerobic stability of silages with 62 and 104 days of storage were evaluated. Forage with high DM showed higher concentrations of acetic acid and neutral detergent fiber (NDF) and higher microbial counts. All inoculants reduced the population of undesirable microorganisms in silages. The LH and LP + LH inoculants stood out for reducing the DM losses of silages with low DM (32.3 and 21.7 g kg-1 DM, respectively), reducing the ethanol concentration with 104 days of storage (6 .01 and 3.36 g kg-1 DM, respectively), reduce the yeast population with 62 days of storage (2.44 and 2.12 log CFU.g-1, respectively) and increase the aerobic stability in the silage with high DM after 62 days of storage (47.75 and 45.50 h, respectively). Silages with low DM had the lowest DM losses (average of 53.0 g kg-1), higher lactic acid production (average of 64.06 g kg-1 DM), and lower pH values (average of 4,55). The profile of the population of prokaryotes in the silage with high DM showed greater abundance about the silage with low DM. The CCMA 0170 (LH) strain is promising for use as an inoculant in silages of elephant grass BRS Capiaçu. Due to the better fermentative profile presented, it is recommended to ensile forage with low DM. Ensilage storage time it affected the fermentative profile, microbial population and particle distribution of silages.

Keywords: *Lactiplantibacillus plantarum*, *Lentilactobacillus hilgardii*, Storage time, Next Generation Sequencing – NGS, aerobic stability.

1. Introduction

Elephant grass is a tropical, perennial grass with a high forage accumulation potential (it can produce 50 t/ha/year of DM), adequate nutritional value, acceptability by different animal species, vigor, and persistence, in addition to the low incidence of pests and diseases, reasons that make it one of the most important forage species, being cultivated in almost all tropical and subtropical regions (Pereira et al., 2021). Although there are many elephant grass cultivars used in Brazil, the BRS Capiaçu cultivar, developed by the elephant grass breeding program of Embrapa Gado de Leite, has stood out for its high forage yield and quality, ease of mechanical harvesting, and resistance to falling over. Due to its high productivity and morphological characteristics, Capiaçú grass has been used mainly in silage, allowing greater use of its productive potential, especially in the rainy season (Daniel et al., 2019).

Despite their qualities, tropical grasses, when harvested for ensilage at the time when they have the best nutritional value, have a high moisture content and low concentration of water-soluble carbohydrates (WSC) (Bernardes et al., 2018). Thus, strategies such as wilting or application of additives are often necessary to reduce the risk of unwanted fermentations and effluent production (Daniel et al., 2019).

Biological inoculants are the most used technology to assist in the fermentation of silages (Wilkinson & Muck, 2019). These products often contain lactic acid bacteria (LAB) to supplement the epiphytic LAB and help ensure proper fermentation in the silo (Muck, 2010). In Brazil, homofermentative LAB-containing inoculants have consistently improved the fermentation parameters of tropical grass silages, whose main objective is to ensure a rapid decline in pH at the beginning of fermentation due to the larger production of lactic acid. In contrast, inoculant classes containing mandatory heterofermentative LAB or a combination of strains are scarce and show inconsistent results (Rabelo, 2016).

As much as the class of inoculants containing homofermentative LAB is recommended for tropical grasses, recent studies of inoculant selection have shown that more than choosing an inoculant by observing only its class is needed. After all, several other factors are involved in the effectiveness of an inoculant, with these effects being dependent on the strains, which, even belonging to the same species, have different metabolic behaviors (Carvalho et al., 2021).

Elephant grass BRS Capiaçu is a recently introduced cultivar. Research related to the effect of DM adjustment and inoculants on this forage's microbiological and fermentative profile is scarce in the literature. As this cultivar reaches maturity, the DM content also increases, but there is a reduction in the nutritional value of the plant, making it relevant to

evaluate the ideal cutting moment in which the plant presents a balance between DM and nutritional value and even what extent it influences the microbial community.

In addition, the species *Lentilactobacillus hilgardii* has shown excellent results in silages of other forages, proving to be a promising species for use in inoculants, which has not yet been explored in elephant grass silage. In this way, evaluating the effects of new strains selected in this cultivar becomes relevant to serve as a basis for further research and to be an alternative for roughage supplementation of good quality for use in production systems. This study aimed to evaluate the effect of new inoculants on elephant grass cultivar BRS Capiaçu silages with different DM contents and storage times.

2. Material and methods

2.1. Elephant grass harvest

The elephant grass (*Pennisetum purpureum* Schum.) cultivar BRS Capiaçu was grown on the Reunidas ACP & Filhos farms in Carmo do Rio Claro – MG. Forage was harvested and chopped by a self-propelled forage harvester (Claas Jaguar 860) with the theoretical particle size set to 19 mm. Two different DM contents were defined in which the forages were harvested, called: 1) low DM, with 90 days of regrowth, cut 30 cm from the ground and with a height of 3.2 m, and 2) high DM, with 110 days of regrowth, cut 50 cm from the ground and with a height of 3.6 m (Table 1).

Table 1. Characteristics of elephant grass forage cultivar BRS Capiaçu harvested with different dry matter (DM) contents.

Forage harvested with low DM		Forage harvested with high DM	
Regrowth time	90 days	Regrowth time	110 days
Plant height	3.2 m	Plant height	3.6 m
Cutting height	30 cm	Cutting height	50 cm

2.2. Inoculants used and silage preparation

The evaluated strains were CCMA 1394 – *Lactiplantibacillus plantarum* (LP), CCMA 0170 – *Lentilactobacillus hilgardii* (LH), and their combination (LP + LH). These LABs are deposited in the Agricultural Microbiology Culture Collection (CCMA) at the Federal

University of Lavras, Brazil. The strains were selected for reducing DM losses and increasing elephant grass silage's aerobic stability (Amaral et al., 2020).

The inoculants were cultivated in MRS broth (Man Rogosa and Sharpe - MERCK®). Subsequently, 150 ml of the inoculant was mixed with 50 ml of distilled water and applied to the forage, resulting in 13.3 L per ton. The average population of inoculated LAB was 8.84 ± 0.12 log of colony forming units (CFU)/g of forage of each LAB, and, in the LH + LP treatment, half of this population of each strain was applied. The same volume of distilled water was applied to the forage used as a control.

The silage was compacted in experimental silos (plastic containers with a volume of 30L) to an approximate density of 600kg/m^3. The silos were sealed, weighed, and stored, protected from rain and sunlight. After 62 and 104 days, the silos were weighed and opened. Samples were taken and fractionated to analyze the chemical composition, particle distribution, in vitro DM digestibility, quantification of the population of microorganisms, metataxonomic analysis, pH analysis, fermentation products, and aerobic stability.

2.3. Particle distribution, in vitro DM digestibility, and chemical composition

Particle size estimation was performed using the wet samples through a Penn State Particle Size Separator set (Kononoff et al., 2003a) containing three sieves with 19, 8, and 4 mm diameters and a bottom.

The samples were dried in an oven with forced ventilation at 55°C for 72 hours and ground in a Wiley mill (1 mm circular sieve), as the Association of Official Analytical Chemists - AOAC (1990) recommended.

The in vitro digestibility of DM (IVDDM) was evaluated following the method of Tilley and Terry (1963), adapted by Holden (1999), in which filter bags containing buffer solution and ruminal fluid were kept incubated in a Daisy incubator (ANKOM Technology Corp.; Macedon, NY, USA).

Determining dry matter (DM) content followed the methodology proposed by AOAC (1990). Neutral detergent fiber (NDF) analysis was performed without the addition of amylase, using sodium sulfite according to Van Soest et al. (1991) using an ANKOM 200 Fiber Analyzer (ANKOM Technology Corp., Macedon, NY, USA).

Water-soluble carbohydrates (WSC) were determined in the silage aqueous extract using phenol/sulfuric acid (DUBOIS et al., 1956).

2.4. Microbial count, metabolites, ammoniacal nitrogen, and pH

Microbial analyses, fermentation products and pH value were evaluated from an aqueous extract. This extract was prepared by homogenizing (20 min in an orbital shaker) 30 g of fresh silage in 270 ml of sterile peptone water (0.1%).

For the analysis of microorganisms, the extract was subjected to serial decimal dilutions from 10^{-1} to 10^{-6}, and subsequently, 0.1 ml aliquots of each dilution were spread in triplicate on the culture media. LABs were quantified in MRS medium (MERCK®) added with nystatin (4 ml/l) and incubated at 36 °C for 48 hours. Yeasts and filamentous fungi were quantified in DRBC medium (Dicloran Rose Bengal Chloramphenicol - HYMEDIA®).

Yeasts were incubated at 28°C for 48 hours, and filamentous fungi at 28°C for 120 hours. Enterobacteria were quantified in VRBGA medium (Violet Red Bile Glucose Agar - KASVI®) and incubated at 37 °C for 24 h. Mesophilic microorganisms and aerobic spore-forming bacteria (ASB) were quantified in Nutrient Agar medium (HYMEDIA®). Mesophiles were incubated at 37°C for 24 hours. For the ASB count, the samples were previously pasteurized at 80°C for 10 minutes to cause the death of the vegetative cells. After plating, they were incubated at 37°C for 24 hours.

A 2 ml aliquot of the aqueous extract was acidified with 10 μL of sulfuric acid (H_2SO_4) 50% (v/v) and frozen for later analysis of lactic, acetic, propionic, succinic, and butyric acids and alcohols, ethanol and 1, 2-propanediol by high-performance liquid chromatography (HPLC) according to Carvalho et al. (2017). A fraction of the aqueous extract was separated and frozen for later analysis by the method of Chaney and Marbach (1962) for the determination of ammoniacal nitrogen ($N\text{-}NH_3$). The pH was determined using a digital potentiometer (Digimed Analytica, model DM20®).

2.5. Aerobic stability

After each storage time, 5 kg silage samples from each treatment were placed in plastic bags and kept in a room with monitored ambient temperature. Inside each bag (approximately in the central position), a Data Logger (Impac, model MI-IN-D-2-L) was positioned, recording the temperature every 30 minutes for seven days. Aerobic stability was considered when the silage temperature remained stable until it increased by 2 ºC about the ambient temperature after opening the silo.

2.6. Metataxonomic analysis

The silage samples were centrifuged, and the obtained pellet was used for genomic DNA extraction. DNA was isolated using the modified phenol/chloroform protocol described by Bashir et al. (2015). After extraction, DNA integrity was assessed by agarose gel electrophoresis, and DNA concentration and purity were determined by QIAxpert (Qiagen, USA).

Amplification of the V3–V4 region of the 16S rRNA gene was performed following the protocol described by Klindworth et al. (2013). The primer pairs used were S-D-Bact-0341-B-S-17, 5' -CCTACGGGNGGCWGCAG-3' and S-D-Bact-0785-a-A-21, 5' -GACTACHVGGGT ATCTAATCC-3'. The DNA was sent to the Molecular Diagnostic Laboratory of the Federal University of Lavras (LabMol/UFLA) for sequencing.

The reads were processed using the Qiime2 program. Forward and reverse sequences were imported and merged and reads smaller than 240 base pairs were removed. Quality control was performed using the Q score >30. OTUs were generated by the NOVO method and grouped with 99% similarity, as chimerical and unclassified sequences were removed from the analyses.

Taxonomic identifications were made using the taxonomic Silva databases (https://www.arb-silva.de/). Diversity and Random Forests analyses were performed on the MicrobiomeAnalyst platform (https://www.microbiomeanalyst.ca/).

The ANOVA statistical test was performed with a significance level of 0.05 probability to evaluate the statistical difference between the alpha diversity indices. A PERMANOVA analysis was performed using a PCoA as a sorting method for the beta diversity index. The non-parametric test based on phylogenetic trees called Random Forest was used for the differential abundance analysis.

2.7. Experimental design and statistical analysis

The experiment was conducted in a completely randomized design (CRD) with a factorial arrangement (4 × 2 × 2), with four possibilities for inoculants (control - without inoculant, LH, LP, and LH + LP), two storage times (62 days and 104 days) and two forage DM contents (high and low) with four replications, totaling 64 experimental units. The evaluation of the silage aerobic stability was carried out separately for each storage time and followed the same design with a factorial arrangement (4 × 2), with four possibilities for inoculants (control - without inoculant, LH, LP, and LH + LP), and two forage DM

concentrations (high and low). The data were submitted for analysis of variance using the SISVAR® computational package (Analysis of Variance for Balanced Data) (Ferreira, 2008). Means were compared using the Scott-Knott test.

3. Results

3.1 Elephant grass cv. BRS Capiaçu data before ensiling

The chemical and microbiological composition of BRS Capiaçu elephant grass harvested with different DM contents is shown in Table 2. The forage harvested with low DM had a DM content of 167.7 $g.Kg^{-1}$ of fresh matter (FM), an NDF concentration of 725.66 $g.Kg^{-1}$ of DM and a WSC concentration of 17 $g.Kg^{-1}$ from DM. The forage harvested with high DM had a DM content of 242.6 $g.Kg^{-1}$ of MF, an NDF concentration of 766.16 $g.Kg^{-1}$ of DM and a WSC concentration of 14.83 $g.Kg^{-1}$ of DM (Table 2).

Succinic acid concentrations were higher in high DM, while lactic acid, acetic acid, and ethanol concentrations were lower (Table 2). The population of microorganisms evaluated was similar between the two DM contents, with a slight difference, showing lower numerical values for low DM (Table 2). The forage harvested with low DM had a larger particle size, with 90.3% of the particles retained on the 8 mm sieve, compared to the forage harvested with high DM (83.4%) (Table 2).

Table 2: Chemical and microbiological composition and particle distribution of BRS Capiaçu elephant grass before ensiling harvested with different dry matter contents.

Variable	Low dry matter	High dry matter
pH	6.2	6.2
Dry matter ($g.Kg^{-1}$ FM)	167.7	242.6
Concentration ($g.Kg^{-1}$ DM)		
Neutral detergent fiber	725.66	766.16
Water-soluble carbohydrates	17.00	14.83
Lactic acid	11.21	10.24
Acetic Acid	55.94	25.36
succinic acid	12.40	15.19
Ethanol	6.27	4.22
Count (log $UFC.g^{-1}$ forage)		

Lactic acid bacteria	9.18	9.31
Enterobacteria	5.64	6.08
Yeasts	6.07	6.20
Filamentous fungi	2.53	2.63
Mesophilic microorganisms	8.06	8.95
ASB*	2.42	2.51
Particle distribution (%)		
19mm	50.39	24.36
8mm	39.88	59.09
4mm	7.59	12.78
Bottom	2.14	3.77

*Aerobic spore-forming bacteria

3.2. Effect of inoculant addition, DM content, and storage time

The DM content affected all characteristics evaluated, except the in vitro digestibility of DM, the population of filamentous fungi and yeast (Table 3). On average, silages with high DM showed more significant DM losses, lower WSC concentration, higher NDF concentration, and higher pH (Table 4). However, there was an interaction between DM and storage time and between DM and microbial inoculant for some variables (Table 3).

In silages with low DM, losses increased from 62 to 104 days, while in silages with high DM, there was no increase in losses from 62 to 104 days (Table 5). On average, all inoculants reduced losses about the control (Table 4), but there was an interaction with the DM content (Table 3). The LH and LP + LH inoculants reduced DM losses in both low DM and high DM silage. The addition of LP, however, reduced losses only in silages with high DM, and in these, it was more efficient than LH and LP + LH (Table 6).

There was an interaction between DM and inoculant ($P=0.023$) on NDF concentrations (Table 3). In silages with high DM, inoculation with LH resulted in a lower concentration of NDF when compared to the other inoculants. In low DM, the inoculants had no effect (Table 6).

There was an interaction ($P<0.001$) between the DM content and the inoculant and between DM and storage time on DM digestibility (Table 3). At 62 days of storage, silage with low DM showed greater digestibility (53.31%) than silage with high DM (48.28%). At 104 days of storage, there was no statistical difference between treatments (Table 5). In silages with low DM, there was a drop in digestibility from 62 to 104 days of storage (53.31% and 49.76%, respectively). There was no significant effect in silages with high DM (Table 5). Silages with

low DM inoculated with LP+LH and the control showed greater digestibility. Silages with high DM, all inoculated silages showed greater digestibility than the control (Table 6). Silages inoculated with LP and LH showed greater digestibility at high DM than at low DM. The control showed greater digestibility at low DM, and silages inoculated with LP+LH did not differ in DM concentration (Table 6).

WSC concentrations were influenced by DM and storage time (Table 3), being higher in silages with low DM and 62 days of storage (Table 4).

For N-NH3 concentration, there was an influence of the factors DM, inoculant, and storage time (Table 3). Silages with high DM had a higher concentration of N-NH_3 compared to silages with low DM. Silages inoculated with the LP inoculant showed the highest concentrations of N-NH_3. With the increase in silage storage time, there was a decrease in N-NH_3 concentrations (Table 4).

Evaluating the main factor, on average, silages with low DM and 104 days of storage had lower pH, with no effect of the inoculant (Table 4). There was, however, an interaction between DM, storage time, and inoculant (P=0.04) on pH values (Tables 3 and 7). In silage with high DM at 62 days of storage, inoculation with LP resulted in a higher pH (5.0), and in silage at 104 days, inoculation with LH resulted in silage with a higher pH (4.87), with there was no difference between the other inoculants and the control. The inoculation of silages with low DM did not result in significant differences in pH values at the two storage times (Table 7). After 62 days of storage, there was a difference in pH values between high and low DM only for silages inoculated with LP. Inoculation with this strain caused the pH of silages with low DM to be lower than that of silages with high DM. At 104 days, the pH values of low DM silages inoculated with LP, LH, and the control were lower than those of high DM silages inoculated with these inoculants (Table 7).

Silages with high DM had a higher concentration of acetic and propionic acid and a lower concentration of lactic acid, succinic acid, and ethanol (Table 4). Lactic acid concentrations were affected by the interaction of DM and storage time and by the interaction between inoculant and DM (P<0.001) (Table 3). Silages with low DM showed the highest concentrations of lactic acid compared to those with high DM at 62 days and 104 days. The concentration of lactic acid in silages with low DM increased from 62 to 104 days (from 61.20 to 66.93 $g.kg^{-1}$ DM), and in silages with high DM, there was a reduction in concentration (from 32.00 to 25.51 $g.kg^{-1}$ DM) (Table 5). Silages with low DM inoculated with LP, LP + LH, and the control had the highest concentrations of lactic acid (70.53, 67.97, and 65.75 $g.kg^{-1}$ DM,

respectively). Silages with high DM inoculated with LH, LP + LH, and the control had the highest lactic acid concentrations (32.29, 32.21, and 30.82 $g.kg^{-1}$ DM, respectively).

Acetic acid concentrations were influenced by the interaction between inoculant, DM, and storage time (Table 3). There was no difference in the concentration of acetic acid in silages with low DM at 62 days of storage. However, at 104 days, silages with low DM inoculated with LH and LP + LH showed a higher concentration of acetic acid (26.99 and 27.15 $g.kg^{-1}$ DM, respectively) (Table 7). In silages with high DM, after 62 days of storage, inoculation with LH and LP + LH resulted in a higher concentration of acetic acid. Using the inoculants evaluated in silages with high DM at 104 days resulted in silages with a higher concentration of acetic acid when compared to the control silage. The concentration of acetic acid in silages with high DM increased from 62 to 104 days of storage in the inoculated silages, and in the control silage, the concentration of this acid also increased during this period in silages with low DM inoculated with LH and with LP + LH.

Silages from plants with high DM, longer storage time, and inoculated plants had a higher propionic acid concentration (Table 4). However, interactions were observed between the DM concentration and the inoculant ($P=0.032$) and between DM and storage time ($P<0.001$) on propionic acid concentrations (Table 3). At 62 days and 104 days, propionic acid concentrations were higher in silage with high DM (29.29 and 38.02 $g.kg^{-1}$ DM, respectively) compared to silage with low DM (2.07 and 3 .29 $g.kg^{-1}$ DM, respectively) (Table 5). In silage with high DM, propionic acid concentration increased from 62 to 104 days (from 29.29 to 38.02 $g.kg^{-1}$ DM). There was no significant difference in silage with low DM (Table 5).

All silages (inoculated and control) formed a single group, with no differences regarding propionic acid concentration in the silage with low DM. However, in the high DM, the inoculated silages had the highest propionic acid concentrations (34.37, 35.70, and 33.97 $g.kg^{-1}$ DM, respectively) (Table 6). In all treatments, there was a higher concentration of propionic acid in silages with high DM compared to silages with low DM (Table 6).

The inoculant factors, storage time, and DM content affected the concentrations of succinic acid in the silage ($P<0.021$). On average, the silages inoculated with LH had 0.22 g kg-1 DM more succinic acid than the other silages (Table 4). Low DM silages had 0.90 g kg-1 DM more succinic acid than high DM silages. The longer storage time (104 days) favored the presence of this acid in elephant grass silage (0.41 g kg-1 DM plus) (Table 4)

Silages with high DM, longer storage time, and inoculated with inoculants containing LH had lower ethanol concentrations (Table 4). There was a tendency for interaction between inoculant and DM ($P=0.073$) and inoculant and storage period ($P<0.001$) (Table 3). Silages

with low DM inoculated with LP + LH showed lower ethanol concentrations (6.85 $g.Kg^{-1}$ DM). There was no significant difference in silages with high DM (Table 6). The 104-day-old silages inoculated with LP and the control had the highest ethanol concentration (10.68 and 10.95 $g.Kg^{-1}$ DM, respectively), the silages inoculated with LP + LH had the lowest concentration (3.36 $g.Kg^{-1}$ DM). Silages aged 62 days did not differ significantly between the inoculants and the control. There was a drop in ethanol concentrations in silages inoculated with LH and LP + LH from 62 days to 104 days of storage (from 11.06 to 6.01 36 $g.Kg^{-1}$ DM and 11.84 to 3.36 $g.Kg^{-1}$, respectively). There was no significant effect for silages inoculated with LP and the control (Table 8).

Table 3: Probability of the statistical model effects on the variables analyzed in elephant grass silage cv. BRS Capiaçu ensiled with low or high dry matter (DM), storage time (time) of 62 days or 104 days, and bacterial inoculants composed of *Lactiplantibacillus plantarum*, *Lentilactobacillus hilgardii* or a combination of these bacteria.

Variable	P-value						
	Dry matter (DM)	Time (T)	Inoculant (I)	DM × T	DM × I	T×I	DM × T × I
DM loss	<0.001	<0.001	<0.001	0.050	0.005	0.311	0.364
Neutral detergent fiber	<0.001	0.183	0.675	0.881	0.023	0.835	0.632
Water-soluble carbohydrates	0.001	<0.001	0.281	0.102	0.816	0.788	0.249
Ammonia nitrogen (N-NH_3)	<0.001	0.001	0.035	0.331	0.193	0.384	0.384
DM digestibility	0.440	0.011	<0.001	<0.001	<0.001	0.071	0.389
pH	<0.001	<0.001	0.093	0.005	0.040	0.023	0.040
Lactic acid	<0.001	0.803	0.004	<0.001	<0.001	0.651	0.820
Acetic Acid	<0.001	<0.001	<0.001	<0.001	0.034	0.620	0.014
Propionic acid	<0.001	<0.001	0.027	<0.001	0.032	0.573	0.466
succinic acid	<0.001	<0.001	0.021	0.290	0.061	0.224	0.086
Ethanol	0.027	<0.001	0.004	0.754	0.073	<0.001	0.443
Lactic acid bacteria	<0.001	<0.001	0.115	0.014	0.022	0.031	0.100
Mesophilic microorganisms	<0.001	<0.001	<0.001	0.671	0.419	<0.001	0.362
ASB*	<0.001	0.002	0.008	0.835	<0.001	0.001	0.014
Yeasts	0.061	0.050	0.134	0.399	0.827	0.004	0.475
Filamentous fungi	0.373	0.756	<0.001	0.978	0.342	0.335	0.047
19mm particles	<0.001	<0.001	0.057	0.001	0.161	0.130	0.123
8mm particles	<0.001	<0.001	0.121	0.021	0.073	0.050	0.104
4mm particles	<0.001	0.161	0.016	<0.001	0.260	0.526	0.730
Bottom	<0.001	<0.001	0.069	0.020	0.252	0.144	0.064

*Aerobic spore-forming bacteria.

Table 4: Effect of adding bacterial inoculants to elephant grass silage cv. BRS Capiaçu harvested with low or high dry matter (DM) after 62 and 104 days of storage.

Variable	Inoculant				DM[2]		Storage time		Standard error
	Control	LP	LH	LP + LH	Low	High	62 days	104 days	
DM loss (g.Kg^{-1} DM)	72.8a	46.8b	49.4b	41.3b	53.0b	70.9a	43.2b	62.0a	0.73
pH	4.57	4.67	4.67	4.64	4.55b	4.73a	4.71a	4.56b	0.06
Concentration (% DM)									
Ammoniacal Nitrogen	0.20b	0.22a	0.19b	0.19b	0.14b	0.25a	0.21a	018b	0.01
DM digestibility	47.29c	51.11b	51.79b	53.13a	50.80	50.55	51.54a	49.43b	1.13
Concentration (g.Kg^{-1} DM silage)									
Neutral detergent fiber	767.3	766.0	765.0	769.6	752.8b	781.3a	765.1	768.9	0.56
Water-soluble carbohydrates	3.00	3.29	2.81	2.84	3.31a	2.65b	3.57a	2.40b	0.38
Lactic acid	48.29a	45.12b	42.15b	50.09a	64.06a	28.76b	46.6	46.22	3.01
Acetic Acid	17.85b	18.89b	21.19a	21.28a	11.08b	28.53a	16.95b	22.65a	0.96
Propionic acid	16.70b	18.30a	19.43a	18.25a	2.68b	33.66a	15.68b	20.66a	1.19
succinic acid	2.33b	2.39b	2.59a	2.40b	2.88a	1.98b	2.22b	2.63a	0.12
Ethanol	10.65a	10.14a	8.53b	7.60b	9.93a	8.52b	10.71a	7.75b	1.21
Population (log UFC.g^{-1} silage)									
Lactic acid bacteria	7.23	7.22	7.31	7.18	6.68b	7.79a	7.63a	6.84b	0.07
Mesophilic microorganisms	6.04b	6.91a	6.88a	6.89a	6.18b	7.18a	6.87a	6.49b	0.11
ASB*	3.31a	3.28a	2.97b	3.24a	2.79b	3.61a	3.07b	3.32a	0.14
Yeasts	2.62	2.42	2.41	2.29	2.53	2.34	2.53	2.33	0.19
Filamentous fungi	2.70a	2.14b	2.18b	2.18b	2.26	2.34	2.31	2.29	0.13
Fresh matter percentage									
19mm particles	39.61	44.33	39.8	38.01	55.02a	25.85b	46.61a	34.26b	3.30
8mm particles	48.61	46.29	50.44	50.97	38.34b	59.82a	43.66b	54.49a	2.95
4mm particles	8.84a	7.30b	7.35b	8.69a	5.54b	10.55a	7.74	8.34	0.85
Bottom	2.94	2.08	2.41	2.33	1.09b	3.78a	1.98b	2.89a	0.45

Lowercase letters in the lines compare inoculants, DM, and storage times in case of significant difference ($p<0.05$) using the Scott-Knott test. *Aerobic spore-forming bacteria. [1]LAB strains *Lactiplantibacillus plantarum* CCMA 1394 (LP), *Lentilactobacillus hilgardii* CCMA 0170 (LH) and the combination of these bacteria (LP+LH). [2]Low (16.77%) or high (24.26%) dry matter content.

Silages with higher DM content had, on average, higher counts of LAB, mesophilic microorganisms, and ASB (7.79 x 6.68 log CFU.g^{-1}, 7.18 x 6.18 log CFU.g^{-1}, 3 .61 x 2.79 log UFC.g^{-1}, respectively) (Table 4). The LAB population was influenced by the interaction between inoculant and DM (P=0.022), inoculant and storage time (P=0.031), and DM and storage time (P=0.014) (Table 3). In both storage times, the LAB population was higher in silages with high DM compared to those with low DM (8.14 x 7.13 log CFU.g^{-1} at 62 days and 7.44 x 6.24 log UFC.g^{-1} at 104 days) (Table 5). Both in silages with low DM and in silages with high DM, the LAB population decreased from 62 days to 104 days (Table 5).

There was a higher LAB count in silages with low DM inoculated with LH and the control (6.75 and 6.78 log CFU.g^{-1}, respectively). In silages with high DM, there was no significant difference between treatments. In all treatments, the LAB population was higher in silages with high DM compared to silages with low DM (Table 6).

After 62 days of storage, silages inoculated with LP and LH showed a higher LAB count than the other treatments (7.68 and 7.65 log CFU.g^{-1}, respectively). After 104 days, there was no significant difference between treatments (Table 8). In all treatments, the LAB population increased at 104 days compared to 62 days (Table 8).

The population of mesophilic microorganisms was influenced by the interaction between inoculants and storage time (Table 3). The control silages had the lowest counts of mesophilic microorganisms at both storage times (Table 8). Silages inoculated with LP and the control had the mesophile population reduced at 104 days (from 7.06 to 6.77 log CFU.g^{-1} and 6.46 to 5.62 log CFU.g^{-1}, respectively). In the other treatments, there was no significant difference (Table 8).

The interaction of inoculant, DM, and storage time (P=0.014) significantly affected the ASB population (Table 3). In silages with low DM and 62 days of storage, the control showed the highest ASB count (3.15 log CFU.g^{-1}) than the average of inoculated silages. With 104 days, there was no significant difference between the silages (Table 7). Silages with high DM at 62 days of storage inoculated with LH had the lowest ASB count (2.94 log CFU.g^{-1}), and after 104 days of storage, silages with high DM inoculated with LP had the highest count (4.36 log CFU.g^{-1}) (Table 7).

With 62 days of storage in all treatments, there was a higher count of ASB in silages with high DM compared to silages with low DM. With 104 days in silages inoculated with LP, LP +

LH, and the control, the count was also higher in silages with high DM. There was no significant difference for silages inoculated with LH (Table 7).

Only silages with low DM inoculated with LH showed a significant difference in the ASB count between storage times. The population increased from 62 days to 104 days of storage (2.44 to 3.24 log CFU.g-1). In silages with high DM, only those inoculated with LP showed a significant difference between storage times. The ASB population increased from 62 days to 104 days of storage (3.45 to 4.36 log $CFU.g^{-1}$) (Table 7).

There was only a significant effect on the interaction of inoculant and storage time on the yeast population (Table 3). Control silages with low DM showed the highest yeast count (2.99 log $CFU.g^{-1}$), and silages with high DM showed no significant difference between treatments (Table 8). Only in the control silages was a reduction in the yeast population from 62 days to 104 days (0.75 log $CFU.g^{-1}$ less) (Table 8).

The count of filamentous fungi was influenced by the interaction between inoculant, DM, and storage time (P=0.047) (Table 3). Silages with low DM and 62 days of storage did not show a significant difference between treatments. However, with 104 days, the control showed a higher count of filamentous fungi (2.95 log $CFU.g^{-1}$). In silages with high DM, with 62 days of storage, the control also presented the highest count (2.91 log $CFU.g^{-1}$). There was no significant difference between treatments in silages with high DM at a storage time of 104 days (Table 7). Only in the control silage was an increase in the count of filamentous fungi from 62 to 104 days. Silages with low and high DM did not differ with 62 days of storage between treatments, with 104 days only the control had a significant difference between DM, in which the population of filamentous fungi was higher in low DM (2.95 log $CFU.g^{-1}$) about high MS (2.28 log $CFU.g^{-1}$) (Table 7).

Adding inoculants changed the percentage of particles retained on the 4mm sieve (P=0.016) (Table 3). The control and LP + LH treatments had a higher percentage of particles on the 4mm sieve (Table 4).

The DM and storage time interaction influenced the distribution of particles on the 19, 8, and 4 mm sieves and on the bottom (Table 3). On the 19 mm sieve, silage with low DM had a higher proportion of particles compared to silage with high DM both at 62 days (64.07% at low DM and 29.15% at high DM) and at 104 days (45.98% in low DM and 22.55% in high DM). After 62 days, silages with low and high DM decreased the proportion of particles on the 19 mm sieve

from 62 days (64.07% and 29.15%, respectively) to 104 days (45.98% and 22.55 %, respectively) (Table 5).

On the 8mm sieve, silage with high DM had a higher proportion of particles than silage with low DM at 62 days (56.18 and 31.15%, respectively) and 104 days (63.46% and 45. 52%, respectively). Silages with low and high DM increased the proportion of particles from 62 days (31.15% and 56.18%, respectively) to 104 days of storage (45.52% and 63.46%, respectively) (Table 5).

The distribution of particles on the 4 mm sieve in silage with high DM had a higher proportion of particles than in silage with low DM at 62 days (11.07% at high DM and 4.42% at low DM) and at 104 days (10.02% in high DM and 6.67% in low DM) (Table 5). In silage with low DM, the proportion of 4mm particles was lower at 62 days (4.42%) than at 104 days (6.67%). There was no significant difference for silage with high DM (Table 5).

Silage particles with high DM deposited at the bottom of the sieve had a higher proportion compared to silage with low DM at 62 days (3.59% at high DM and 0.36% at low DM) and at 104 days (3.97% in high MS and 1.82% in low MS) (Table 5). In silage with low DM, the proportion of particles at the bottom of the sieve was lower at 62 days (0.36%) than at 104 days (1.82%). There was no significant difference for silage with high DM (Table 5).

Table 5: Effect of the interaction between dry matter concentration (DM) and storage time on DM loss (g kg^{-1} DM), digestibility (% DM), population of lactic acid bacteria (log $UFC.g^{-1}$), concentrations of lactic acid and propionic acid (g kg^{-1} DM) and particle distribution (%) in elephant grass silage cv. BRS Capiaçu harvested with low (16.77%) or high (24.26%) dry matter (DM) concentration and ensiled at 62 days and 104 days.

Variable	Storage time	DM*		Standard error
		Low	High	
DM loss	62 days	26.9bB	59.5a	0.73
	104 days	53.0bA	70.9a	
Digestibility	62 days	53.31aA	48.28b	1.13
	104 days	49.76B	50.58	
Lactic acid	62 days	61.20aB	32.00bA	3.01
	104 days	66.93aA	25.51bB	
Propionic acid	62 days	2.07b	29.29aB	1.19
	104 days	3.29b	38.02aA	
Lactic acid bacteria	62 days	7.13bA	8.14aA	0.07
	104 days	6.24bB	7.44aB	
19mm particles	62 days	64.07aA	29.15bA	3.30
	104 days	45.98aB	22.55bB	
8mm particles	62 days	31.15bB	56.18aB	2.95
	104 days	45.52bA	63.46aA	
4mm particles	62 days	4.42bB	11.07a	0.85
	104 days	6.67bA	10.02a	
Bottom	62 days	0.36bB	3.59a	0.45
	104 days	1.82bA	3.97a	

Lowercase letters in the lines compare the MS (M), and capital letters in the columns compare the storage times in case of significant difference ($p<0.05$) using the Scott-Knott test. *Low dry matter concentration (16.77%) or high (24.26%).

Table 6: Effect of the interaction between bacterial inoculant and dry matter (DM) content on DM loss (g kg^{-1} DM), concentrations of neutral detergent fiber, lactic and propionic acid (g kg^{-1} DM), and population of lactic acid bacteria (log UFC.g^{-1}) in elephant grass silage cv. BRS Capiaçu.

Variable	DM**	Inoculant*				Standard error
		Control	LP	LH	LP + LH	
DM loss	Low	60.2aB	45.5aA	32.3bB	21.7bB	0.73
	High	85.4aA	48.1cA	66.5bA	60.9bA	
Digestibility	Low	52.61aA	49.34bB	48.05bB	53.20a	1.13
	High	41.97bB	52.88aA	52.78aA	53.07a	
Neutral detergent fiber	Low	750.4B	748.7B	758.5B	753.2B	0.56
	High	784.2aA	783.3aA	771.5bA	786.0aA	
Lactic acid	Low	65.75aA	70.53aA	52.00bA	67.97aA	3.01
	High	30.82aB	19.70bB	32.29aB	32.21aB	
Propionic acid	Low	2.82B	2.22B	3.17B	2.52B	1.19
	High	30.60bA	34.37aA	35.70aA	33.97aA	
Ethanol	Low	11.79a	11.35a	9.73a	6.85b	1.21
	High	9.50	8.93	7.34	8.35	
Lacti acid bacteria	Low	6.78aB	6.60bB	6.75aB	6.61bB	0.07
	High	7.69A	7.84A	7.87A	7.76A	

Lowercase letters in the lines compare the inoculants (I), and capital letters in the columns compare the DM in case of significant difference ($p<0.05$) using the Scott-Knott test. *LAB strains *Lactiplantibacillus plantarum* CCMA 1394 (LP), *Lentilactobacillus hilgardii* CCMA 0170 (LH), and the combination of these bacteria (LP+LH). **Low (16.77%) or high (24.26%) dry matter content.

Table 7: Effect of the interaction between bacterial inoculant and dry matter concentration (DM) and storage time on pH values, acetic acid concentration (g.Kg^{-1} DM), and populations of filamentous fungi and aerobic bacteria that form spores (ASB) (log UFC.g^{-1}), in silages of elephant grass cv. BRS Capiaçu.

Storage time	Inoculantt[1]								Standard error
	Control		LP		LH		LP + LH		
	DM[2]								
	Low	High	Low	High	Low	High	Low	High	
	pH								
62 days	4.60*	4.65B	4.65Ŧ*	5.00AŦ*	4.72	4.65B*	4.70*	4.72B	0.06
104 days	4.40Ŧ*	4.62BŦ	4.35Ŧ*	4.67BŦ*	4.45Ŧ	4.87AŦ*	4.50*	4.62B	
	Ácido acético								
62 days	8,91Ŧ	20,42BŦ*	9,36Ŧ	22,15BŦ*	10,53Ŧ*	26,99AŦ*	10,12Ŧ*	27,15AŦ*	0,96
104 days	11,61bŦ	30,48BŦ*	10,30bŦ	33,75AŦ*	14,67aŦ*	32,58AŦ*	13,11aŦ*	34,73AŦ*	
	BAFES								
62 days	3,15Ŧa	3,74ŦA	2,64Ŧb	3,45*ŦA	2,44*Ŧb	2,94ŦB	2,47Ŧb	3,76ŦA	0,14
104 days	2,90Ŧ	3,44ŦC	2,69Ŧ	4,36*ŦA	3,24*	3,26C	2,82Ŧ	3,89ŦB	
	Fungos filamentosos								
62 days	2,68	2,91*A	2,10	1,92B	2,22	2,08B	2,09	2,08B	0,13
104 days	2,95Ŧa	2,28*Ŧ	2,33b	2,21	2,10b	2,31	2,05b	2,50	

Capital letters in the lines compare treatments at high DM, and lowercase letters compare treatments at low DM within each inoculant level and storage time in case of significant difference ($p<0.05$) by the Scott-Knott test. Ŧ compare the different DM levels in the same inoculant and at the same storage time in case of significant difference ($p<0.05$) by the Scott-Knott test. * compares storage times in the same inoculant and DM in case of significant difference ($p<0.05$) using the Scott-Knott test. [1]LAB strains *Lactiplantibacillus plantarum* CCMA 1394 (LP), *Lentilactobacillus hilgardii* CCMA 0170 (LH), and the combination of these bacteria (LP+LH). [2] Low (16.77%) or high (24.26%) dry matter content.

Table 8: Effect of the interaction between bacterial inoculant and storage time on ethanol concentration (g kg-1 DM), the population of lactic acid bacteria, mesophilic microorganisms, and yeasts (log UFC.g-1), and distribution of 8mm (%) in elephant grass silage cv. BRS Capiaçu.

Variable	Storage time	Inoculant*				Standard error
		Control	LP	LH	LP + LH	
Ethanol	62 days	10.61A	9.32A	11.06A	11.84A	1.21
	104 days	10.68aA	10.95aA	6.01bB	3.36cB	
Lactic acid bacteria	62 days	7.54bA	7.68aA	7.75aA	7.57bA	0.07
	104 days	6.92B	6.76B	6.87B	6.80B	
Mesophilic microorganisms	62 days	6.46bA	7.06aA	7.00a	6.97a	0.11
	104 days	5.62bB	6.77aB	6.77a	6.81a	
Yeasts	62 days	2.99aA	2.57b	2.44b	2.12b	0.19
	104 days	2.24B	2.27	2.38	2.45	
8mm particles	62 days	41.65B	42.86B	42.36B	47.79B	2.95
	104 days	55.57A	49.73A	58.52A	54.15A	

Lowercase letters in the lines compare the inoculants (I), and capital letters in the columns compare the storage times (T) in case of significant difference ($p<0.05$) by the Scott-Knott test. *LAB strains *Lactiplantibacillus plantarum* CCMA 1394 (LP), *Lentilactobacillus hilgardii* CCMA 0170 (LH), and the combination of these bacteria.

3.3. Aerobic stability, maximum temperature, and highest temperature time.

There was a significant effect of inoculants and DM content on aerobic stability after opening the silage after 62 days of storage ($P<0.04$) (Table 9). Silages inoculated with LH and LP + LH showed the highest aerobic stability (39.94 and 38.56 hours, respectively) compared to silages inoculated with LP and the control (27.96 and 32.12 hours, respectively). Silage with high DM had greater aerobic stability (39.86 hours) than silage with low DM (29.44 hours) (Table 9). The maximum temperature that the silages reached during aerobic exposure was influenced by the inoculants ($P<0.008$) and DM concentrations ($P<0.001$). On average, the control silage reached a temperature of 35.20 ºC, the highest compared to the inoculated ones. Silages with low DM showed a higher maximum temperature (36.07°C) compared to those with high DM (29.58°C) (Table 9). The time the silage reached maximum temperature after 62 days of storage was influenced by the interaction between inoculant and DM ($P<0.01$). In silages with high DM inoculated with LP, LH, and LP + LH, the time to reach the maximum temperature (242.00, 312.62, and 298.12 hours,

respectively) was longer than the control (164.50 hours) (Table 9). In all treatments, the time to reach maximum temperature was longer in silages with high DM (Table 9).

After opening the silage at 104 days, aerobic stability was influenced only by DM concentration ($P<0.001$). Silage with high DM had greater aerobic stability (193.16 hours) compared to silage with low DM (36.19 hours) (Table 10). The maximum temperature reached by the silage after 104 days of storage was influenced by the inoculants ($P<0.046$) and the DM concentration ($P<0.001$). Silage with high DM inoculated with LH reached the other treatments' lowest temperature (25.95 ºC). There was no significant effect between treatments in silage with low DM. Silage with low DM reached the highest temperature (39.39 ºC) compared to silage with high DM (28.43 ºC) (Table 10).

The time the silage reached maximum temperature after 104 days of storage was influenced only by the DM concentration ($P<0.001$). Silage with low DM reached maximum temperature in less time (48.25 hours) than silage with high DM (209.59 hours) (Table 10).

Table 9: Effect of the interaction between bacterial inoculant and dry matter content (DM) on aerobic stability, maximum temperature, and time to reach maximum temperature (Tmax Time) in elephant grass silage cv. BRS with 62 days of storage.

Variable	DM**	Inoculant (I)*				Average	Standard error	P-value		
		Control	LP	LH	LP + LH			I	DM	I x DM
Aerobic stability (h)	Low	30.5	23.25	32.12	31.62	29.44B	4.42	0.043	0.003	0.485
	High	33.50	32.69	47.75	45.50	39.86A				
	Average	32.12b	27.96b	39.94a	38.56a					
Maximum temperature (ºC)	Low	37.12	33.67	36.35	37.12	36.07A	1.14	0.008	<0.001	0.175
	High	33.27	28.10	27.82	29.12	29.58B				
	Average	35.20a	30.89b	32.09b	33.12b					
Maximum temperature time (h)	Low	51.12B	48.12B	55.12B	55.25B	52.41B	27.88	0.0485	0.070	<0.001
	High	164.50bA	242.00aA	312.62aA	298.12aA	254.31A				
	Average	107.81	145.06	183.87	176.69					

Lowercase letters in the lines compare the inoculants (I), and capital letters in the columns compare the DM in case of significant difference ($p<0.05$) using the Scott-Knott test. *LAB strains *Lactiplantibacillus plantarum* CCMA 1394 (LP), *Lentilactobacillus hilgardii* CCMA 0170 (LH), and the combination of these bacteria (LP+LH). **Low (16.77%) or high (24.26%) dry matter content.

Table 10: Effect of the interaction between bacterial inoculant and dry matter content (DM) on aerobic stability, maximum temperature, and time to reach maximum temperature (Tmax Time) in elephant grass silage cv. BRS Capiaçu with 104 days of storage.

Variable	DM**	Inoculant (I)*				Average	Standard error	P-value		
		Control	LP	LH	LP + LH			Control	LP	LH
Aerobic stability (h)	Low	27.00	37.37	41.25	39.12	36.19B	24.24	0.2297	<0.001	0.3774
	High	170.62	181.75	250.87	169.37	193.16A				
	Average	98.81	109.56	146.06	104.25					
Maximum temperature (ºC)	Low	39.70	39.42	39.37	39.07	39.39A	0.82	0.0467	<0.001	0.0882
	High	30.65a	28.47a	25.95b	28.65a	28.43B				
	Average	35.17	33.95	32.66	33.86					
Maximum temperature time (h)	Low	35.00	49.37	54.25	54.37	48.25B	16.65	0.4449	<0.001	0.6354
	High	197.62	226.62	223.75	190.37	209.59A				
	Average	116.31	138.00	139.00	122.37					

Lowercase letters in the lines compare the inoculants (I), and capital letters in the columns compare the DM in case of significant difference ($p<0.05$) using the Scott-Knott test. *LAB strains *Lactiplantibacillus plantarum* CCMA 1394 (LP), *Lentilactobacillus hilgardii* CCMA 0170 (LH), and the combination of these bacteria (LP+LH). **Low (16.77%) or high (24.26%) dry matter content.

3.4. Diversity of prokaryotes

The relative abundance of the main phyla and genera present in silages inoculated with LP, LH, LP+LH, and the control is represented in Figures 1 and 2, respectively. The percentage of all genera identified in the different treatments evaluated can be seen in the supplementary table.

The presence of microorganisms from the Firmicutes phylum was predominant in all treatments, followed by the Proteobacteria phylum. Among the main genera in the treatments, the *Lactobacillus* was predominant, reaching 77.71% of the entire silage population with high DM inoculated with LP (Supplementary Table). The genus *Weissella* was the second most abundant in silage, reaching 23.71% of the population of low DM silage inoculated with LP and 22.39% of the low DM control silage (Supplementary Table).

Except for the control, the presence of microorganisms of the genus *Caproiciproducens* increased with storage time in silages with low DM, reaching 20.36% of the total population of silages inoculated with LH (Supplementary Table). In the control silage with low DM, there was a significant increase in storage time in the population of microorganisms of the genus *Acetobacter*, reaching 15.01% of the total population (Supplementary Table).

The genus *Pediococcus* showed greater abundance in control silage with high DM (3.10%). However, with storage time, there was a reduction in its quantification in all treatments (Supplementary Table). The genus *Clostridium* had greater abundance in silages with low DM inoculated with LH and LP (6.72 and 4.81%, respectively) in the last storage period (104 days) (Supplementary table).

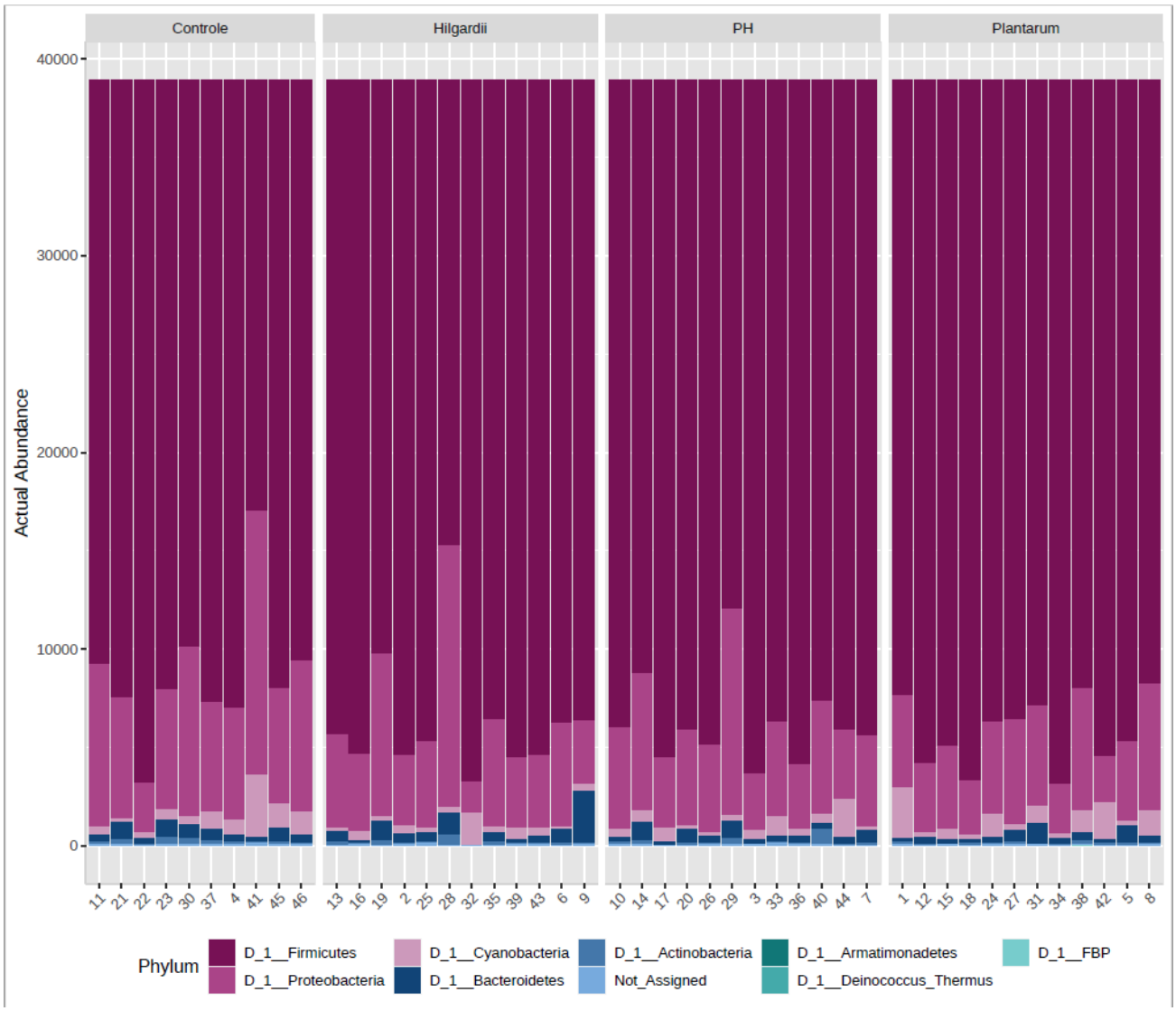

Figure 1: Relative abundance at the level of Phyla in silages inoculated with *Lentilactobacillus hilgardii*, *Lactiplantibacillus plantarum*, *Lentilactobacillus hilgardii* + *Lactiplantibacillus plantarum* (PH) and the control.

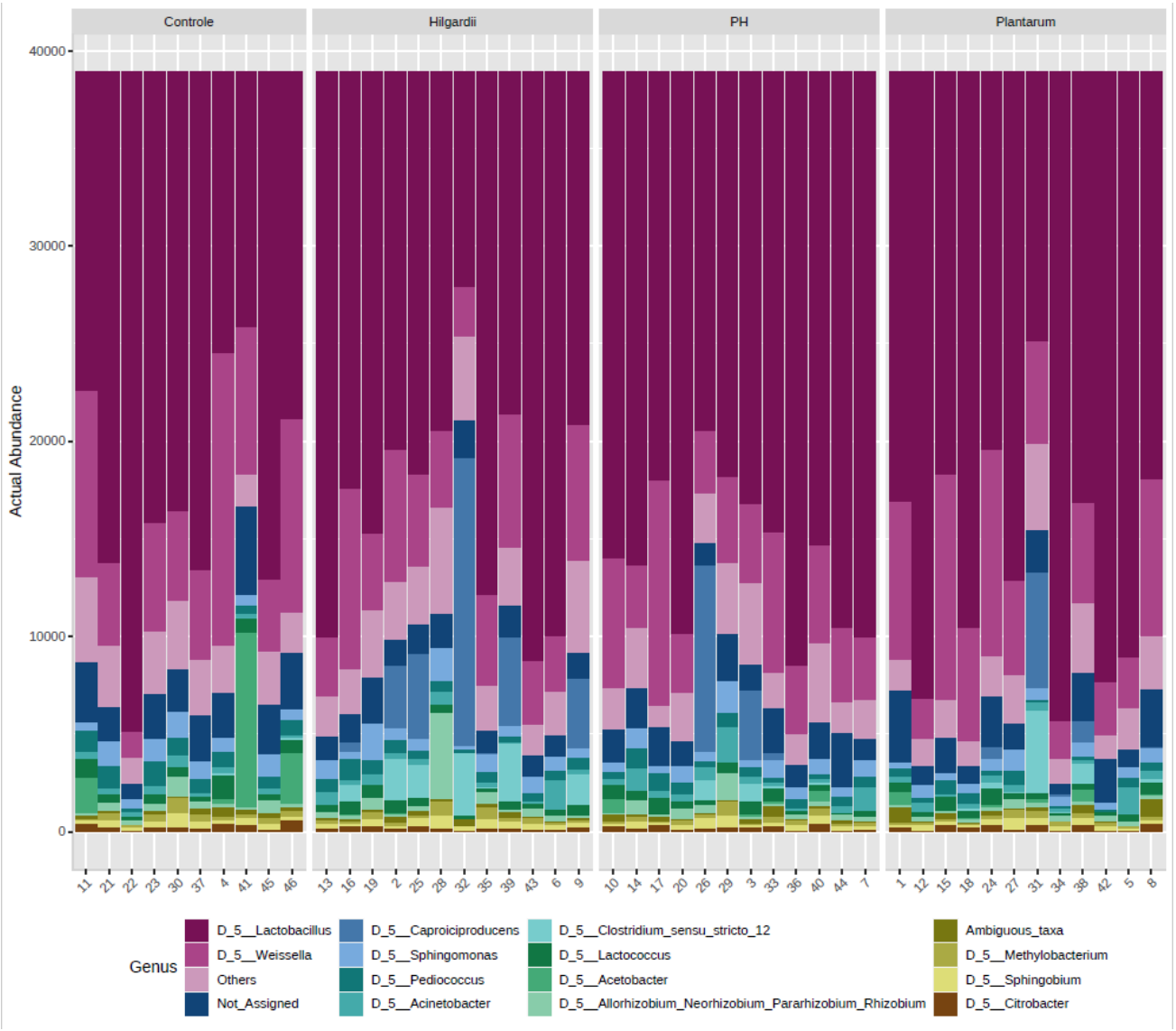

Figure 2: Relative abundance of the 15 most abundant genera in silages inoculated with *Lentilactobacillus hilgardii*, *Lactiplantibacillus plantarum*, *Lentilactobacillus hilgardii* + *Lactiplantibacillus plantarum* (PH) and the control.

The alpha diversity between silages with inoculants and the control is represented in Figure 3, and between silages with different DM concentrations is represented in Figure 4. This diversity reflects the number and abundance of species within a community.

The abundance of prokaryotes present in silages with inoculants and the control did not differ statistically according to the Shannon (P=0.38) and Chao 1 (P=0.72) indices (Figure 3). However, there was a statistical difference in the abundance of prokaryotes between silages

with low and high DM according to the Shannon and Chao 1 indices (P<0.05) (Figure 4). The silage with high DM showed a greater abundance of prokaryotes.

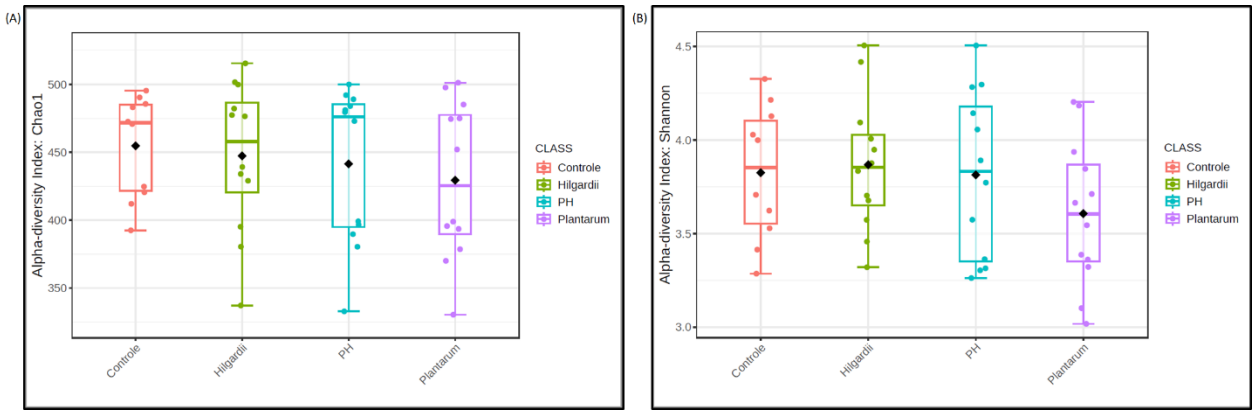

Figure 3: Alpha diversity for treatments with different inoculants. (A) Chao 1 index (P=0.72). (B) Shannon index (P=0.38).

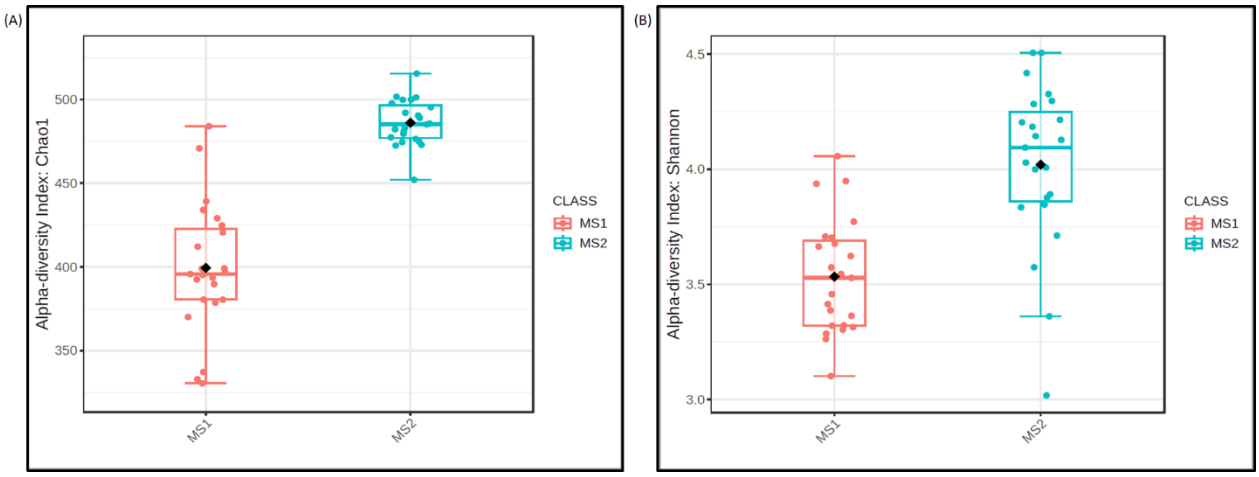

Figure 4: Alpha diversity for silages with low dry matter (DM1) and high dry matter (DM2). (A) Chao 1 index (P<0.05). (B) Shannon index (P<0.05).

The beta diversity between silages with inoculants and the control and between silages with different DM contents is represented in Figure 5. This diversity is related to the multiplicity and abundance between the genera of microorganisms in each treatment.

Using the Bray-Curtis index, a significant difference was observed between the silages with the inoculants and the control (P=0.005) and between the different DM contents (P=0.001).The diversity of prokaryotes in silage inoculated with LH and LP+LH was closer and more restricted than inoculated with LP and the control (Figure 5-A). The content of DM

influenced the diversity of microorganisms present in silages (P=0.001). Silages with low DM showed a microbial community markedly different from silages with high DM (Figure 5-B).

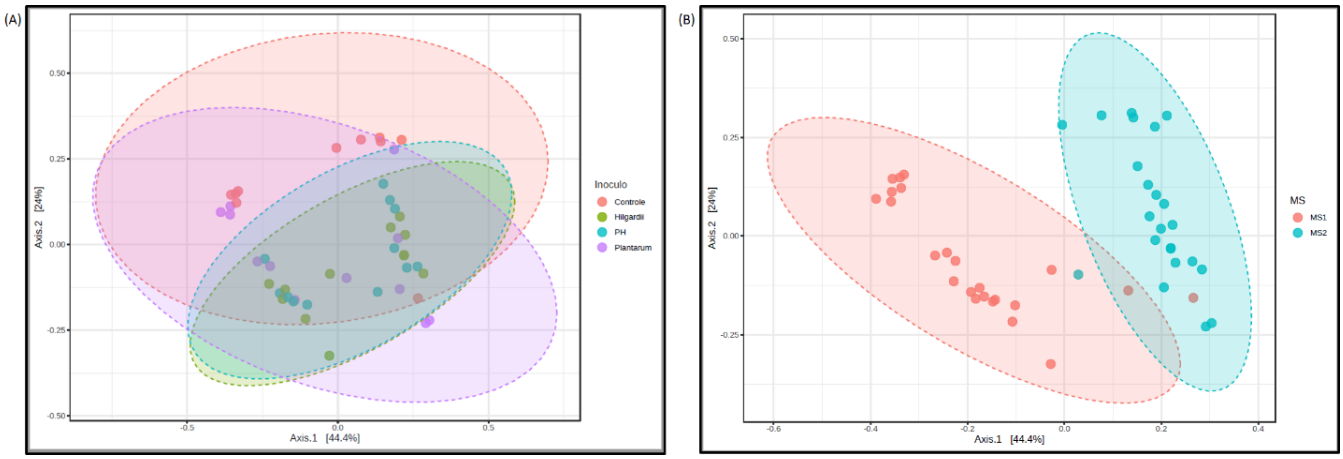

Figure 5: Beta diversity. Permutational multivariate analysis of variance (PERMANOVA), based on the Bray-Curtis index. (A) Inoculant treatments (P=0.005). (B) Treatments with different dry matter contents (MS1 = low DM, DM2 = high DM) (P=0.001).

Figure 6 represents the relationship between each inoculant and the control with the abundance of each genus of prokaryotes. Figure 7 represents the relationship between DM concentrations and the abundance of each genus. Both relationships were analyzed using the Random Forest method, an algorithm used to make predictions. It randomly creates several decision trees and combines them to generate a result.

Among the genera most identified in silage studies, *Klebsiella*, *Enterobacter*, *Pediococcus*, and *Citrobacter* were more abundant in control silage. *Clostridium* was more abundant in silage inoculated with LH, and the genera *Bacillus* and *Lactobacillus* were more abundant in silage inoculated with LP (Figure 6).

It is possible to observe in Figure 7 that silage with high DM (DM2) showed greater diversity compared to silage with low DM (DM1). Most genera observed are in greater quantity in silage with high DM.

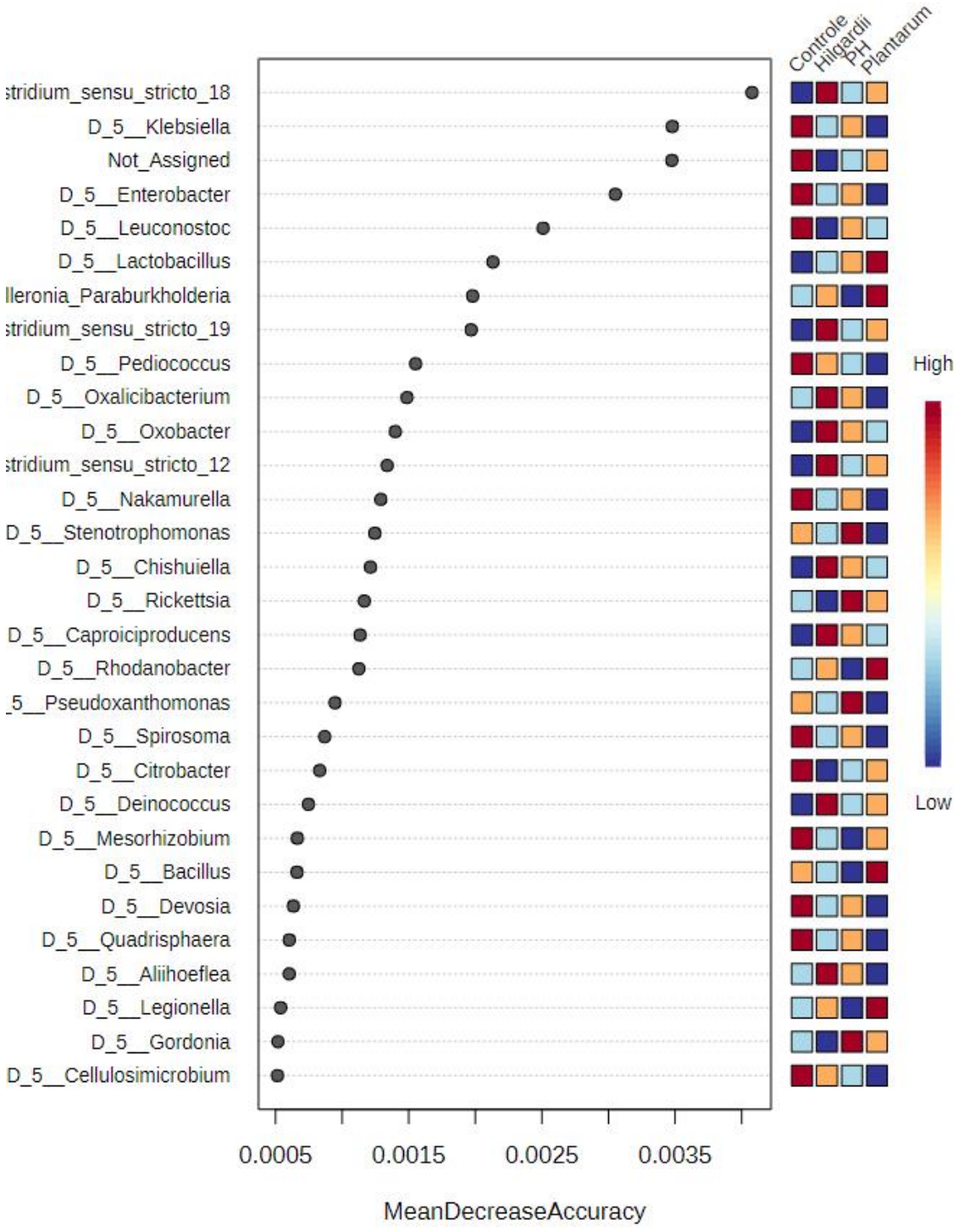

Figure 6: Variation in the abundance of genera in the inoculants and the control, generated using the Random Forest method. Blue indicates low abundance, and red indicates high abundance.

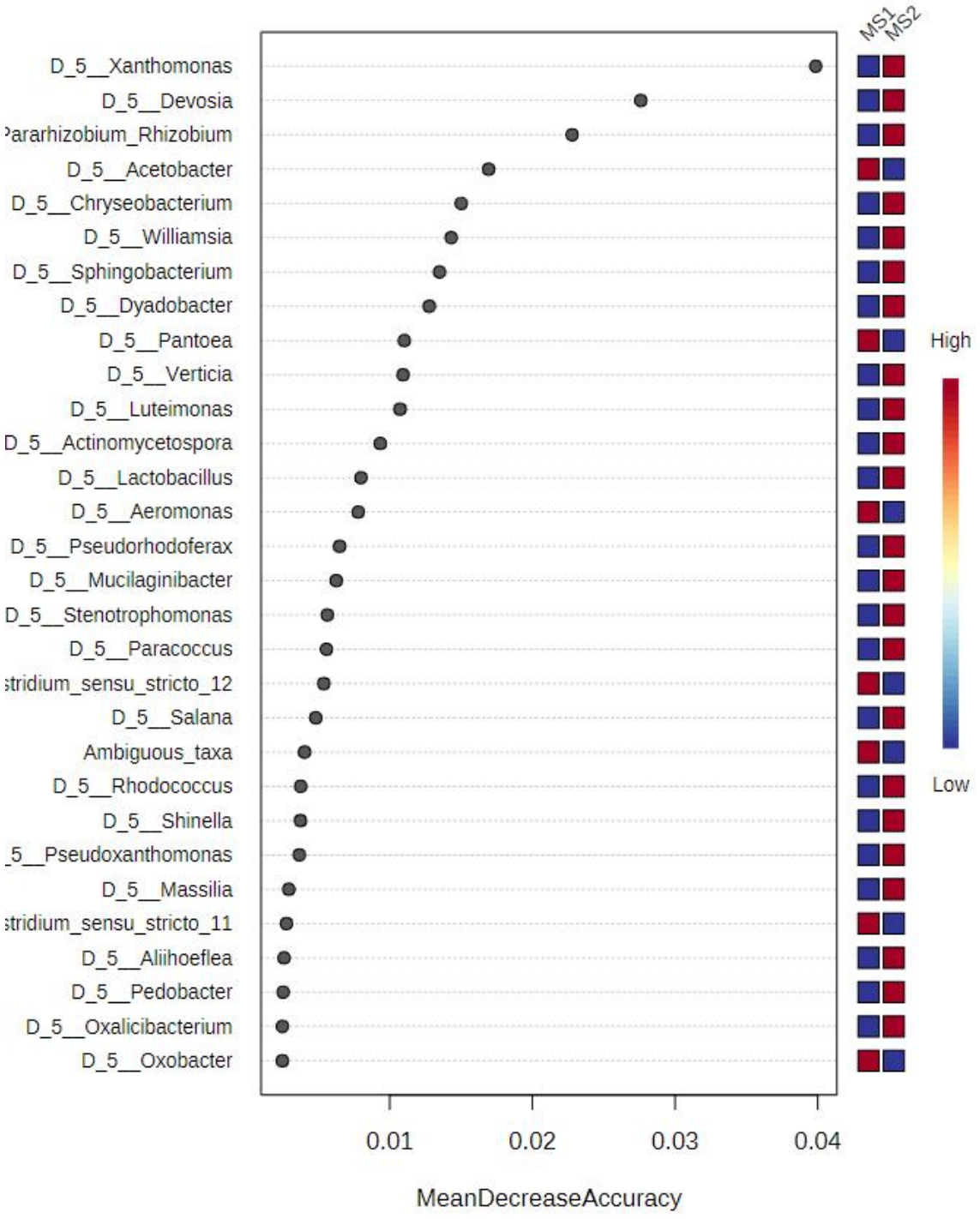

Figure 7: Variation in the abundance of genera in silages with low DM (MS1) and high DM (MS2), generated using the Random Forest method. Blue indicates low abundance, and red indicates high abundance.

4. Discussion

As for the metabolites detected in forage plants before ensiling, the concentration of acetic acid was considerably high, especially in plants harvested with low DM, reaching almost 6% of the total DM (55.94 $g.Kg^{-1}$ DM). According to Kung Jr. et al. (2018), acetic acid is inversely related to DM concentration. Excessively high concentrations of acetic acid are more frequently detected in very moist forages when ensiled (greater than 70%), which can cause unwanted natural fermentations carried out mainly by enterobacteria (McDonald et al., 1991), and the longer the time interval between harvesting and sealing, the higher the concentration of this acid (Weiss et al., 2016).

In this study, forage plants presented low DM content (17.66 and 24.26%) with humidity greater than 70% before being ensiled. During this time of the aerobic phase (between harvesting and sealing), this metabolite may have been produced mainly by enterobacteria, which act at this stage. Enterobacteria were detected in considerable numbers in forage (between 5.64 and 6.08 log $CFU.g^{-1}$ forage).

The LAB population in forage plants before ensiling (9.18 and 9.31 log $CFU.g^{-1}$ forage) was much higher than the average population of this bacterium commonly reported in fresh plants before ensiling, which according to Pahlow et al. (2003) is between 10 and 100000 $CFU.g^{-1}$ of forage (or 1 to 6 log $CFU.g^{-1}$ of forage), since, under aerobic conditions, other aerobic or facultative aerobic microorganisms at this stage of ensiling are commonly higher than LAB. Amaral et al. (2020) observed an initial LAB population of 3.58 log $CFU.g^{-1}$ in fresh BRS Capiaçu elephant grass forage, a much lower count than in this study. The high LAB population before ensiling may be related to the growth of other bacteria in the MRS culture medium and those belonging to this group. Although MRS is specific for the growth of LAB, some studies show that other bacteria can also grow in this culture medium (Carvalho et al.,2017 and Amaral et al., 2020).

The yeast count in the two forages with different DM concentrations was higher than those reported by Amaral et al. (2020) for the same elephant grass cultivar by Ferreira et al. (2013) for cv. Napier and Pereira et al. (2007) for cv. Cameroon, on the other hand, the population of filamentous fungi was lower than those reported by these authors.

DM losses associated with silage fermentation are mainly through carbon dioxide production, with the amount of this loss depending on the dominant microbial species and fermented substrates (Borreani et al., 2018). DM losses were lower in silages with low DM inoculated with the inoculants LH and LH+LP. Both inoculants contain the strain CCMA 0170

(*Lentilactobacillus hilgardii*), which, although it is an obligatory heterofermentative species, in previous studies also showed effectiveness in reducing DM losses for corn, sugar cane, and elephant grass cv. BRS Capiaçu silages (Ávila et al., 2014; Carvalho et al., 2014; Reis et al., 2017; Amaral et al. 2020).

In the two storage periods (62 and 104 days), silage with low DM had lower losses than silage with high DM, which may be related to the initial concentration of water-soluble carbohydrates (WSC) in the forage. In silage with low DM, lactic acid production, the primary metabolite produced by LAB from sugars, was higher than in silage with high DM. In addition, the initial concentration of CSA in the forage plant with low DM before ensiling was superior to the forage plant with high DM.

In tropical grasses, the concentration of available WSC is essential so that LAB rapidly decreases pH through the production of lactic acid. Otherwise, other microorganisms can play a significant role in fermentation. This work observed a high population of enterobacteria and yeasts in the forage before ensiling. Enterobacteria are often in higher populations than LAB at the beginning of silage fermentation (Borreani et al., 2018) and, therefore, if there is no rapid reduction in pH, they can compete with LAB for available sugars and produce the main product, acetic acid, causing more significant DM losses (Muck, 2010), while yeast can produce ethanol from glucose and contribute to increased DM losses (Borreani et al., 2018).

After the storage period, the NDF concentration was higher in silage with high DM, which was expected, given that the forages already showed this difference at harvest. An increase in NDF concentration was observed in both silages compared to fresh forage. According to Ávila et al. (2014), this increase in the fibrous fraction probably occurs due to the metabolism of WSC during fermentation and the synthesis of ethanol by yeast, generating CO2 that is lost.

Silage with high DM inoculated with LH showed a smaller increase in NDF concentration compared to the other inoculants and the control. Although the reasons for this smaller increase are not very clear, some strains of the species *L. hilgardii*, which, in addition to being characterized by producing acetic acid, are also capable of producing other compounds, such as phenylacetic and 4-hydroxyphenyl acetic acids (Valerio et al ., 2004) that can reduce the metabolism of yeast, one of the microorganisms responsible for the increase in DM losses. We are still determining, however, whether this strain produces these compounds.

According to Kung Jr. et al. (2018), high moisture silages generally have higher concentrations of soluble N and NH3-N than drier silages as they present a more robust general fermentation. However, in this study, silage with low DM presented lower concentrations of NH_3-N than silage with high DM. The production of NH_3-N is related to plant and microbial proteolytic processes that lead to changes in nitrogen compounds in silages. Higher levels of these metabolites may be related to the proteolytic activity of unwanted microorganisms (Kung Jr., 2018), which may justify the highest concentration in silages with high DM, which showed more significant DM losses.

The pH range in the control silage in this study (4.5) was higher than that reported for the same cultivar by Amaral et al. (2020) (3.9) in smaller experimental silos. Studies carried out with silages from elephant grass cultivars have demonstrated variation in the final pH value of the silage, as reported by Zanine et al. (2010), Ferreira et al. (2013), and Cardoso et al. (2016) (4.0; 4.7; 4.4, respectively).

The inoculants were not able to reduce the pH of the silages. Silage with high DM inoculated with LP inoculant after 62 days of storage showed the highest pH, possibly related to the low concentration of lactic acid detected in this silage. During the storage period, lactic acid is the acid with the highest concentration in silages and is the one that most contributes to the decline in pH (Kung Jr. et al., 2018). The *L. plantarum* strain appeared not to have adapted to the characteristics of the silage with high DM after 62 days of storage. It could not overgrow and dominate fermentation or may even have negatively affected this interaction since the concentration of lactic acid was lower than that of control silage (without inoculant).

Silages with low DM reduced pH with storage time. The same did not occur in silages with high DM. After 104 days of storage, the pH of silage with low DM was lower than that of silage with high DM. This drop is related to the concentration of lactic acid, which increased with storage time in silages with low DM and reduced in silages with high MS.

In this study, lactic acid concentrations in silages with high DM (ensilated with 242.6 $g.Kg^{-1}$ DM) were lower than those reported by Amaral et al. (2020) and by Jesus et al. (2021) in BRS Capiaçu elephant grass silages ensiled with 222 and 228.5 $g.Kg^{-1}$ DM, respectively, but in silages with low DM (ensiled with 167.7 $g.Kg^{-1}$ DM), the concentrations were higher. The difference in lactic acid concentration between silages ensiled at different DM concentrations was also observed by Cezário et al. (2015) in silages of *Brachiaria brizantha* cv. Marandu, with 30 and 75 days of regrowth, the authors also observed that silages ensiled with a lower regrowth

age had the highest concentrations of lactic acid, which is probably related to the greater availability of WSC.

As expected, silages with low DM inoculated with the LH inoculant showed the lowest lactic acid concentration. The species *L. hilgardii* is obligatory heterofermentative, and from 1 mol of glucose, it produces 1 mol of lactic acid, 1 mol of carbon dioxide (CO_2), and 1 mol of ethanol or acetic acid (Muck, 2010). Despite producing acetic acid, it has a pKa of 4.75, higher than that of lactic acid (3.86), and is therefore less efficient in reducing pH (Kung Jr et al., 2018).

Silages inoculated with inoculants containing the heterofermentative species *L. hilgardii* (LH and LP + LH) showed the highest concentrations of acetic acid. Moderate concentrations of this acid in silage may be desirable, as they inhibit yeast, resulting in more excellent stability during air exposure (Kung Jr et al., 2018).

The relationship between the concentrations of acetic acid and lactic acid in silages with high DM was inverse during the storage time, which was not observed in silages with low DM, in which the concentrations of lactic acid and acetic acid increased. Possibly, silages with high DM had lower availability of WSC. During the storage time, due to the scarcity of sugars, metabolic activities occurred in the production of acetic acid from lactic acid due to LAB activity, as this group of microorganisms was high and even higher than the population of silages with low DM.

Parvin and Nishino (2009) ensiled wilted guinea grass forages with 286 and 443 $g.Kg^{-1}$ DM, and after 180 days of ensiling, they observed an increase in the concentration of acetic acid, decreasing its ratio with lactic acid in the silage with DM. Low that had lower concentrations of WSC. According to the authors, this suggests that there was probable production of acetic acid from lactic acid by LAB activity, such as that of the species *L. plantarum*, which is known to metabolize lactic acid into acetic and formic acid under conditions of sugar deficiency (Lindgren et al., 1990).

Propionic acid concentrations in silages with low DM were below 0.3%, and concentrations above this are considered high (Kung Jr. et al., 2018). However, propionic acid concentrations were very high in silages with high DM, exceeding 3% of the total DM. High concentrations of propionic acid in silage may be indicative of the action of propionic acid bacteria that metabolize sugars and lactic acid into acetic and propionic acid (Moon et al., 1983). In this study, although the concentration of lactic acid was reduced and acetic and propionic

acid increased in silages with high DM, it is unlikely that it was a consequence of the action of these bacteria since their presence in silage without having been inoculated is uncommon (Kung Jr et al., 2018). However, most of these bacteria are anaerobic, have low tolerance to acidity, and do not grow well at pH values below 4.8 (Ávila & Carvalho, 2020). Shah et al. (2018) observed propionic acid concentrations that ranged from 0.5 to 0.7% in elephant grass silages inoculated with *Lactobacillus plantarum* and *Pediococcus acidilactici*, however, to date, no data have been found in the literature with such high propionic acid concentrations, such as those found in silages with high DM in this study.

Silages with low DM inoculated with LP + LH showed the lowest ethanol concentrations. Ethanol is the alcohol most found in silages and can be produced by various microorganisms, including heterofermentative LAB (Kung Jr. et al., 2018). It can indicate yeast metabolism and loss of DM (Carvalho et al., 2014). In this study, silages with low DM inoculated with LP + LH showed low DM losses and yeast counts. Conversely, the control had higher ethanol concentrations, higher DM losses, and higher yeast counts after 62 days of storage.

Despite the lower lactic acid concentrations, the LAB population was higher in silage with high DM at both storage times. Santos et al. (2011) evaluated the LAB population in *Brachiaria decumbens* cv. Basilisk with different regrowth ages (30, 40, 50, 60, and 70 days) observed that silages produced from older plants had a higher LAB population. Da Silveira et al. (2021) observed that elephant grass silage cv. Cameroon ensiled with 16 weeks of regrowth showed a higher LAB population than silages ensiled with eight weeks of regrowth, even with lower concentrations of WSC. According to the authors, this variation in the epiphytic population of LAB may be related to environmental factors.

The inoculants could not increase the LAB population in low and high DM silages. The plant's epiphytic population was high enough to guarantee the fermentation process. According to Pahlow et al. (2003), LAB is essential for the silage fermentation process and is the group of microorganisms that vary most in population and can be found in populations of 101 to 106 $CFU.g^{-1}$ in perennial grasses. In this study, the BAL population in the control exceeded 106 $CFU.g^{-1}$.

The reduction in the LAB population from 62 days to 104 days of storage was observed in all treatments. The number of LAB and other viable microorganisms naturally decreases with

storage time due to the drop in pH and accumulation of metabolites produced (Pahlow et al., 2003; Duniére et al., 2013).

Silages inoculated with LH had the lowest ASB counts, which is desirable, as these microorganisms can survive the fermentation process by forming spores and return to activity in the presence of oxygen when the silo is opened and contribute to aerobic deterioration. (Ávila & Carvalho, 2020). This more significant inhibition of ASB by the species *L. hilgardii* may be related to its antibacterial activity. Ganzorig et al. (2016) isolated a strain of *L. hilgardii* in samples from Airag that showed antibacterial activity. Supernatants from strain *L. hilgardii* (Uvu-21) completely inhibited the growth of *E. coli* and *B. subtilis* and showed a wide range of thermal and pH stability in their antibacterial activity. According to the authors, this antibacterial activity probably originates from the peptides produced by this microorganism.

This study's population of yeasts and filamentous fungi was lower than those of Amaral et al. (2020) in the elephant grass cv. BRS Capiaçu silage. The inoculants effectively reduced the yeast population after 62 days of storage, unlike the control, which only reduced after 104 days. The CCMA 0170 strain also inhibited the growth of yeast in sugarcane silage (Carvalho et al., 2014) and elephant grass cv. BRS Capiaçu (Amaral et al., 2020). The inoculants also reduced the population of filamentous fungi in silages with low DM at 104 days of storage and in silages with high DM at 62 days of storage. The reduction in the population of yeasts and filamentous fungi in silage is desirable, as yeasts, in addition to being related to DM losses and greater ethanol production, are important causes of aerobic deterioration by assimilating lactic acid in the presence of oxygen, increasing pH and allowing the growth of other microorganisms that are less tolerant to acidity (Duniére et al., 2013; Kung Jr et al., 2018). Filamentous fungi in silage are associated with oxygen and increased pH values. It can produce mycotoxins as products of their secondary metabolism, which, despite not presenting problems for the conservation of silage, can cause damage to animal health. (Ávila & Carvalho, 2020).

The ideal particle distribution values vary depending on the forage used. The particles retained in the 19 mm sieve are effective in stimulating chewing but are also more prone to selection by ruminants (Kononoff & Heinrichs, 2003). Therefore, they must contain 10 to 20% of the total particles. The 8 mm intermediate sieve must retain between 45 and 75% of the fraction to be homogeneous and efficient in promoting the animal's rumination and salivation. The 4 mm sieve must retain between 30 and 40%, and the bottom must not retain fractions greater than 10% (Heinrichs, 2013). This study retained more than 60% of silage particles with

low DM on the 19 mm sieve mm. The high humidity of the silage influenced the passage of particles through the sieves, overestimating the percentage retained on the 19 mm sieve and underestimating the values on the other sieves. With storage time, the percentage of particles retained in the 19 mm sieve reduced in both silages and increased in the other sieves, suggesting that this reduction may result from metabolic activities during fermentation.

When the silages were opened after 62 days, silages with high DM inoculated with heterofermentative LAB *L. hilgardii* or in combination with *L. plantarum* showed greater aerobic stability, the lower maximum temperature reached and longer time to reach maximum temperature about the control. These were the silages with the highest concentrations of acetic acid after 62 days of storage.

Due to its antifungal properties, acetic acid has been linked to greater aerobic stability in silages. In addition, the species *L. hilgardii* has been shown to produce other antifungal compounds that, in synergism with acetic acid, may be responsible for improved aerobic stability (Kung Jr. et al., 2018).

Silages with high DM at both opening times showed greater aerobic stability and lower maximum temperature reached. The acetic and propionic acid concentrations in these silages were much higher than those found in silages with low DM. These acids are less dissociated than lactic acid. Their minimum inhibitory concentrations against yeasts and fungi are much lower than those of lactic acid (Wilkinson & Davies, 2013).

The lower temperature observed in silages with high DM after aerobic exposure may be related to the lower concentrations of lactic acid in these silages since aerobic deterioration occurs because silage fermentation products, such as lactic acid, are substrates for microbial growth when oxygen is reintroduced into the silage (Pahlow et al., 2003).

As expected, the Firmicutes phylum predominated in all treatments during storage time since the main bacteria involved in lactic acid fermentation in silage belong to this phylum. Furthermore, this phylum also harbors important undesirable genera in silages, such as *Clostridium*, *Bacillus*, and *Listeria*.

The phylum Proteobacteria was the second most abundant in silages. This phylum is mainly composed of the genera *Sphingomonas*, *Pantoea*, *Pseudomonas*, and *Stenotrophomonas* (Ogunade et al., 2018) and was probably present in the forage plant before ensiling, a time when LAB had not yet dominated the fermentation process.

Zhao et al. (2022), evaluating the effects of delayed harvest and storage time of elephant grass silage on the bacterial community, observed that Proteobacteria was the dominant phylum in the elephant grass forage plant, with 69.1% of the relative abundance of the bacterial community. After ensilage, the relative abundance of Proteobacteria decreased while that of Firmicutes increased. According to the authors, this rapid succession of Proteobacteria by Firmicutes during ensiling is attributed to the suppression of aerobic microorganisms (and the prevalence of LAB).

McGarvey et al. (2013) obtained similar results by monitoring the bacterial community before and after ensiling alfalfa forage. They observed that the relative abundance of Proteobacteria reduced drastically (from 89.6 to 26.9%), while that of Firmicutes increased markedly (from 8.1 to 70.6%) after 40 days of ensiling.

The genus *Lactobacillus* was predominant in all treatments, especially in inoculated silages with high DM. This indicates that the inoculated strains of this genus could dominate the silage fermentation process. Microorganisms belonging to this genus are essential for the initial establishment of the acidic environment and contribute to the preservation of silage (Muck, 2013).

The genus *Weissella* was the second genus with the highest relative abundance in the treatments. Most *Weissella* species are obligate heterofermentative bacteria and are present at the beginning of the fermentation process and tend to decrease with storage time, as they are outcompeted by *Lactobacillus* species that are more tolerant to a drop in pH (Graf et al., 2016).

In this study, the presence of the genus *Caproiciproducens* was detected, which, except for the control, increased with storage time in silages with low DM, especially in silages inoculated with LH (20.36%). Although little known in silages, this genus has also been found in other studies in alfalfa and stylo silages (Wang et al., 2019) and mixed ryegrass and corn straw silage (Yan et al., 2019).

The genus Caproiciproducens may be related to CO_2, ethanol, acetic acid, and butyric acid production under anaerobic conditions (Kong et al., 2018). In this study, the loss of DM from silages with low DM increased considerably with storage time, in the same way that microorganisms of this genus increased, leading to the hypothesis that they have collaborated with undesirable fermentations.

The relative abundance of microorganisms of the genus *Acetobacter* in control silage with low DM increased significantly with storage time. This genus is undesirable for silage due

to its ability to degrade lactic acid and acetic acid and produce CO_2 and H_2O, causing DM losses and aerobic deterioration (Carvalho-Estrada et al., 2022).

Silages with high DM showed a greater diversity of prokaryotes than silages with low DM. According to Mendez-Garcia et al. (2015), a drop in pH can reduce microbial diversity in the habitat. Zhao et al. (2022) also observed that, in general, elephant grass silages with low DM had lower prokaryote diversity than silages with high DM and were associated with a drop in pH.

Silages inoculated with LH and LP+LH had lower prokaryotic diversity than the control, and silage inoculated with LP. Ogunade et al. (2018) observed in alfalfa silage that when inoculating them with *L. buchneri* and *L. plantarum* there was an increase in the relative abundance of the predominant genus *Lactobacillus* and a reduction in the diversity of the bacterial community. Therefore, one hypothesis is that the bacteria present in the inoculant were more effective in competing for substrate and dominating the silage fermentation process.

5. Conclusion

All inoculants reduced the population of undesirable microorganisms and reduced DM losses. The LH and LP +LH inoculants stood out for reducing the concentration of ethanol and NH_3-N during storage time. In addition, they increased digestibility and prolonged the aerobic stability of silages with high DM. The heterofermentative strain CCMA 0170 (*L. hilgardii*) with or without the association with the strain CCMA 1394 (*L. plantarum*) in inoculants showed promising results to improve the quality of elephant grass cv. BRS Capiaçu silage.

Storage time affected the fermentation profile, microbial population, and particle distribution of the silages. Silage with high DM harmed the fermentative profile after 104 days of storage. Silage with low DM showed better fermentative characteristics, greater lactic acid production, lower DM losses, and lower pH. Therefore, it is recommended to ensile BRS Capiaçu elephant grass with low DM.

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Supplementary Table: Relative abundance of genera identified in elephant grass cv. BRS Capiaçu silage ensiled with low or high dry matter (DM), storage time (time) of 62 days or 104 days, and bacterial inoculants composed of *Lactiplantibacillus plantarum*, *Lentilactobacillus hilgardii* or a combination of these bacteria.

Gêneros	Controle				*Lentilactobacillus hilgardii*				*Lactiplantibacillus plantarum*				*L. hilgardii + L. plantarum*			
	Low DM		High DM		Low DM		High DM		Low DM		High DM		Low DM		High DM	
	62 dias	104 dias	62 dias	104 dias	62 dias	104 dias	62 dias	104 dias	62 dias	104 dias	62 dias	104 dias	62 dias	104 dias	62 dias	104 dias
Ambiguous_taxa	0.71	0.74	0.50	0.64	0.67	0.85	0.32	0.44	1.85	0.90	0.29	0.24	0.85	0.79	0.39	0.42
Acetobacter	1.90	15.01	0.02	0.01	0.20	0.15	0.01	0.01	0.70	0.96	0.08	0.01	0.84	0.66	0.01	0.01
Achromobacter	0.05	0.01	0.05	0.12	0.01	0.00	0.06	0.13	0.00	0.03	0.01	0.03	0.01	0.01	0.02	0.05
Acidovorax	0.01	0.00	0.03	0.02	0.00	0.01	0.01	0.04	0.01	0.02	0.02	0.02	0.01	0.01	0.02	0.01
Acinetobacter	0.95	0.62	1.02	1.07	0.80	0.46	2.59	1.01	0.83	0.92	2.03	0.65	1.05	1.04	2.50	2.12
Actinomycetospora	0.01	0.01	0.04	0.05	0.01	0.01	0.02	0.03	0.01	0.02	0.02	0.03	0.02	0.01	0.03	0.03
Aeromicrobium	0.01	0.01	0.01	0.01	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.01
Aeromonas	0.03	0.07	0.00	0.01	0.04	0.02	0.00	0.01	0.05	0.02	0.01	0.00	0.04	0.04	0.00	0.00
Aliihoeflea	0.01	0.01	0.02	0.04	0.00	0.00	0.02	0.05	0.00	0.00	0.02	0.02	0.00	0.01	0.04	0.02
Allorhizobium Rhizobium	0.34	0.23	0.96	1.76	0.22	0.25	1.04	4.66	0.19	0.33	0.45	0.95	0.12	0.55	1.22	1.65
Anoxybacillus	0.41	0.00	0.00	0.00	0.03	0.02	0.00	0.01	0.02	0.03	0.00	0.00	0.05	0.01	0.01	0.00
Arthrobacter	0.02	0.00	0.03	0.07	0.01	0.02	0.03	0.02	0.03	0.06	0.03	0.01	0.03	0.55	0.01	0.01
Aureimonas	0.29	0.42	0.49	0.85	0.22	0.23	0.40	0.89	0.19	0.60	0.17	0.51	0.17	0.49	0.47	0.63
Azohydromonas	0.01	0.01	0.01	0.01	0.00	0.01	0.00	0.04	0.01	0.00	0.00	0.02	0.00	0.02	0.00	0.01
Bacillus	0.66	0.02	0.05	0.10	0.08	0.11	0.06	0.03	0.14	0.32	0.05	0.10	0.09	1.15	0.02	0.01
Bdellovibrio	0.07	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.09	0.00	0.01	0.00	0.01	0.01	0.01	0.00
Bordetella	0.03	0.02	0.01	0.01	0.06	0.01	0.02	0.00	0.05	0.04	0.00	0.00	0.05	0.02	0.00	0.00
Bosea	0.01	0.02	0.03	0.06	0.00	0.01	0.02	0.11	0.01	0.02	0.01	0.03	0.01	0.01	0.02	0.02
Bradyrhizobium	0.03	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.02	0.03	0.00	0.00	0.00	0.02	0.00	0.01
Brevibacillus	0.29	0.00	0.00	0.00	0.01	0.02	0.00	0.00	0.02	0.11	0.00	0.00	0.00	0.00	0.00	0.00
Brevundimonas	0.04	0.07	0.08	0.09	0.04	0.02	0.09	0.10	0.04	0.02	0.05	0.04	0.03	0.04	0.11	0.07
Burkholderia	0.15	0.01	0.01	0.00	0.09	0.01	0.03	0.00	0.16	0.05	0.01	0.01	0.03	0.00	0.00	0.00

Caproiciproducens	0.01	0.02	0.00	0.04	6.39	20.36	0.00	0.08	0.09	6.69	0.07	0.19	3.14	8.69	0.00	0.03
Cellulomonas	0.01	0.00	0.02	0.01	0.00	0.00	0.00	0.22	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.01
Cellulosimicrobium	0.02	0.01	0.01	0.02	0.02	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.10	0.00	0.01
Chishuiella	0.05	0.03	0.03	0.03	0.07	0.01	0.13	0.05	0.05	0.05	0.05	0.02	0.09	0.03	0.06	0.07
Chryseobacterium	0.07	0.05	0.41	0.24	0.05	0.08	0.41	0.28	0.03	0.08	0.15	0.18	0.05	0.10	0.40	0.21
Citrobacter	0.83	1.32	0.62	0.50	0.62	0.51	0.52	0.41	0.88	0.96	0.35	0.19	0.74	0.78	0.42	0.32
Clostridium sensu stricto 11	0.04	0.07	0.00	0.00	1.41	3.30	0.00	0.01	0.05	1.33	0.04	0.02	2.42	0.50	0.00	0.00
Clostridium sensu stricto 12	0.08	0.15	0.00	0.01	3.95	6.72	0.00	0.01	0.20	4.81	0.07	0.03	0.78	1.09	0.00	0.01
Clostridium sensu stricto 18	0.00	0.01	0.00	0.00	0.43	0.45	0.00	0.00	0.02	0.04	0.00	0.01	0.00	0.07	0.00	0.00
Clostridium sensu stricto 19	0.00	0.00	0.00	0.00	0.36	1.12	0.00	0.00	0.01	0.78	0.02	0.00	0.05	0.18	0.00	0.00
Comamonas	0.06	0.21	0.06	0.07	0.04	0.04	0.06	0.07	0.07	0.07	0.03	0.04	0.03	0.11	0.07	0.11
Corynebacterium	0.01	0.01	0.01	0.02	0.03	0.01	0.01	0.01	0.02	0.04	0.01	0.01	0.01	0.01	0.01	0.01
Curtobacterium	0.01	0.01	0.08	0.02	0.00	0.02	0.03	0.02	0.01	0.00	0.01	0.02	0.01	0.01	0.03	0.02
Deinococcus	0.01	0.01	0.02	0.01	0.03	0.03	0.00	0.01	0.02	0.03	0.00	0.01	0.01	0.02	0.00	0.01
Devosia	0.03	0.02	0.15	0.29	0.02	0.01	0.17	0.54	0.01	0.02	0.07	0.11	0.01	0.04	0.17	0.19
Dyadobacter	0.01	0.00	0.12	0.06	0.00	0.01	0.03	0.08	0.00	0.00	0.04	0.02	0.00	0.00	0.05	0.04
Ellin6055	0.01	0.04	0.02	0.03	0.01	0.02	0.03	0.01	0.01	0.05	0.01	0.02	0.01	0.01	0.02	0.02
Empedobacter	0.03	0.02	0.07	0.01	0.03	0.04	0.09	0.03	0.02	0.04	0.05	0.02	0.03	0.02	0.06	0.03
Enterobacter	0.13	0.06	0.18	0.11	0.04	0.07	0.19	0.07	0.08	0.07	0.06	0.05	0.06	0.08	0.12	0.07
Enterococcus	0.08	0.01	0.04	0.04	0.01	0.01	0.03	0.02	0.01	0.01	0.02	0.01	0.01	0.02	0.03	0.01
Escherichia_Shigella	0.13	0.09	0.05	0.06	0.10	0.11	0.05	0.05	0.14	0.12	0.05	0.03	0.08	0.09	0.04	0.06
Ferruginibacter	0.01	0.00	0.01	0.01	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.00
Fibrella	0.06	0.06	0.03	0.03	0.05	0.02	0.02	0.03	0.06	0.05	0.02	0.01	0.03	0.04	0.03	0.03
Flavobacterium	0.02	0.01	0.21	0.07	0.02	0.04	0.11	0.08	0.02	0.00	0.04	0.03	0.01	0.02	0.13	0.05
Geodermatophilus	0.03	0.03	0.12	0.05	0.02	0.02	0.08	0.04	0.03	0.02	0.03	0.03	0.04	0.01	0.06	0.06
Gordonia	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.01	0.00	0.00	0.01	0.01	0.00	0.01	0.02	0.01
Herbaspirillum	0.07	0.02	0.03	0.01	0.04	0.02	0.02	0.03	0.03	0.05	0.02	0.01	0.04	0.04	0.02	0.00
Herbiconiux	0.11	0.10	0.16	0.20	0.09	0.06	0.09	0.19	0.10	0.08	0.07	0.09	0.06	0.11	0.12	0.11
Hymenobacter	0.04	0.04	0.17	0.15	0.04	0.04	0.13	0.10	0.04	0.07	0.04	0.07	0.03	0.05	0.10	0.06
Kineococcus	0.03	0.01	0.10	0.02	0.00	0.02	0.04	0.02	0.01	0.01	0.02	0.02	0.01	0.01	0.05	0.03

Klebsiella	0.32	0.25	0.50	0.27	0.14	0.20	0.36	0.20	0.23	0.20	0.19	0.10	0.24	0.29	0.22	0.16
Kosakonia	0.01	0.01	0.07	0.02	0.00	0.02	0.02	0.02	0.00	0.02	0.01	0.02	0.00	0.00	0.02	0.01
Lactobacillus	55.38	39.81	62.09	63.56	50.46	42.24	69.89	64.68	54.50	47.39	77.71	77.60	58.41	56.85	71.17	68.30
Lactococcus	1.87	1.57	1.06	0.77	1.46	0.70	0.92	0.63	1.40	1.00	0.68	0.36	1.75	0.93	0.86	0.57
Larkinella	0.01	0.01	0.07	0.04	0.01	0.01	0.05	0.03	0.02	0.02	0.01	0.02	0.01	0.02	0.04	0.03
Legionella	0.06	0.00	0.01	0.00	0.01	0.00	0.00	0.01	0.07	0.02	0.01	0.00	0.01	0.02	0.00	0.00
Leucobacter	0.00	0.02	0.01	0.03	0.00	0.00	0.01	0.01	0.01	0.00	0.01	0.02	0.00	0.01	0.01	0.02
Leuconostoc	0.67	0.36	1.11	0.76	0.26	0.26	0.50	0.50	0.58	0.32	0.33	0.54	0.38	0.37	0.62	0.67
Luteimonas	0.04	0.01	0.15	0.15	0.01	0.04	0.17	0.10	0.02	0.01	0.13	0.04	0.01	0.03	0.23	0.14
Massilia	0.01	0.01	0.03	0.03	0.00	0.01	0.03	0.04	0.02	0.00	0.02	0.02	0.00	0.02	0.03	0.03
Mesorhizobium	0.01	0.02	0.01	0.02	0.00	0.00	0.00	0.02	0.01	0.01	0.00	0.01	0.00	0.00	0.00	0.01
Methylobacterium	0.31	0.59	0.96	1.22	0.28	0.34	0.63	1.33	0.38	0.69	0.25	0.75	0.19	0.72	0.52	0.88
Morganella	0.01	0.01	0.02	0.00	0.00	0.01	0.01	0.01	0.00	0.01	0.01	0.00	0.01	0.02	0.00	0.00
Mucilaginibacter	0.06	0.02	0.24	0.14	0.02	0.09	0.15	0.14	0.01	0.03	0.06	0.10	0.02	0.06	0.14	0.17
Mycobacterium	0.06	0.02	0.02	0.02	0.06	0.02	0.04	0.06	0.04	0.05	0.02	0.02	0.01	0.01	0.01	0.02
Nakamurella	0.00	0.02	0.03	0.04	0.01	0.00	0.02	0.02	0.01	0.02	0.01	0.01	0.02	0.02	0.02	0.02
Novosphingobium	0.00	0.00	0.03	0.01	0.00	0.01	0.02	0.01	0.01	0.03	0.00	0.01	0.02	0.00	0.02	0.02
Nubsella	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.02	0.01
Ochrobactrum	0.03	0.01	0.03	0.04	0.01	0.00	0.03	0.04	0.00	0.03	0.02	0.01	0.00	0.03	0.02	0.04
Oxalicibacterium	0.01	0.00	0.01	0.02	0.00	0.00	0.03	0.06	0.00	0.01	0.01	0.01	0.00	0.01	0.03	0.03
Oxobacter	0.00	0.01	0.00	0.00	0.16	0.52	0.00	0.00	0.01	0.27	0.01	0.00	0.31	0.09	0.00	0.00
Pantoea	0.32	0.33	0.14	0.14	0.30	0.16	0.17	0.12	0.36	0.35	0.15	0.06	0.44	0.24	0.13	0.11
Paracoccus	0.01	0.01	0.04	0.08	0.00	0.01	0.07	0.10	0.00	0.02	0.02	0.04	0.01	0.01	0.04	0.05
Pectobacterium	0.02	0.01	0.02	0.00	0.02	0.02	0.00	0.00	0.01	0.03	0.01	0.00	0.02	0.02	0.00	0.01
Pediococcus	1.74	1.58	3.10	1.91	1.96	0.75	1.62	1.38	1.31	0.48	1.12	0.83	1.53	0.62	1.81	1.46
Pedobacter	0.02	0.01	0.09	0.06	0.01	0.03	0.06	0.05	0.01	0.01	0.04	0.03	0.02	0.02	0.09	0.05
Pelomonas	0.01	0.11	0.00	0.02	0.01	0.01	0.01	0.02	0.01	0.02	0.01	0.01	0.01	0.02	0.00	0.02
Phyllobacterium	0.02	0.02	0.02	0.03	0.01	0.02	0.01	0.05	0.02	0.04	0.01	0.01	0.01	0.02	0.02	0.01
Prevotella_7	0.00	0.02	0.00	0.01	0.01	0.01	0.00	0.01	0.00	0.74	0.00	0.08	0.03	0.03	0.00	0.00
Prevotella_9	0.08	0.20	0.00	0.01	2.08	0.00	0.01	0.01	0.05	0.03	0.00	0.01	0.00	0.00	0.00	0.00

Providencia	0.02	0.01	0.02	0.02	0.03	0.02	0.02	0.04	0.01	0.01	0.02	0.02	0.01	0.02	0.01	0.03
Pseudocitrobacter	0.04	0.03	0.06	0.04	0.02	0.02	0.05	0.04	0.04	0.03	0.04	0.01	0.02	0.03	0.04	0.02
Pseudomonas	0.06	0.02	0.09	0.05	0.02	0.07	0.08	0.08	0.06	0.04	0.07	0.06	0.02	0.05	0.07	0.06
Pseudorhodoferax	0.00	0.00	0.02	0.02	0.01	0.00	0.01	0.07	0.00	0.01	0.01	0.02	0.00	0.01	0.02	0.02
Pseudoxanthomonas	0.02	0.01	0.11	0.07	0.01	0.02	0.07	0.08	0.02	0.03	0.04	0.06	0.02	0.02	0.12	0.07
Quadrisphaera	0.05	0.02	0.10	0.03	0.04	0.02	0.04	0.02	0.03	0.02	0.01	0.01	0.03	0.04	0.02	0.03
Ralstonia	0.04	0.55	0.03	0.11	0.03	0.15	0.02	0.09	0.06	0.32	0.01	0.04	0.04	0.30	0.02	0.09
Rhodanobacter	0.69	0.05	0.06	0.02	0.91	0.10	0.21	0.02	1.12	0.35	0.11	0.02	0.39	0.05	0.06	0.04
Rhodococcus	0.01	0.00	0.03	0.03	0.00	0.00	0.03	0.04	0.00	0.01	0.01	0.03	0.00	0.01	0.02	0.02
Rhodoferax	0.04	0.01	0.04	0.06	0.01	0.01	0.05	0.04	0.01	0.02	0.02	0.02	0.01	0.02	0.04	0.04
Rhodopseudomonas	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.04	0.00	0.01	0.00	0.01	0.00	0.00	0.01	0.02
Rickettsia	0.00	0.01	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.01	0.00	0.01	0.01	0.01
Rosenbergiella	0.09	0.08	0.10	0.34	0.07	0.05	0.15	0.17	0.07	0.11	0.09	0.11	0.05	0.10	0.13	0.12
Roseomonas	0.04	0.05	0.06	0.09	0.04	0.03	0.08	0.16	0.02	0.04	0.02	0.08	0.04	0.06	0.08	0.08
Salana	0.01	0.01	0.04	0.04	0.00	0.01	0.04	0.02	0.01	0.00	0.04	0.01	0.01	0.01	0.05	0.03
Serratia	0.01	0.03	0.01	0.01	0.01	0.02	0.01	0.01	0.02	0.02	0.00	0.01	0.00	0.01	0.01	0.00
Shinella	0.01	0.00	0.01	0.10	0.01	0.01	0.02	0.15	0.00	0.01	0.02	0.04	0.00	0.05	0.03	0.08
Siphonobacter	0.01	0.00	0.03	0.03	0.01	0.01	0.02	0.04	0.01	0.02	0.01	0.03	0.01	0.01	0.03	0.04
Sphingobacterium	0.06	0.04	0.22	0.23	0.02	0.05	0.32	0.31	0.01	0.05	0.47	0.07	0.03	0.05	0.25	0.17
Sphingobium	0.29	0.50	0.73	1.05	0.31	0.67	0.59	1.13	0.28	0.73	0.33	0.81	0.20	0.82	0.53	0.85
Sphingomonas	1.40	1.38	3.12	2.79	1.13	1.04	3.00	2.83	1.18	1.50	1.33	1.61	1.00	1.71	2.37	2.36
Spirosoma	0.31	0.31	0.60	0.52	0.32	0.14	0.39	0.31	0.24	0.31	0.26	0.25	0.17	0.35	0.44	0.35
Sporosarcina	0.08	0.00	0.01	0.02	0.00	0.02	0.01	0.00	0.01	0.02	0.01	0.00	0.00	0.02	0.00	0.00
Staphylococcus	0.01	0.00	0.01	0.02	0.01	0.02	0.01	0.01	0.03	0.05	0.01	0.02	0.02	0.13	0.02	0.03
Starkeya	0.01	0.02	0.01	0.02	0.01	0.01	0.01	0.03	0.02	0.02	0.01	0.00	0.01	0.01	0.00	0.01
Stenotrophomonas	0.10	0.03	0.16	0.11	0.04	0.03	0.17	0.27	0.05	0.04	0.10	0.05	0.02	0.08	0.31	0.19
Variovorax	0.00	0.02	0.01	0.03	0.00	0.02	0.01	0.07	0.01	0.03	0.00	0.04	0.01	0.03	0.01	0.03
Verticia	0.01	0.00	0.03	0.03	0.00	0.01	0.05	0.08	0.00	0.00	0.03	0.02	0.00	0.01	0.04	0.05
Weissella	22.12	22.39	12.60	11.05	19.64	12.01	8.33	10.10	23.71	18.00	8.89	8.14	19.03	13.19	8.13	9.99
Williamsia	0.01	0.02	0.05	0.06	0.00	0.01	0.04	0.05	0.00	0.01	0.01	0.03	0.00	0.01	0.03	0.06

Xanthomonas	0.02	0.00	0.25	0.23	0.00	0.01	0.23	0.30	0.00	0.00	0.08	0.11	0.00	0.02	0.19	0.17
Outros	5.38	9.67	5.40	6.35	3.55	4.62	4.09	3.67	7.42	6.35	2.48	3.64	4.23	4.62	3.92	5.44