MARCOS RENAN LIMA LEITE

ALTERAÇÕES NA ESTRUTURA E DIVERSIDADE DA COMUNIDADE BACTERIANA DA RIZOSFERA DE CANA-DE-AÇÚCAR APÓS APLICAÇÃO DE SILÍCIO

TERESINA - PI

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Tese apresentada à Universidade Federal do Piauí, como parte das exigências do Programa de Pós-Graduação em Agronomia, na área de concentração de Produção Vegetal, como requisito para obtenção do título de Doutor em Agronomia.

Orientador

Prof. Dr. Francisco de Alcântara Neto

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APROVADA em 27 de janeiro de 2023.

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TERESINA – PI

2023

Aos meus pais José e Seli, em cuja simplicidade residem suas maiores riquezas. As minhas irmãs, Andréia, Paula e Cléia, pela cumplicidade e incentivo constante.

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“A crítica válida presta um favor ao cientista”

Carl Sagan

RESUMO

A fertilização com silício (Si) no cultivo da cana-de-açúcar, tem promovido benefícios que resultam em maiores rendimentos de colmo e açúcar. A interação do Si com os microrganismos do solo pode aumentar potencialmente a disponibilidade de nutrientes, a fixação biológica de N, e contribuir na maior tolerância a estresses bióticos e abióticos. O uso de fontes fluidas de Si tem-se mostrado eficaz no fornecimento desse elemento. Este estudo propôs uma investigação aprofundada do efeito da fertilização com Si fluido na comunidade bacteriana da rizosfera de cana-de-açúcar. Assim, dois ensaios foram realizados, um em condições de campo e o outro em casa de vegetação. No ensaio de campo foi avaliado o efeito da adubação com Si sobre as comunidades microbianas na rizosfera de três genótipos de cana-de-açúcar durante dois ciclos (planta e soca). No ensaio em casa de vegetação avaliou-se o efeito de duas fontes fluidas de Si (silicato de sódio e silicato de potássio) sobre as comunidades microbianas da rizosfera na fase inicial de crescimento da cultura. Ambos os ensaios utilizaram o sequenciamento do gene 16S rRNA. Os resultados demonstraram diferentes padrões na abundância de filos entre a rizosfera da cana planta e soca. A fertilização com Si e os genótipos de cana-de-açúcar influenciaram a diversidade e composição da comunidade microbiana na rizosfera, enriquecendo-a com alguns grupos bacterianos, como fixadores de N e outros promotores de crescimento. A aplicação de silicato de sódio teve um efeito mais pronunciado na comunidade procariótica do que o silicato de potássio, o que resultou em uma mudança na estrutura da comunidade. O fornecimento de silicato de sódio levou a um aumento na abundância relativa de Proteobacteria e Bacteriodetes, enquanto o silicato de potássio promoveu um enriquecimento de Chloroflexi e Acidobacteriota. As respostas distintas das fontes de Si sobre a comunidade microbiana, pode ser atribuída à composição de cada fertilizante, que possivelmente promoveram alterações no perfil de exsudação radicular, recrutando grupos específicos de bactérias que desempenham importantes funções na rizosfera da cana-de-açúcar. Novos estudos são recomendados para avaliar o microbioma da rizosfera de cana-de-açúcar fertilizada com Si, bem como o impacto de grupos promotores de crescimento e fixadores de N no crescimento, desempenho e rendimento da cana-de-açúcar.

Palavras-chave: *Saccharum officinarum*, interação microrganismos plantas, ecologia microbiana, silicato de sódio e potássio, bioinformática.

ABSTRACT

Fertilization with silicon (Si) in sugarcane cultivation has promoted benefits that result in higher stalk and sugar yields. The interaction of Si with soil microorganisms can potentially increase nutrient availability, biological N fixation, and contribute to greater tolerance to biotic and abiotic stresses. The use of Si fluid sources has been shown to be effective in supplying this element. This study proposed an in-depth investigation of the effect of fluid Si fertilization on the bacterial community of the sugarcane rhizosphere. In this way, two tests were carried out, one in field conditions and the other in a greenhouse. In the field trial, the effect of Si fertilization on microbial communities in the rhizosphere of three sugarcane genotypes during two cycles (cane plant and ratoon) was evaluated. In the greenhouse test, the effect of two fluid sources of Si (sodium silicate and potassium silicate) on the microbial communities of the rhizosphere in the initial phase of culture growth was evaluated. Both assays used 16S rRNA gene sequencing. The results showed different patterns in the abundance of phyla between the cane plant and ratoon rhizosphere. Si fertilization and sugarcane genotypes influenced the diversity and composition of the microbial community in the rhizosphere, enriching it with some bacterial groups, such as N fixers and other growth promoters. Sodium silicate application had a more pronounced effect on the prokaryotic community than potassium silicate, which resulted in a change in community structure. The supply of sodium silicate led to an increase in the relative abundance of Proteobacteria and Bacteriodetes, while potassium silicate promoted an enrichment of Chloroflexi and Acidobacteriota. The distinct responses of Si sources on the microbial community can be attributed to the composition of each fertilizer, which possibly promoted changes in the root exudation profile, recruiting specific groups of bacteria that play important roles in the sugarcane rhizosphere. Further studies are recommended to evaluate the rhizosphere microbiome of sugarcane fertilized with Si, as well as the impact of growth promoting groups and N fixers on growth, performance and yield of sugarcane.

Keywords: *Saccharum officinarum*, plant microorganisms interaction, microbial ecology, sodium and potassium silicate, bioinformatics.

SUMÁRIO

1 INTRODUÇÃO GERAL

A cana-de-açúcar (*Saccharum* spp.) é uma cultura de grande importância socioeconômica, cultivada frequentemente sob monocultura de longo prazo em regiões tropicais e subtropicais do mundo, sendo capaz de sintetizar e armazenar altas concentrações de sacarose no colmo, matéria prima utilizada para produção de açúcar e etanol (MARTÍNI et al., 2020).

O Brasil se destaca como maior produtor mundial de cana-de-açúcar, com área colhida de 8,3 milhões de ha e produção de 585,2 milhões de toneladas na safra 2021/22 (CONAB, 2022). A maior parte das áreas com cana-de-açúcar utilizam sistemas convencionais de produção, com cultivos sucessivos em uma mesma área e uso intensivo de agroquímicos (inseticidas, fungicidas, herbicidas, entre outros) e fertilizantes minerais, principalmente em regiões com predominância de solos ácidos e com baixa fertilidade natural (BORDONAL et al., 2018). Nesse contexto, há uma crescente preocupação relacionada aos possíveis impactos ambientais gerados do cultivo, em especial a degradação da qualidade do solo, relacionada às mudanças das características físico-químicas (MARASCA et al., 2016, CANISARES et al., 2019) e diminuição da diversidade da comunidade microbiana do solo (NIU et al., 2021; PANG et al., 2021).

Esse cenário tem impulsionado o desenvolvimento de pesquisas voltadas para uma agricultura mais sustentável. Nos últimos anos a fertilização com silício (Si) tem demonstrado grande relevância no cultivo da cana-de-açúcar (MAJUMDAR et al., 2020; VERMA et al., 2020; TEXEIRA et al., 2022). O Si é considerado um elemento benéfico e está associado a diferentes benefícios às plantas, como maior estabilidade e fortalecimento estrutural da planta, aumento da eficiência no uso da água, além de estimular a absorção de nutrientes, que resulta em incrementos na produção de colmos e açúcar (ZHU e GONG, 2014; GUERREIRO et al., 2016; FRAZÃO et al., 2020).

O uso de Si na forma fluida, à exemplo do silicato de sódio e silicato de potássio, têm-se mostrado uma alternativa eficaz, por permitir a redução das doses do elemento e otimizar a performance de absorção pelas plantas (ZHOU et al., 2018; OLIVEIRA et al., 2021; ROCHA et al., 2022). Isso deve ocorrer especialmente com seu fornecimento via solo na forma fluida, pois promove aumento da absorção pelas raízes

da cana-de-açúcar (TEXEIRA et al., 2020; 2021; 2022) e de outras espécies (ROCHA et al., 2022; TEXEIRA et al., 2022).

Estudos recentes têm demonstrado que a interação Si e microrganismos na região da rizosfera, pode melhorar melhora a saúde do solo, por meio de alterações na estrutura da comunidade bacteriana, que pode promover aumento da diversidade de bactérias promotoras de crescimento, associadas à resistência a estresses, crescimento, desempenho e rendimento de diversas culturas (DAS et al., 2019; LIN, et al., 2020; WANG et al., 2020; VERMA et al., 2022).

Deng et al. (2021) observaram que a fertilização com metassilicato de sódio anidro, promoveu o enriquecimento da rede de bactérias da rizosfera, e aumentou significativamente a abundância relativa de grupos associados ao filo Proteobacteria, cujo alguns membros são promotoras de crescimento vegetal. No entanto, ainda é necessário um melhor entendimento sobre o emprego de diferentes fontes fluidas de Si e seus efeitos sobre o crescimento inicial da cana-de-açúcar e sobre a dinâmica das comunidades microbianas associadas a rizosfera de diferentes genótipos e ciclos de crescimento. Essas respostas são importantes, haja visto que estudos com outras culturas demonstraram que existe efeito dos genótipos e estádio da planta sobre a comunidade bacteriana associada à rizosfera (FERNÁNDEZ-BACA et al., 2021; LI et al., 2021; MOROENYANE et al., 2021).

Neste estudo, foram levantadas as seguintes hipóteses: (i) a adubação com Si influencia as comunidades microbianas da rizosfera associadas a diferentes genótipos de cana-de-açúcar; (ii) a estrutura das comunidades microbianas diferem entre o primeiro ano (cana-planta) e o segundo ano (cana-soca) de cultivo da cana-de-açúcar e (iii) diferentes fontes fluidas de Si promovem respostas diferenciadas na estrutura das comunidades associadas a rizosfera da cana-de-açúcar durante o estádio inicial de desenvolvimento.

Os resultados deste estudo permitirão avançar ainda mais no campo da nutrição da cana-de-açúcar, bem como relacionar os efeitos do Si fluido na estrutura e na composição da comunidade microbiana associados a resposta de diferentes genótipos e ciclos de cultivo. Essas informações ainda não exploradas pela pesquisa permitirão uma melhor compreensão do sistema produtivo da cana-de-açúcar e o uso mais sustentável dos recursos naturais, reduzindo os impactos ao meio ambiente.

Diante do exposto, objetivou-se avaliar o efeito da adubação com Si sobre as comunidades microbianas na rizosfera de genótipos de cana-de-açúcar cultivados em solo tropical, durante dois ciclos: planta e soca, e utilizando duas fontes fluidas de Si, por meio do sequenciamento do gene 16S rRNA.

2 REVISÃO DE LITERATURA

2.1 O cultivo de cana-de-açúcar no Brasil

A busca por fontes de energia renovável tem incentivado o desenvolvimento de uma nova matriz energética capaz de atender à crescente demanda de energia global, por meio de uma produção sustentável, e ao mesmo tempo, evitar a competição com a produção de alimentos (CARVALHO-NETTO et al., 2014).

Nesse cenário, a cana-de-açúcar (*Saccharum* spp.) tem se destacado como uma das principais culturas agrícolas de forte impacto econômico no mundo, devido sua capacidade de sintetizar e armazenar altas concentrações de sacarose no colmo, sendo matéria prima para produção de açúcar e etanol (MARTÍNI et al., 2020).

O Brasil é o maior produtor mundial de cana-de-açúcar e um dos principais fornecedores de açúcar e etanol no mundo (FAO, 2021a). Tal feito, se dá principalmente em decorrência do país possuir, em geral, grandes áreas cultiváveis aliada às condições edafoclimáticas favoráveis ao cultivo (CAVALCANTI et al., 2019).

Na safra de 2021/22 a produção nacional de cana-de-açúcar totalizou 585,2 milhões de toneladas em uma área colhida de 8,3 milhões de hectares, com produtividade média de 70.357 kg ha^{-1}. O Sudeste do Brasil foi a região com maior participação e respondeu por 62,7% (366.929,9 mil t) do total de produção, seguida da região Centro-Oeste, responsável por 22,4% (131.370,3 mil t), do Nordeste com 8,7% (51.062,1 mil t), Sul com 5,5% (31.961,6 mil t) e o Norte com apenas 0,7% (3.855,53 mil t) do total de produção (CONAB, 2022).

A cana-de-açúcar é uma cultura típica de clima quente e úmido, e isso faz com que se adapte bem ao clima de muitas regiões do Brasil (CARVALHO et al., 2015). O Nordeste é caracterizado como uma região promissora ao desenvolvimento do setor Sucroenergético do país, com expansão das áreas de cultivo de cana-de-açúcar (ANDRADE JUNIOR et al., 2018). No entanto, alguns fatores limitam a produção de

cana-de-açúcar nessa região, que possui áreas de cultivo com predominância de solos arenosos, com baixa fertilidade e baixa capacidade de armazenamento de água (CAVALCANTI et al., 2019).

No Nordeste é comum a ocorrência de irregularidades pluviométricas, que culminam em longos períodos de escassez hídrica no solo, considerado um dos principais fatores abióticos que mais geram impactos negativos sobre a produção de biomassa total da cana-de-açúcar, inibindo o crescimento, desenvolvimento e taxa de assimilação fotossintética de CO_2 (MONTENEGRO e RAGAB, 2012; VERMA et al. 2019; PAIXÃO et al., 2020). Os estádios de perfilhamento e crescimento pleno são as fases mais sensíveis ao déficit hídrico, pois coincidem com as de maior demanda de água pela planta (DINH et al., 2017). Para atingir altas produtividades, a cana-de-açúcar requer de 1.500 a 2.500 mm de água/ano, distribuída de maneira uniforme ao longo da estação de cultivo (FAO, 2021b).

Outro fator crucial na produção de cana-de-açúcar é disponibilidade de nutrientes no solo. A cana-de-açúcar é uma cultura com alta demanda de nutrientes, cujas quantidades extraídas do solo variam de acordo com tipo de solo, disponibilidade de nutrientes, variedade e métodos de cultivo. Em geral, as extrações dos nutrientes se encontraram na ordem decrescente para macronutrientes de K>N>Ca>Mg>S>P (MAEDA, 2009).

A adubação mineral é uma das principais práticas responsáveis pelos incrementos de produtividade da cana-de-açúcar e que mais interferem na qualidade da cultura (KORNDORFER, 1994). A maior parte dos estudos sobre adubação mineral em cana-de-açúcar têm se concentrado nos principais macronutrientes, como nitrogênio, fósforo e potássio (TRIVELIN et al., 2013; CRUSCIOL et al., 2020).

No entanto, alguns estudos sobre nutrição de cana-de-açúcar têm demonstrado que o silício (Si), é capaz de mitigar estresses bióticos e abióticos, além de ser considerado um bioestimulante para o crescimento, desempenho e rendimento das plantas superiores (VERMA et al., 2020; MAJUMDAR e PRAKASH, 2020; THIND et al., 2020).

2.2 Silício no cultivo de cana-de-açúcar

O Si é o segundo elemento mais abundante na crosta terrestre, muito embora, sua forma prontamente absorvida pelas plantas, que ocorre na solução do solo como ácido silícico ou ácido monossilícico [$Si(OH)_4$] também chamado de Si solúvel ou biodisponível, seja encontrada em pequenas concentrações (normalmente variando de 0,1 a 2,0 mM (pH < 9)) e tenha sua disponibilidade afetada pelo pH, temperatura, teor de matéria orgânica, presença de óxidos de ferro e alumínio e concentração do elemento na solução (EPSTEIN, 1994; KORNDÖRFER, 2006; MALI e AERY, 2009).

As plantas diferem em sua capacidade de acumular Si, sendo classificadas como acumuladoras (teor de Si > 1 g kg^{-1}), intermediárias (0,5 a 1 g kg^{-1} Si), e não acumuladoras (< 0,5 g kg^{-1} Si) (MA et al., 2001). As espécies pertencentes a família *Poaceae* são consideradas acumuladoras de Si, por possuírem transportadores específicos LSi1 que favorece a absorção do elemento pela cultura (MITANI et al., 2008) a exemplo da cana-de-açúcar, considerada a cultura que mais absorve o elemento (300−700 kg de Si ha^{-1}) e a segunda mais responsiva, atrás apenas do arroz (BARKER E PILBEAM, 2007; LIANG et al., 2015; SAHEBI et al., 2015).

Na cana-de-açúcar, o Si é absorvido mais do que qualquer outro nutriente (ANDERSON, 1991), sendo depositado na forma de sílica gel nas folhas e colmos, que atua como uma barreira física aliviando a ação de insetos (CAMARGO et al., 2014; ATENCIO et al., 2018), doenças (CAMARGO et al., 2020), além de reduzir a perda de água por transpiração (VERMA et al., 2020). O Si ainda melhora a capacidade fotossintética da planta, sendo considerado um importante regulador enzimático na síntese, retenção e armazenamento de açúcar (MEYER e KEEPING, 2000).

Diversos estudos têm demonstrado o papel do Si na estabilidade e fortalecimento estrutural da cana-de-açúcar, no equilíbrio de nutrientes, além de aumentar a eficiência no uso da água, mantendo o turgor celular e regulando a taxa de transpiração e de atividades de enzimas antioxidantes, o que resulta em incrementos significativos na produção de colmos e açúcar devido a maior eficiência fotossintética da planta (ZHU e GONG, 2014; GUERREIRO et al., 2016; NAEEM et al., 2018; MCCRAY e SHANGNING, 2018; OLIVEIRA et al., 2019; FRAZÃO et al., 2020).

Por ser uma cultura que responde positivamente à adubação com Si, sob condições específicas a cana-de-açúcar é capaz de remover quantidades significativas do elemento do solo (MEYER e KEEPING, 2000). Segundo Savant et al. (1999), um dos fatores para o declínio da fertilidade de canaviais é a absorção contínua de Si pela soca da cana-de-açúcar sem reposição do elemento, justificando a importância da fertilização com Si em áreas de cultivo intensivo de cana. Além disso, alguns estudos reforçam a necessidade de aplicação de Si, principalmente em áreas com cultivos de cana de sequeiro, onde foram confirmadas deficiências regulares de Si, quando comparadas com áreas irrigadas (VAN DER LAAN e MILES, 2010).

Em estudo recente Zang e Guan (2022), demonstraram que a redução de Si no solo também gera impactos importantes na estrutura e função de comunidades bacterianas do solo. Essa é uma informação de grande relevância, pois sabe-se que as comunidades microbianas do solo desempenham papel importante na manutenção das funções do agrossistema, e afetam os ciclos biogeoquímicos dos nutrientes e sua disponibilidade para as plantas.

2.3 Comunidade microbiana da rizosfera associada a planta

Estudos sobre microrganismos associados as plantas têm possibilitado a exploração da diversidade microbiana do solo, além de desvendar o seu papel no crescimento, desenvolvimento, tolerância à estresses e maior aproveitamento de nutrientes pelas plantas. No solo, os microrganismos desempenham um papel fundamental na transformação e translocação dos nutrientes para as plantas, além disso, por serem altamente sensíveis à contaminantes, são utilizados como importante indicador das mudanças nas propriedades físicas e químicas do solo e da qualidade ambiental (JACOBY et al., 2017; KUANG et al., 2018).

Avanços no campo das interações planta-microrganismos demonstram que as plantas são capazes de moldar seu microbioma da rizosfera, região estreita do solo influenciada pelos exsudatos radiculares, que pode conter até 10^{11} células microbianas por grama de raiz e mais de 30.000 espécies procarióticas. Já foi demosntrado que sob cultivo em um mesmo solo, espécies diferentes de plantas hospedam comunidades microbianas específicas (MENDES et al., 2008; BERENDSEN et al., 2011; EGAMBERDIEVA et al.; 2012).

Na cultura da cana-de-açúcar, por exemplo, foi demonstrado que algumas bactérias isoladas da rizosfera se mostraram capazes de realizar a fixação de nitrogênio em condições de campo, por meio de um ecossistema microbiano autossustentável (DÖBEREINER et al., 1972; URQUIAGA et al., 2012).

As comunidades bacterianas são influenciadas pela sazonalidade, tipo de vegetação e manejo do solo (SILVA et al., 2019). O tipo de vegetação atua como um determinante primário na estrutura das comunidades microbianas, seja através da liberação de exsudatos radiculares, fonte de energia para a microbiota do solo, seja pela contribuição na quantidade e qualidade da serapilheira, influenciando na heterogeneidade das comunidades microbianas (BRESOLIN et al., 2010).

No entanto, o entendimento sobre as comunidades microbianas sempre foi limitado, uma vez que as técnicas convencionais de cultivo são restritas a menos de 1% dos microrganismos presentes no solo (YUN et al., 2004). Assim, o desenvolvimento de técnicas da biologia molecular possibilitou um maior conhecimento sobre as comunidades microbianas (SCHOLER et al., 2017), incluindo aquelas que não eram possíveis de serem acessadas através de técnicas tradicionais, devido a impossibilidade de cultivo em meio de cultura.

Nos últimos anos, abordagens baseadas em *amplicon* visando regiões variáveis de marcadores específicos como 16S e ITS (espaçador transcrito interno, do inglês “internal transcribed spacer”) têm sido empregadas em diversos estudos para descrever a composição das comunidades microbianas do solo (XU et al., 2018; JOCHUM et al., 2019; LEE et al., 2021). O gene 16S rRNA é utilizado na avaliação da diversidade de procariotos (bactérias e arqueas), enquanto as regiões ITS do RNA ribossômico é utilizado na avaliação da diversidade fúngica (SCHÖLER et al., 2017).

O emprego de plataformas de sequenciamento de alto rendimento, à exemplo do Illumina MiSeq, tem sido uma ferramenta importante e possibilitado a análise da composição da comunidade microbiana, o que pode ser ver verificado em diversos estudos (SUN et al., 2016; XU et al., 2017; CHEN et al., 2020). Essas plataformas têm alcançado um alto número de sequências (*reads*), capazes de revelar a diversidade de comunidades microbianas complexas (PARAMESWARAN, 2010).

Nesse sentido, o uso conjunto de técnicas da bioinformática tem sido fundamental para interpretação de dados metagenômicos (ZHANG et al., 2014) em

razão do grande volume e complexidade, sendo uma ferramenta importante para uma compreensão mais abrangente da estrutura da comunidade microbiana, ou seja, da sua composição, riqueza, diversidade e potencial biotecnológico (Zheng et al., 2019).

2.4 Silício associado a comunidade bacteriana da rizosfera

Nos últimos anos, pesquisas voltadas para o estudo dos efeitos da fertilização com silício sobre as comunidades microbianas do solo, associado a resistência de diversos estresses, vem sendo desenvolvidas para diversas culturas de interesse agronômico (LI et al., 2019; LIN et al., 2020; WANG et al., 2020; SONG et al., 2021; ZANG e GUAN et al., 2022).

Em estudo com a cultura acelga pakchoi (*Brassica chinensis L.)*, Wang et al. (2020) avaliaram os efeitos do Si na comunidade bacteriana de um solo contaminado com metais pesados, e reportaram mudanças significativas das estruturas da comunidade bacteriana do solo sob as maiores doses do elemento. Os autores sugerem que a fertilização com Si pode ter desempenhado papel importante na redução da pressão de metais pesados presentes no solo. A aplicação de silício ainda melhorou significativamente o rendimento da acelga pakchoi, além de reduzir o teor de metais pesados na planta. Além disso, bactérias associadas ao funcionamento metabólico do solo de carbono/nitrogênio, incluindo *Nitrospirae, Flavobacteriias, Anaerolineae* e *Bacillales* tiveram maior abundância nos tratamentos fertilizados com Si.

Na cultura do pepino (*Cucumis sativus* L.), a fertilização com silicato de sódio aumentou a resistência das plantas à murcha de *Fusarium*, além de aumentar a abundância e diversidade das comunidades bacterianas do solo (ZHOU et al., 2018). Segundo os autores o aumento da resistência à doença foi mais associado as alterações nas comunidades microbianas mediadas pelo Si do que da inibição direta do elemento no crescimento do patógeno.

Em estudo com a cultura do tomate Wang et al. (2020) inocularam um solo com *Ralstonia Solanacearum* que causa a murcha bacteriana, e verificaram que o tratamento com 2,0 mM de Si reduziu o índice de doença em 19,18% a 52,7% em comparação com plantas não tratadas com o elemento. Além disso, o Si influenciou fortemente a composição da comunidade microbiana da rizosfera, regulando um total

de 63,7% das unidades taxonômicas operacionais bacterianas (OTUs) e 43,8% das OTUs fúngicas. Segundo os autores a resistência mediada pelo Si foi associado a mudanças na quantidade de microrganismos e na atividade enzimática do solo.

No cultivo de trigo em solo contaminado com cádmio (Cd), Song et al. (2021) reportaram efeitos benéficos do Si, atuando na redução de Cd no solo e nas plantas. O fornecimento de Si também alterou a comunidade microbiana que desempenhou papel substancial no aumento do rendimento do trigo. A fertilização com Si também promoveu o aumento na abundância de Acidobactérias e Thaumarchaeota, que por sua vez diminuíram a disponibilidade de Cd do solo.

Song et al. (2021) verificaram que o retorno da palha de arroz ao solo aumentou significativamente o Si biodisponível, bem como alterou significativamente a abundância da comunidade microbiana do solo, cuja análise de redundância demonstrou que o status de Si explicou significativamente 12% da variação da comunidade bacteriana do solo. Os resultados reportados sugerem que a comunidade e a diversidade de bactérias do solo interagem com a mobilidade do Si e resultam no equilíbrio de várias fontes de nutrientes para impulsionar o ciclo biológico do Si no agroecossistema.

Em estudo recente Yuan et al., (2022) evidenciaram que a fertilização com Si na cana-de-açúcar, promoveu significativamente a abundância bacteriana em compartimentos distintos da planta (folha, caule, raiz, rizosfera). Segundo os autores o fornecimento de Si tem o potencial de aumentar a manutenção da diversidade bacteriana, bem como a riqueza e coexistência de bactérias nos sistemas solo-planta, proporcionando uma maior diversidade funcional que resulta em efeitos positivos no crescimento das plantas.

Deng et al., (2021) avaliando a cana-de-açúcar durante o estádio de crescimento inicial do colmo, fertilizada com metassilicato de sódio anidro (Na_2SiO_3) como fonte de Si, demonstraram por meio de uma análise da rede de associação que a aplicação de Si enriqueceu a rede bacteriana da rizosfera, gerando impactos positivos sobre o crescimento da cultura. O Si promoveu um aumento significativo na abundância relativa de Proteobacterias, e segundo os autores esse pode ser o grupo dominante no crescimento da cana-de-açúcar fertilizada com Si.

No entanto, para a cultura da cana-de-açúcar, o efeito do Si biodisponível sobre a comunidade microbiana da rizosfera ainda é pouco compreendido, principalmente quando associados a resposta de diferentes genótipos e ciclos de cultivo.

Contudo, ainda são incipientes informações a respeito do efeito da fertilização de fontes distintas de Si fluido sobre a estrutura da comunidade bacteriana da rizosfera de cultivares de cana-de-açúcar, no estádio inicial de desenvolvimento da planta, e durante dois ciclos de cultivo (cana-planta e cana-soca).

REFERÊNCIAS

ANDERSON, D. L. Soil and leaf nutrient interactions following application of calcium silicate slag to sugarcane. **Fertilizer Research** v. 30, p. 9–18, 1991.

ANDRADE JUNIOR, A.S.; BASTOS, E.; RIBEIRO, V.; LOPES, A.D.S.; SILVA, M.V. P.; SILVA, J. Produtividade de colmos e eficiência do uso de água em cultivares de cana-de-açúcar em gotejamento subsuperficial. Embrapa Meio-Norte - **Comunicado Técnico** (INFOTECA-E), 2018.

ATENCIO, R.; GOEBEL, F.; GUERRA, A. Effect of silicon and nitrogen on *Diatraea tabernella* dyar in sugarcane in Panama. **Sugar Tech**, v. 21, p. 113–121, 2019.

BARKER, A. V., and PILBEAM D. J. 2007. Handbook of Plant Nutrition. Boca Raton, FL: Taylor & Francis. Chapman, H. D., and P. F. Pratt. 1961. **Methods of Analysis for Soil, Plant and Water**. Riverside, CA: Division of Agriculture Science, California University.

BERENDSEN, R. L.; PIETERSE, C. M.J.; BAKKER, P. A. H. M. The rhizosphere microbiome and plant health. **Trends in Plant Science**, v. 17, n. 8, p. 478-486, 2012.

BORDONAL, R. O.; CARVALHO, J. L. N.; LAL, R.; FIGUEREDO, E. B.; OLIVEIRA, B. G.; LA SCALA JUNIOR, N. Sustainability of sugarcane production in Brazil. A review. **Agronomy for Sustainable Development**, v. 38, n.13, 2018.

BRESOLIN, J. D.; BUSTAMANTE, M.M.C; KRÜGER, R. H.; SILVA, M.R.S.S.; PEREZ, K.S. Structure and composition of bacterial and fungal community in soil under soybean monoculture in the Brazilian Cerrado. **Brazilian Journal of Microbiology**, v. 41, n. 2 p. 391-403, 2010.

CAMARGO, M. S.; COUTINHO, I. D.; LOURENÇO, S. A.; SOARES, M. K. M.; COLNAGO, L. A.; APPEZZATO-DA-GLÓRIA, B.; CAVALHEIRO, A. J.; AMORIM, L. Potential prophylactic role of silicon against brown rust (*Puccinia melanocephala*) in sugarcane. **European Journal of Plant Pathology**, v.157 n.1 p. 77-88, 2020.

CAMARGO, M. S.; KORNDÖRFER, G. H.; FOLTRAN, D. E. Silicon absorption and stalk borer incidence by sugarcane varieties in two ratoons. **Bioscience Journal**, v. 30, n. 5, p. 1304-1313, 2014.

CANISARES, L.P; CHERUBIN M.R.; SILVA, L.F.S.; FRANCO, A.L.C.; COOPER, M.; MOONEY, S. J.; CERRI, C.E.P. Soil microstructure alterations induced by land use change for sugarcane expansion in Brazil. **Soil Use Management**, v.36, p. 189– 199, 2020.

CARVALHO, A. L.; MENEZES, R. S. C.; NÓBREGA, R. S.; PINTO, A. S.; OMETTO, J. P. H. B.; VON RANDOW, C.; GIAROLLA, A. Impact of climate changes on potential sugarcane yield in Pernambuco, Northeastern region of Brazil. **Renewable Energy**, v. 78, p. 26-34, 2015.

CARVALHO-NETTO, O. V.; BRESSIANI, J.A.; SORIANO, H. L.; FIORI, C. S.; SANTOS, J. M.; BARBOSA, G. V.; XAVIER, M. A.; LANDELL, M. G.; PEREIRA, G. A. The potential of the energy cane as the main biomass crop for the cellulosic industry. **Chemical and Biological Technologies in Agriculture**, v. 1, p. 1–20, 2014.

CAVALCANTI, R. Q.; ROLIM, M. M.; LIMA, R. P.; TAVARES, U. E.; PEDROSA, E. M.R.; GOMES, I. F. Soil physical and mechanical attributes in response to successive harvests under sugarcane cultivation in Northeastern Brazil, **Soil and Tillage Research**, v. 189, p. 140-147, 2019.

CHEN, Y.; BONKOWSKI, M.; SHEN, Y.; GRIFFITHS, B. S.; JIANG, Y.; WANG, X.; SUN, B. Root ethylene mediates rhizosphere microbial community reconstruction when chemically detecting cyanide produced by neighbouring plants. **Microbiome**, v.8, 2020.

CONAB – Companhia Nacional de Abastecimento. **Cana-de-açúcar**, V.6, Safra 2021/2022, N.4 – Quarto levantamento, Abril 2022. Disponível em: https://www.conab.gov.br/info-agro/safras/cana/boletim-da-safra-de-cana-de-acucar/item/download. Acesso em: 17 de abril de 2022

CRUSCIOL, C. A. C.; ARRUDA, D. P.; FERNANDES, A. M.; ANTONANGELO, J. A.; ALLEONI, L. R. F.; NASCIMENTO, C. A. C.; MCCRAY, J. M. Methods and extractants to evaluate silicon availability for sugarcane. **Scientific Reports**, v. 8, n.1, p. 1-14, 2018.

CRUSCIOL, C. A. C.; SORATTO, R. P.; CASTRO, G. S. A.; COSTA, C. H. M.; FERRARI NETO, J. Aplicação foliar de ácido silícico estabilizado na soja, feijão e amendoim. **Revista Ciência Agronômica**, v.44, n.2, p. 404-410, 2013.

CRUSCIOL, C.; CAMPOS, M.; MARTELLO, J. M.; ALVES, C. J.; NASCIMENTO, C.; PEREIRA, J.; CANTARELLA, H. Organomineral fertilizer as source of P and K for sugarcane. **Scientific Reports**, v. 10, n.5398, 2020.

DAS, S.; KIM, G. W.; HWANG, H. Y.; VERMA, P. P.; KIM; P. J. Cropping with slag to address soil, environment, and food security. **Frontiers in Microbiology**, v.10, p.1320, 2019.

DENG, Q.; YU, T.; ZENG, Z.; ASHRAF, U.; SHI, Q.; HUANG, S.; LIAN, T.; CHEN, J.; MUZAFFAR, W.; SHEN, W. Silicon application modulates the growth, rhizosphere soil characteristics, and bacterial community structure in sugarcane. **Frontiers in Plant Science**, v.12, n.710139, 2021.

DINH, T.H.; WATANABE, K.; TAKARAGAWA, H.; NAKABARU, M.; KAWAMITSU, Y. Photosynthetic response and nitrogen use efficiency of sugarcane under drought stress conditions with different nitrogen application levels. **Plant Production Science**, v. 20, p. 412–422, 2017.

DOBEREINER, J.; DAY, J. M.; DART, P. J. Nitrogenase activity in the rhizophere of sugarcane and some other tropical grasses. **Plant Soil** v. 37, p. 191–196, 1972.

EGAMBERDIEVA, D.; KAMILOVA, F.; VALIDOV, S.; GAFUROVA, L.; KUCHAROVA, Z.; LUGTENBERG, B. High incidence of plant growth-stimulating bacteria associated with the rhizosphere of wheat grown on salinated soil in Uzbekistan. **Environmental Microbiology**, v.10, n. 1, p. 1-9, 2008.

EPSTEIN E (1994) The anomaly of silicon in plant biology. **Proc. Natl. Acad. Sci. USA**, v. 91, p. 11–17.

EPSTEIN, E. Annual Review of Plant Physiology and Plant Molecular Biology. **Silicon**, v. 50, p. 641-664, 1999.

FAO (2021a). **The agricultural production indices**. Food and Agricultural Organisation of the United Nations. Disponível em: https://www.fao.org. Acesso em: 17 março de 2022.

FAO (2021b). **Land & water: sugarcane**. Food and Agricultural Organisation of the United Nations. Disponível em: https://www.fao.org. Acesso em: 17 março de 2022.

FERNÁNDEZ-BACA, C. P.; RIVERS, A. R.; KIM, W.; IWATA, R.; MCCLUNG, A. M., ROBERTS, D. P.; REDDY, V. R., BARNABY, J. Y., Changes in rhizosphere soil microbial communities across plant developmental stages of high and low methane emitting rice genotypes. **Soil Biology and Biochemistry**, v. 156, 2021.

FRAZÃO, J. J.; PRADO, R. M.; SOUZA JÚNIOR, J. P.; ROSSATTO, D. R. Silicon changes C:N:P stoichiometry of sugarcane and its consequences for photosynthesis, biomass partitioning and plant growth. **Scientific Reports**, v.10, n.1, p. 1-10, 2020.

GUERRIERO, G.; HAUSMAN, J.F.; LEGAY, S. Silicon and the Plant Extracellular Matrix. **Frontiers in Plant** Science, v.7, n.463, p. 1-8, 2016.

HAYNES, R.J. A Contemporary Overview of Silicon Availability in Agricultural Soils. **Journal of Plant Nutrition and Soil Science**, v. 177, p. 831-844, 2014.

HILL G. T.; MITKOWSKI, N. A.; ALDRICH-WOLFE, L.; EMELE, L. R.; JURKONIE, D. D.; FICKE, A.; MALDONADO-RAMIREZ, S.; LYNCH, S.T.; NELSON, E. B. Methods for assessing the composition and diversity of soil microbial communities. **Applied Soil Ecology**, v. 15, n. 1, p. 25-36, 2000.

JACOBY, R.; PEUKERT, M.; SUCCURRO, A.; KOPRIVOVA, A.; KOPRIVA, S. The Role of Soil Microorganisms in Plant Mineral Nutrition – Current Knowledge and Future Directions. **Frontiers in Plant Science**, v. 8, 2017.

JOCHUM, M. D.; MCWILLIAMS, K. L.; PIERSON, E. A.; JO, Y. K. Host-mediated microbiome engineering (HMME) of drought tolerance in the wheat rhizosphere. **PLoS ONE**, v.14, n.12, p. 1–15, 2019.

KORNDÖRFER, G. H. **A importância da adubação na qualidade da cana de açúcar**: SÁ, M. E. de; BUZZETI, S. (Coordenadores). Importância da adubação na qualidade dos produtos agrícolas. São Paulo: Ícone, 1994. cap. 7, p. 133-142.

KORNDÖRFER, G. H. **Elementos benéficos. In: Nutrição mineral de plantas**. FERNANDES, M. S. (Ed.). Viçosa: SBCS, 2006. 432p.

KUANG, S.; SU, Y.; WANG, H.; YU, W.; LANG, Q.; MATANGI, R. Soil microbial community structure and diversity around the aging oil sludge in yellow river delta as determined by High-Throughput Sequencing. **Journal Archaea**, v.18, p. 1-10, 2018.

LAANE, H. M. The Effects of the application of foliar sprays with stabilized silicic acid: an overview of the results from 2003-2014. **Silicon**, v.9, p. 803–807, 2017.

LEE, S. M.; KONG, H. G.; SONG, G. C.; CHOONG-MIN, R. Disruption of Firmicutes and Actinobacteria abundance in tomato rhizosphere causes the incidence of bacterial wilt disease. **ISME Journal**, v. 15, p. 330–347, 2021.

LI Y.; ZHANG, Z.; LIU, W.; KE, M.; QU, Q.; ZHOU, Z.; LU, T.; QIAN, H. Phyllosphere bacterial assemblage is affected by plant genotypes and growth stages. **Microbiological Research**, v. 248, 2021.

LI, M.; WANG, Q.; LIU, Z.; PAN, X.; ZHANG, Y. Silicon application and related changes in soil bacterial community dynamics reduced ginseng black spot incidence in *Panax ginseng* in a short-term study. **BMC Microbiology**, v. 19, n. 263, 2019.

LIANG, Y.; NIKOLIC, M.; BÉLANGER, R.; GONG, H.; SONG, A. 2015. **Silicon in Agriculture**. Dordrecht: Springer.

LIN, W.; JIANG, N.; PENG, L.; FAN, X.; GAO, Y.; WANG, G.; CAI, K. Silicon impacts on soil microflora under *Ralstonia Solanacearum* inoculation. **Journal of Integrative Agriculture**, v.19, n.1, p. 251–264, 2020.

MA, J.F.; MIYAKE, Y.; TAKAHASHI, E. Silicon as a beneficial element for crop plants. In: DATNOFF, L.E.; SNYDER, G.H.; KORNDÖRFER, G.H. **Silicon in agriculture**. Amsterdam: Elsevier, 2001. p.17-39.

MAEDA, A. S. Adubação nitrogenada e potássica em socas de cana de açúcar com e sem queima em solos de cerrado. 2009. 110f. **Tese de doutorado** (Doutorado em Agronomia) - Faculdade de Engenharia do Campus de Ilha Solteira, Universidade Estadual Paulista, Ilha Solteira, 2009.

MAJUMDAR, S. and PRAKASH, N. B. An overview on the potential of silicon in promoting defence against biotic and abiotic stresses in sugarcane. **Journal of Soil Science and Plant Nutrition**, v. 20, p.1969–1998, 2020.

MALI, M., and AERY, N.C. Effect of silicon on growth, biochemical constituents, and mineral nutrition of cowpea. **Communications in Soil Science and Plant Analysis**. v.40, n.7-8, p. 1041– 1052, 2009.

MARASCA, I.; FERNANDES, B. B.; CATERINA, G. L.; DENADAI, M. S.; LANÇAS, K. P. Chemical properties of ultisol in different tillage systems under sugarcane cultivation. **Energia na Agricultura**, v.31, n. 2, p. 200-206, 2016.

MARTÍNI, A. F.; VALANI, G. P.; BOSCHI, R. S.; BOVI, R. C.; SIMÕES DA SILVA, L. F.; COOPER, M. Is soil quality a concern in sugarcane cultivation? A bibliometric review. **Soil and Tillage Research**, v. 204, p. 1-8, 2020.

MCCRAY, J. M. and SHANGNING, J. I. Sugarcane yield response to calcium silicate on Florida mineral soils. **Journal of Plant Nutrition**, v.41, p1–12, 2018.

MENDES, R.; KRUIJT M.; BRUIJN, I.; DEKKERS, E.; VAN DER VOORT, M.; SCHNEIDER, J. H. M.; PICENO, Y. M.; DESANTIS, T. Z.; ANDERSEN, G. L.; BAKKER, P. A. H. M.; RAAIJMAKERS, J. M. Deciphering the rhizosphere microbiome for disease-suppressive bacteria. **Science**, v. 332, n. 6033, p. 1097-1100, 2011.

MEYER, J. H.; KEEPING, M. G. Review of research into the role of silicon for sugarcane production. **Proc. South Afr. Sug. Technol. Assoc**., v. 74, p. 29–40, 2000.

MITANI, N.; YAMAJI N.; MA J. F. 2008. Characterization of Substrate Specificity of a Rice Silicon Transporter Lsi1. **European Journal of Physiology**, v.456, n.4, p. 679–686, 2008.

MONTENEGRO, S., and RAGAB, R. Impact of possible climate and land use changes in the semiarid regions: A case study from North Eastern Brazil. **Journal of Hydrology**, v.55, p. 434-435, 2012.

MOROENYANE, I.; MENDES, L. W.; TREMBLAY, J.; TRIPATHI, B.; YERGEAU, E. Plant compartments and developmental stages modulate the balance between niche-based and neutral processes in soybean microbiome. **Microbial Ecology**, v. 82, p. 416-428, 2021.

NAEEM, A.; SAIFULLAH, ZIA-UR-REHMAN, M.; AKHTAR, T.; ZIA, M. H.; ASLAM, M. Silicon nutrition lowers cadmium content of wheat cultivars by regulating transpiration rate and activity of antioxidant enzymes. **Environmental Pollution**, v.242, p. 126–135, 2018.

NIU, H.; PANG, Z.; FALLAH, N.; ZHOU, Y.; ZHANG, C.; HU, C.; LIN, W.; YUAN, Z. Diversity of microbial communities and soil nutrients in sugarcane rhizosphere soil under water soluble fertilizer. **PLoS ONE**, v. 16, n. 1:e0245626, 2021.

OLIVEIRA, R.L.L., PRADO, R. M., FELISBERTO, G.; CHECCHIO, M. V.; GRATÃO, P. L. Silicon Mitigates Manganese Deficiency Stress by Regulating the Physiology and Activity of Antioxidant Enzymes in Sorghum Plants. **Journal of Soil Science and Plant Nutrition**, v.19**,** p. 524–534, 2019.

PAIXÃO, J.S.; CASAROLI, D.; BATTISTI, R; EVANGELISTA, A. W. P.; ALVES JÚNIOR, J.; MESQUITA, M. Characterizing Sugarcane Production Areas Using Actual Yield and Edaphoclimatic Condition Data for the State of Goiás, Brazil. **International Journal of Plant Production,** v.14, p. 511–520, 2020.

PANG, Z.; TAYYAB, M.; KONG, C.; LIU, Q.; LIU, Y.; HU, C.; HUANG, J.; WENG, P.; ISLAM, W.; LIN, W.; YUAN, Z. Continuous Sugarcane Planting Negatively Impacts Soil Microbial Community Structure, Soil Fertility, and Sugarcane Agronomic Parameters. **Microorganisms***,* v.9, n.10, 2021.

PILON, C.; SORATTO, R. P.; BROETTO, F.; FERNANDES, A. M. Foliar or soil application of silicon alleviate water-deficit stress of potato plants. **Agronomy Journal**, v. 106, p. 2325-2334, 2014.

ROCHA, J. R.; MELLO PRADO, R.; CASSIA PICCOLO, M. Mitigation of water deficit in two cultivars of *Panicum maximum* by the application of silicon. **Water, Air, & Soil Pollution**, v.233, n. 63, 2022.

SAHEBI, M.; HANAFI, M. M.; SITI NOR AKMAR, A.; RAFII, M. Y.; AZIZI, P.; TENGOUA, F.; NURUL, M. A. J.; SHABANIMOFRAD, M. Importance of silicon and mechanisms of biosilica formation in plants. **Biomedical Research International**, v.16, 2015.

SANTOS, L.C.N.; TEIXEIRA, G.C.M.; PRADO, R.M.; ROCHA, A. M. S.; PINTO, R. C. S. Response of pre-sprouted sugarcane seedlings to foliar spraying of potassium silicate, sodium and potassium silicate, nanosilica and monosilicic acid. **Sugar Tech**, v.22, p. 773–781, 2020.

SAVANT, N. K.; KORNDÖRFER, G. H.; SNYDER, G. H.; DATNOFF, L. E. Silicon nutrition and sugarcane production: A review. **Journal of Plant Nutrition**, v. 12, n. 22, p. 1853 – 1903, 1999.

SCHÖLER, A.; JACQUIOD, S.; VESTERGAARD, G.; SCHULZ, S.; SCHLOTER, M. Analysis of soil microbial communities based on amplicon sequencing of marker genes. **Biology and Fertility of Soils**, v. 53, p.485–489, 2017.

SILVA, M. R. S. S.; CASTRO, A. P.; KRÜGER, R. H.; BUSTAMANTE, M. Soil bacterial communities in the Brazilian Cerrado: Response to vegetation type and management. **Acta Oecologica**, v. 100, n.103463, 2019.

SONG, A.; LI, Z.; LIAO, Y.; LIANG, Y.; WANG, E.; WANG, S.; LI, X.; BI, J.; SI, Z.; LU, Y.; NIE, J.; FAN, F. Soil bacterial communities interact with silicon fraction transformation and promote rice yield after long-term straw return. **Soil Ecology Letters**, v. 3, p. 395–408, 2021b.

SONG, L.; LI, Z.; WANG, E.; XU, D.; WANG, S.; BI, J.; WANG, H.; JEYAKUMAR, P.; LI, Z.; FAN, F. Supplying silicon alters microbial community and reduces soil cadmium bioavailability to promote health wheat growth and yield. **Science of The Total Environment**, v. 796, 2021a.

SUN, F-L.; FAN, L-L.; XIE, G-J. Effect of copper on the performance and bacterial communities of activated sludge using Illumina MiSeq platforms. **Chemosphere**, v. 156, 2016.

TEIXEIRA, G. C. M.; MELLO PRADO, R.; OLIVEIRA, L. T.; SOUZA, J. V. C.; ROCHA, A. M. S. Silicon fertigation with appropriate source reduces water requirement of maize under water deficit. **Plant Soil**, v. 477, p. 83–97, 2022.

TEIXEIRA, G. C. M.; MELLO PRADO, R.; ROCHA, A. M. S.; SANTOS, L. C. N.; SARAH, M. M. S.; GRATÃO, P. L.; FERNANDES, C. Silicon in pre-sprouted sugarcane seedlings mitigates the effects of water deficit after transplanting. **Journal of Soil Science and Plant Nutrition**, v. 20, p. 849–859, 2020.

TEIXEIRA, G. C. M.; MELLO PRADO, R.; ROCHA, A. M. S.; SOUSA JUNIOR, G. S.; GRATÃO, P. L. Beneficial effect of silicon applied through fertigation attenuates damage caused by water deficit in sugarcane. **Journal of Plant Growth Regulation**, v. 41, p. 3255–3270, 2022.

TEIXEIRA, G. C. M.; MELLO PRADO, R.; ROCHA, A. M. S. Low absorption of silicon via foliar in comparison to root application has an immediate antioxidant effect in mitigating water deficit damage in sugarcane. **Journal of Agronomy and Crop Science**, v. 208, p. 805– 814, 2022.

THIND, S.; HUSSAIN, I., ALI, S.; HUSSAIN, S.; RASHEED, R.; ALI, B.; HUSSAIN, H. A. Physiological and Biochemical Bases of Foliar Silicon-Induced Alleviation of Cadmium Toxicity in Wheat. **Journal of Soil Science and Plant Nutrition**, v. 20, p. 2714–2730, 2020.

TRIVELIN, P. C. O.; FRANCO, H. C. J.; OTTO, R.; FERREIRA, D. A.; VITTI, A. C.; FORTES, C.; FARONI, C. E.; OLIVEIRA, E. C. A.; CANTARELLA, H.; Impact of sugarcane trash on fertilizer requirements for São Paulo, **Brazil. Sci. Agric**. v. 70, p. 345–352, 2013.

URQUIAGA, S.; XAVIER, R. P.; MORAIS, R. F.; BATISTA, R. B.; SCHULTZ, N.; LEITE, J. M.; BODDEY, R. M. Evidence from field nitrogen balance and 15N natural abundance data of the contribution of biological N2 fixation to Brazilian sugarcane varieties. **Plant and Soil**, v. 356, p. 5–21, 2012.

VAN DER LAAN, M. and MILES, N. Nutrition of the South African sugar crop: Current status and long-term trends. **Proc S Afr Sug Technol Ass**, v. 83, p. 195-204, 2010.

VERMA, K. K.; LIU, X.; WU, K.; SINGH, R. K.; SONG, Q-Q.; MALVIYA, M. K.; SONG, X-P.; SINGH, P.; VERMA, C. L.; LI, Y-R. The impact of silicon on photosynthetic and biochemical responses of sugarcane under different soil moisture levels. **Silicon**, v. 12, p. 1355–1367, 2020.

WANG, B.; CHU, C.; WEI, H.; ZHANG, L.; AHMAD, Z.; WU, S.; XIE, B. Ameliorative effects of silicon fertilizer on soil bacterial community and pakchoi (*Brassica chinensis*

L.) grown on soil contaminated with multiple heavy metals. **Environmental Pollution**, v.267, p. 1-10, 2020.

XU, J.; ZHANG, Y.; ZHANG, P.; TRIVEDI, P.; RIERA, N.; WANG, Y.; LIU, X.; FAN, G.; TANG, J.; COLETTA-FILHO, H. D.; CUBERO, J.; DENG, X.; ANCONA, V.; LU, Z.; ZHONG, B.; ROPER, M. C.; CAPOTE, N.; CATARA, V.; PIETERSEN, G.; VERNIÈRE, C.; AL-SADI, A. M.; LI, L.; YANG, F.; XU, X.; WANG, J.; YANG, H.; JIN, T.; WANG, N. The structure and function of the global citrus rhizosphere microbiome. **Nature Communications**, v. 9, n. 4894, 2018.

XU, X.; ZHANG, Z.; HU, S.; RUAN Z.; JIANG, J.; CHEN, C.; SHEN, Z. Response of soil bacterial communities to lead and zinc pollution revealed by Illumina MiSeq sequencing investigation. **Environmental Science and Pollution Research**, v. 24, p. 666–675, 2017.

YUN, J.; KANG, S.; PARK, S.; YOON, H.; KIM, M-J.; HEU, S.; RYU, S. Characterization of a Novel Amylolytic Enzyme Encoded by a Gene from a Soil-Derived Metagenomic Library. **Applied and Environmental Microbiology**, v. 70, n. 12, p. 7229-7235, 2004.

ZHANG L. and GUAN A. Y. Consistent responses of soil bacterial communities to bioavailable silicon deficiency in croplands. **Geoderma**, v.408, n.115587, 2022.

ZHANG, J.; KOBERT, K.; FLOURI, T.; STAMATAKIS, A. PEAR: a fast and accurate Illumina paired-end read merger. **Bioinformatics**, v. 30, n. 5, p. 614-620, 2014.

ZHENG, Q.; HU, Y.; ZHANG, S.; NOLL, L.; BÖCKLE, T.; DIETRICH, M.; HERBOLD, C. W.; EICHORST, S. A.; WOEBKEN, D.; RICHTER, A.; WANEK, W. Soil multifunctionality is affected by the soil environment and by microbial community composition and diversity. **Soil Biology and Biochemistry**, v. 136, 2019.

ZHOU, X.; SHEN, Y.; FU, X.; WU, F. Application of sodium silicate enhances cucumber resistance to fusarium wilt and alters soil microbial communities. **Frontiers in Plant Science**, v. 9, n. 624, p. 1–12, 2018.

ZHU, Y., and H. GONG. Beneficial effects of silicon on salt and drought tolerance in plants. **Agronomy for Sustainable Development**, v.34, n.2, p.455-472, 2014.

ZHU, Y., and H. GONG. Beneficial effects of silicon on salt and drought tolerance in plants. **Agronomy for Sustainable Development**, v.34, n.2, p.455-472, 2014.

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CAPÍTULO I – Silicon application influences the prokaryotic communities in the rhizosphere of sugarcane genotypes

Abstract

Silicon (Si) is beneficial to sugarcane production and its application in the soil can influence the microbial communities in the rhizosphere of sugarcane. However, it is unclear how the microbial communities in the rhizosphere of different sugarcane genotypes respond to Si application. This study assessed the effect of Si application on the prokaryotic communities in the rhizosphere of three sugarcane genotypes (RB021754, RB036066, RB92579) during the stages of plant cane and ratoon, through the 16S rRNA gene sequencing. The composition of prokaryotic communities varied by comparing genotypes and Si application in both plant cane and ratoon. In the sugarcane plant, the relative abundance of Planctomycetes and Gemmatimonadetes decreased in the rhizosphere of genotype RB021754, while the relative abundance of Proteobacteria increased in the rhizosphere of genotype RB92579 with Si application. Regarding ratoon cane, the relative abundance of Acidobacteria, Thaumarchaeota, and Planctomycetes increased in the rhizosphere of genotypes RB021754, RB036066, and RB92579, respectively, after Si application. At the genus level, *Catenulispora, Sphingoaurantiacus, Luteitalea*, and *Flavisolbacter* presented a positive correlation with Si application, while *Alloprevotella, Gaiella, Sporomomusa*, and *Tahibacter* presented a negative correlation. Also, there was an enrichment of N-fixers and plant growth-promoter microbes after Si application. This study has shown that Si application affected prokaryotic diversity and changed the abundance of specific microbial groups, which varied according to different genotypes.

Keywords: *Saccharum officinarum*, plant microbes interaction, microbial ecology

1 Introduction

Sugarcane (*Saccharum* spp. hybrids) is an important crop for both food and energy production and plays a vital role in the economies of countries in South America, Asia, and Oceania (Sozinho et al., 2018). One of the key uses of sugarcane is in the production of ethanol, which is considered a sustainable biofuel option because it can greenhouse gas emissions by 85 % compared to gasoline (Oliveira et al., 2020). Given these benefits, sugarcane is particularly important to Brazil, which is one of the top producers of sugar and ethanol globally (Doriguel et al., 2018). In addition, Brazil presents the potential to increase the production of sugarcane by about 4 % annually until 2024, which corresponds to 884 megatons (Heinrichs et al., 2017). This increase will occur due to the increase of cultivated areas associated with modern cultivars and soil practices, such as the application of fertilizers. Particularly, the application of fertilizers is important since sugarcane presents great demand for nutrients from the soil to allow the plants to produce large biomass (Oliveira et al., 2017). However, studies about chemical fertilizers for sugarcane have focused on the main macronutrients such as nitrogen, phosphorus, and potassium (Trivelin et al., 2013; Crusciol et al., 2020). On the other hand, some non-essential nutrients can be important to confer higher production of sugarcane, such as silicon (Si). This nutrient is beneficial for plants since it can mitigate abiotic and biotic stresses by regulating several physiological, biochemical, and molecular mechanisms (Ranjan et al., 2021).

The application of Si can enhance sugarcane growth, resulting in increased sugarcane yields (Verma et al., 2020; Martíni et al., 2020). In sugarcane, Si promotes several benefits, such as increased efficiency in the water use by plants, production of antioxidant enzymes, and stimulation of the absorption of other nutrients, which results in increased production of stalks and sugar (Guerriero et al., 2016; McCray and Shangning, 2018; Naeem et al., 2018; Oliveira et al., 2019; Frazão et al., 2020).

Although some studies have reported the effects of Si on plants, this element can promote changes in the microbial communities associated with the plant rhizosphere (Das et al., 2019; Lin et al., 2020; Wang et al., 2020; Zhang and Guan, 2022). In addition, the application of Si can change the chemical properties of the soil, driving an indirect response of the microbial communities. Recent studies have found that the application of Si contributed to changes in the microbial community in the rhizosphere

(Deng et al., 2021; Zhang and Guan, 2022). For instance, Zhang and Guan (2022) applied Si in soil under rice and observed significant changes in the microbial communities with the enrichment of Actinobacteria; while in sugarcane, Deng et al. (2021) observed enrichment of groups affiliated to the Proteobacteria phylum associated with Si application. On the other hand, a recent study has shown that the soil microbial community can interact with Si affecting its mobility and transformation in soils, which contributes to increasing the Si bioavailability to plants (Song et al., 2021).

Despite the findings of a recent study by Deng et al. (2021) on the impact of Si application on the microbial community in the rhizosphere of sugarcane, there remains a gap in knowledge regarding the effect of Si on the prokaryotic community in the rhizosphere of various genotypes and growth stages of sugarcane. Such information is important because several studies have shown that different plant genotypes and growth stages drive differently the prokaryotic community assembly in the rhizosphere (Mendes et al., 2018; Moroenyane et al., 2021). In this study, we hypothesized that the Si application influences the rhizosphere prokaryotic communities associated with different genotypes of sugarcane comparing both plant cane and ratoon. To address this hypothesis, we aimed to assess the prokaryotic communities through the 16S rRNA gene sequencing in the rhizosphere of plant cane and ratoon of different genotypes with and without Si application.

2 Material and methods

2.1 Experimental field

The experimental field belongs to Usina Comvap located in the municipality of União, Piaui, Brazil (04°52’56” S, 42°52’58” W; 68 m). The experiment was conducted during the season 2019/2020 (sugarcane plant) and 2020/2021 (sugarcane ratoon). The regional climate is dry tropical with annual average temperatures of 30 °C and rainfall of 1,500 mm. The soil of the area is classified as a Fluvisol (8% clay, 31% silt, and 61% sand).

2.2 Treatments and Experimental Design

The experimental design consisted of three sugarcane genotypes (RB021754, RB036066, RB92579) and two treatments with Si (with and without Si application) under a factorial scheme (3x2), using a randomized block design with four replicates. These treatments were applied to sugarcane plants and sugarcane ratoon. The sugarcane genotypes were provided by the Sugarcane Breeding Program of the Federal University of Piauí (PMGCA/UFPI) linked to the Inter-University Network for the Development of the Sugarcane Industry (RIDESA).

Before planting, soil chemical analysis was performed according to the method described by Raij et al. (2001) and the results are shown in Table S1. The area was also prepared with subsoiling, plowing, and harrowing operations, and then furrows were opened at a depth of 0.40 m, with a 2-row furrower spaced 1.4 m apart.

The experimental plots presented 84 m2 area (six lines of 10 m each), with 56 m2 of usable area for plant collection and evaluation, and rows spaced of 1.4 m. The planting was carried out manually considering an average of 18 buds per linear meter. Fertilizers for planting and covering were carried out based on soil analysis and fertilization recommendations for sugarcane according to Martinez et al. (1999).

The source of soluble Si used was sodium silicate (Na_2SiO_3), at a concentration of 2.0 mmol L^{-1}. The product is characterized by being fluid and containing 4.6 % of total Si and 3.7 % of highly available soluble Si. The silicon was sprayed at the bottom of the furrow at the time of planting (plant cane) and applied to the soil close to the regrowth of the clump (first ratoon). A solution volume of 200 L ha^{-1} was used and the pH was adjusted to 12.7 through the addition of potassium hydroxide, aiming to increase the availability of monomeric chemical species of Si (H_4SiO_4). In the control treatments (without Si), water was applied to plants.

2.3 Biometric Parameters

At 130 days after emergence (DAE), the number of tillers per plot was quantified. The stem height (SH) and stem diameter (SD) of six plants were also determined at the time of harvest, which occurred 340 DAE. To measure the SH, a tape graduated in

centimeters was to measure the distance from the base of the plant to the apex of the last fully expanded leaf. The SD was measured in the middle third of each culm using a digital caliper.

2.4 Production and technological parameters

After 12 months from both plant cane and ratoon cycle, respectively, the yield of stalks per hectare (tons of cane/hectare, TCH) was determined by manual harvesting of the cane in the useful area of each plot, with the total fresh weight per plot being quantified with a digital scale and then estimated to hectare. Sugarcane juice quality (total recoverable sugar – TRS), as well as sugar yield (kg per hectare - SY) were determined according to the methodology proposed by Liu et al. (2016).

2.5 Silicon Determination

Silicon content in the shoot was determined according to the method described by Kraska and Breitenbeck (2010) and the reading was performed using the colorimetric method in a spectrophotometer at 410 nm (Korndörfer et al., 2004). Si accumulation in the shoot of the plants was calculated based on Si content and dry mass.

2.6 Rhizosphere sampling

Rhizospheric soil sampling was carried out at 120 days after emergence in plant cane, and at 120 days after the first cut in ratoon cane, periods corresponding to the final stage of tillering and beginning of stem elongation. For both sampling times, we sampled the same plants in the first (plant) and second (ratoon) years. For the rhizospheric soil sampling, four plants per plot were sampled and the soil adhering to the roots was collected and mixed to form a composite sample per plot. The soil samples were sieved (2 mm mesh) and stored at -20 ◦C before analysis. Soil samples collected 20 cm distant from the sugarcane roots were considered bulk soil.

2.7 DNA extraction and sequencing

Soil DNA was extracted from 0.5 g (total humid weight) of soil using the Powerlyzer Power Soil DNA Isolation Kit (MoBIO Laboratories, Carlsbad, CA, USA), according to the manufacturer's instructions. The DNA extraction was performed in triplicate for each soil sample. The quality and concentration of the extracted DNA were determined using NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, USA).

The V4 region of the 16S rRNA gene was amplified with region- specific primers (515F/806R) (Caporaso et al., 2011). Each 25 µL PCR reaction contained the following: 12.25 µL of nuclease-free water (Certified Nuclease-free, Promega, Madison, WI, USA), 5.0 µL of buffer solution 5× (MgCl2 2 Mm), 0,75 µL de solution of dNTP's (10 mM), 0,75 µL of each primer (515 YF 40 µM e 806 R 10 µM), 1.0 unit of Platinum Taq polymerase High Fidelity in a concentration of 0,5 µL (Invitrogen, Carlsbad, CA, USA), and 2.0 µL of template DNA. Moreover, a control reaction was performed by adding water in place of DNA. The conditions for PCR were as follows: 95 ºC for 3 min to denature the DNA, with 35 cycles at 98 ºC for 20 s, 55 ºC for 20 s, and 72 ºC for 30 s, with a final extension of 3 min at 72 ºC to ensure complete elongation. This PCR step included Illumina sequencing adapters and dual-index barcodes using the Nextera XT indices to discriminate each sample in the pool.

After indexing, the PCR products were cleaned up using Agencourt AMPure XP – PCR purification beads (Beckman Coulter, Brea, CA, USA), according to the manufacturer's manual, and quantified using the dsDNA BR assay Kit (Invitrogen, Carlsbad, CA, USA) on a Qubit 2.0 fluorometer (Invitrogen, Carlsbad, CA, USA). Once quantified, the equimolar concentrations of each library were pooled into a single tube. After quantification, the molarity of the pool was determined and diluted to 2 nM, denatured, and then diluted to a final concentration of 8.0 pM with a 20 % PhiX (Illumina, San Diego, CA, USA) spike for loading into the Illumina MiSeq sequencing machine (Illumina, San Diego, CA, USA).

Sequence data were processed using QIIME 2 version 2021.4. Firstly, the sequences were demultiplexed and quality control was carried out using DADA2 (Callahan et al., 2017), using the consensus method to remove any remaining chimeric and low-quality sequences. The 16S rRNA sequencing approach generated

approximately 6.103,000 reads with <10 % of chimera. After trimming, 5.438,403 high-quality sequences remained with an average of 139.400 sequences per sample.

Afterward, samples were rarefied to 80.450 sequences (Supplementary Fig. S1), following the number of the lowest sample, and singletons and doubletons were removed. The taxonomic affiliation was performed using a pre-trained Naïve Bayes classifier with the Python sklearn code, using the Silva database v. 132 at 97 % similarity (Quast et al., 2013), and the generated matrix was further used for statistical analyses. The sequences are submitted to the NCBI Sequence Read Archive under the identification PRJNA808057.

2.8 Data analysis

The statistical analyses were performed comparing bulk soil and the rhizosphere of the three distinct sugarcane genotypes, but for some analysis, we grouped the genotypes to compare between plant cane and ratoon, and between with and without silicon application. We first checked the data for normal distribution and homogeneity of variance using the Shapiro-Wilk and Levene's tests, respectively. Then, we assessed the prokaryotic community structure using the Principal Component Analysis (PCA) constructed in Canoco 5 (Biometrics, Wageningen, The Netherlands). To test whether each plant genotype and treatment harbored a significantly different microbial community we used PERMANOVA (Anderson, 2001). Diversity measurements of richness and Shannon were calculated based on the taxonomic matrix at the OTU level and compared using Tukey's HSD test in PAST 4.01software (Hammer et al., 2001). The differential abundance of prokaryotic groups at the phylum and genus level was compared using the Statistical Analysis of Metagenomic Profile (STAMP) software (Parks and Beiko, 2010). P-values were calculated using a two-sided Tukey-Kramer test, and corrections were made using Benjamini-Hochberg's false discovery rate (Benjamini and Hochberg, 1995). To explore the relationships between microbial groups at the phylum with plant parameters, and between microbial genera with silicon application, we calculated Spearman's rank correlation coefficients using the ‘multtest’ package in R (R Development Core Team, 2007), and corrections were made using the Benjamini-Hochberg FDR. Finally, we used the FAPROTAX database (Louca et al., 2016) to predict the relevant potential functions of the community. For this, we used

as input a table of frequency of taxa at the genus level and converted it into a putative functional table, and compared the treatments considering functions related to nitrogen, carbon, and sulfur cycles.

3 Results

3.1 Biometric, productive, and technological parameters of sugarcane

The biometric (stem height and diameter), productive (stalk yield), and technological parameters (total recoverable sugar and sugar yield) of sugarcane varied according to different genotypes and Si applications. In the plant cane, the genotypes RB036066 presented higher biometry, while RB021754 presented higher productivity (stalk yield), as compared to RB92579. However, the values of technological parameters (total recoverable sugar and sugar yield) were higher in RB92579 and RB021754 (Table 1).

Table 1. Biometric, productive, and technological parameters of sugarcane cultivars

Cultivars	SH (m)	SD (mm)	SY (t ha^{-1})	SC (Pol %)	TRS (t ha^{-1})
Plant					
RB021754	3.60 a	26.53 ab	164.70 a	12.64 a	128.79 a
RB036066	3.63 a	27.58 a	159.08 ab	11.40 b	118.45 b
RB92579	3.21 b	25.87 b	154.40 b	13.00 a	132.59 a
Ratoon					
RB021754	2.97 b	24.37 b	143.52 a	14.17 a	137.68 a
RB036066	3.25 a	26.94 a	142.65 ab	11.51 c	125.55 b
RB92579	3.18 a	25.47 ab	139.78 b	13.52 b	137.68 a

SH: stem height, SD: stem diameter, SY: stalk yield, SC: sucrose content, TRS: total recoverable sugar
Means followed by the same letters in each column do not differ statistically by the Tukey test ($P< 0.05$).

In ratoon cane, RB036066 presented higher biometry and productivity than RB021754 and RB92579, respectively. RB021754 presented the highest values of all technological parameters. When compared to Si fertilization, no differences were observed in the plant cane (Table 2). However, Si fertilization improved the biometry, production, and quality (technological parameters) of ratoon cane.

Table 2. Biometric, productive, and technological parameters of sugarcane with and without silicon fertilization

Si	SH (m)	SD (mm)	SY (t ha^{-1})	SC (Pol %)	TRS (t ha^{-1})
Plant					
0%	3.46 a	26.35 a	157.33 a	12.31 a	126.50 a
100%	3.50 a	26.97 a	161.45 a	12.38 a	126.72 a
Ratoon					
0%	2.93 b	23.86 b	134.66 b	13.32 b	135.72 b
100%	3.23 a	25.99 a	148.63 a	14.37 a	144.04 a

SH: stem height, SD: stem diameter, SY: stalk yield, SC: sucrose content, TRS: total recoverable sugar
Means followed by the same letters in each column do not differ statistically by the Tukey test (P< 0.05).

3.2 Prokaryotic community structure and diversity

We first compared the microbial community structure between bulk soil and the rhizosphere of plant cane and ratoon. The analysis showed that each of the three treatments harbored a distinct community structure (PERMANOVA F = 12.4, P = 0.0001). Then, we split the analysis between plant cane and ratoon. The principal component analysis (PCA) explained 17.2% and 22.6% of the total variation in microbial OTUs in the rhizosphere of plant cane and ratoon, respectively (Figure 1A, C). In both periods, PCA separated the prokaryotic communities found in bulk soil and sugarcane rhizosphere, independent of genotype and application of Si (PERMANOVA P < 0.05). Interestingly, there was not a clear separation in the prokariotic communities comparing genotypes and fertilization with Si in both plant cane and ratoon. In general, the microbial richness and diversity increased in the rhizosphere as compared to bulk soil (Fig. 1B, D; P <0.05). When comparing treatments with and without Si application in sugarcane, the prokaryotic communities found in the rhizosphere of genotype RB021754 (G1) with the application of genotype were less diverse, in sugarcane plant, while they were richer in the genotype RB92579 (G3), in ratoon cane.

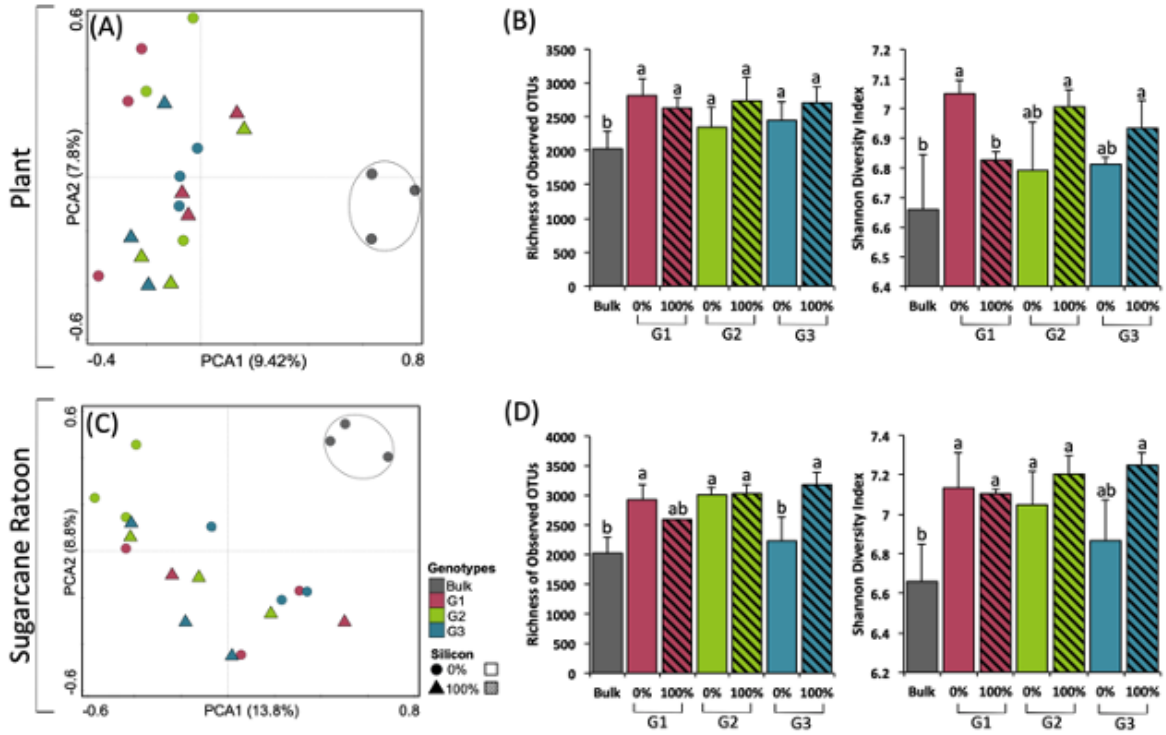


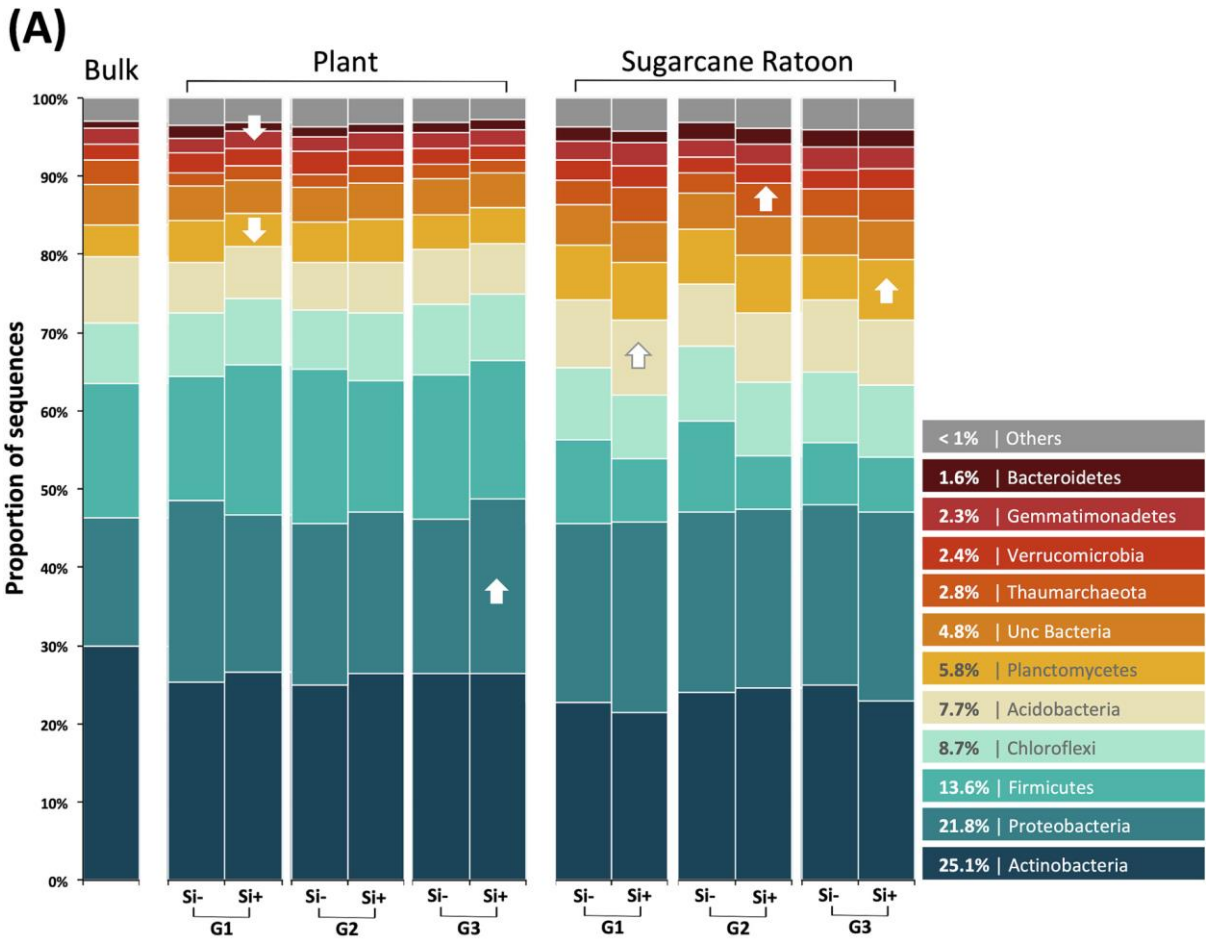
Figure 1. Prokaryotic community (bacteria and archaea) structure and diversity in bulk soil and rhizosphere of different sugarcane genotypes and stages, i.e., plant cane (first year) and ratoon cane (second year). The taxonomic profiling was based on the 16S rRNA gene affiliated to the Silva database at 97 % of similarity. Principal component analysis (PCA) showing the structure of the communities in (A) plant cane and (C) ratoon cane. The dashed line indicates a grouping based on PERMANOVA ($P < 0.05$). (B-D) Richness and diversity measurements based on OTUs level. Different lower-case letters indicate significant differences based on Tukey's HSD test ($P < 0.05$). G1 =RB021754, G2 =RB036066, G3 =RB92579. Si- =no Si application; Si+=100 % of Si application.

3.3 Prokaryotic community composition

The prokaryotic communities, i.e., bacteria and archaea, consisted of the 11 most abundant phyla (abundance >1 %), being Actinobacteria (25.1 % of total sequences), Proteobacteria (21.8 %), Firmicutes (13.6 %), Chloroflexi (8.7 %), and Acidobacteria (7.7 %) in the rhizosphere and bulk soil (Fig. 2A). Although the PCA analysis showed no clear differences between treatments, when considering the effect of genotypes and

the application of Si, the results showed that the application of Si changed the abundance of specific groups as compared to unfertilized treatments. In the sugarcane plant, the abundance of Planctomycetes and Gemmatimonadetes decreased in the rhizosphere of genotype RB021754 (G1), while the abundance of Proteobacteria increased in the rhizosphere of genotype RB92579 (G3) with the application of Si. Regarding ratoon cane, the abundance of Acidobacteria, Thaumarchaeota, and Planctomycetes increased in the rhizosphere of genotypes RB021754 (G1), RB036066 (G2), and RB92579 (G3), respectively, with Si application.

As reported above, there were different patterns in the abundance of phyla comparing the rhizosphere of plant cane and ratoon. Thus, Acidobacteria, Planctomycetes, Thaumarchaeota, Proteobacteria, and Chloroflexi were enriched in the sugarcane plant, while Firmicutes, Actinobacteria, and Dependentiae were enriched in ratoon cane (Figure 2B)



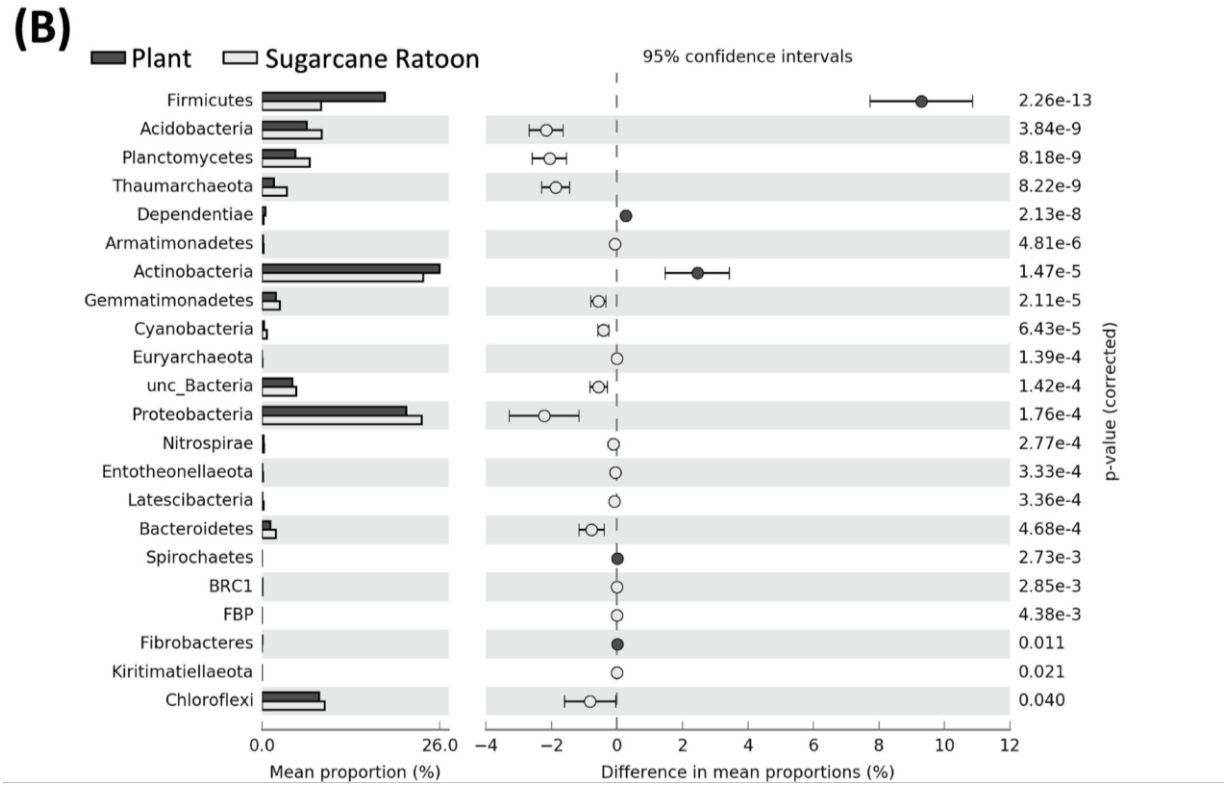


Figure 2. (A) Distribution of the most abundant phyla in the bulk soil and rhizosphere of different sugarcane genotypes and stages, i.e., plant cane (first year) and ratoon cane (second year) based on the 16S rRNA gene. The arrows within the graph indicate a significant increase or decrease of specific phyla ($P < 0.05$). (B) Scatter-plot showing the differential abundance of phyla between the rhizosphere of plant cane and ratoon cane. P-values were calculated using a two-sided Tukey-Kramer test, and corrections were made using Benjamini-Hochberg FDR. Si- = no Si application; Si+ = 100 % of Si application.

At a deeper taxonomical level, we compared the effect of Si application on the microbial groups in plant cane and ratoon (Figure 3). In the sugarcane plant, *Acidothermus* and *Solirubrobacter*, which belong to Actinobacteria, were enriched in the rhizosphere with Si application, while Saccharimonadales (Patescibacteria), Chthniobacteraceae (Verrucomicrobia), and *Flavisolibacter* (Bacteroidetes) were enriched in the unfertilized rhizosphere (Figure 3A). Regarding ratoon cane, Nitrososphaeraceae (Thaumarcheota), Gammaproteobacteria (Proteobacteria), and *Pirellula* (Planctomycetes) were enriched in the rhizosphere with Si application, while

Bacillus and Bacillales, which belong to Firmicutes, were enriched in the unfertilized rhizosphere (Figure 3B).

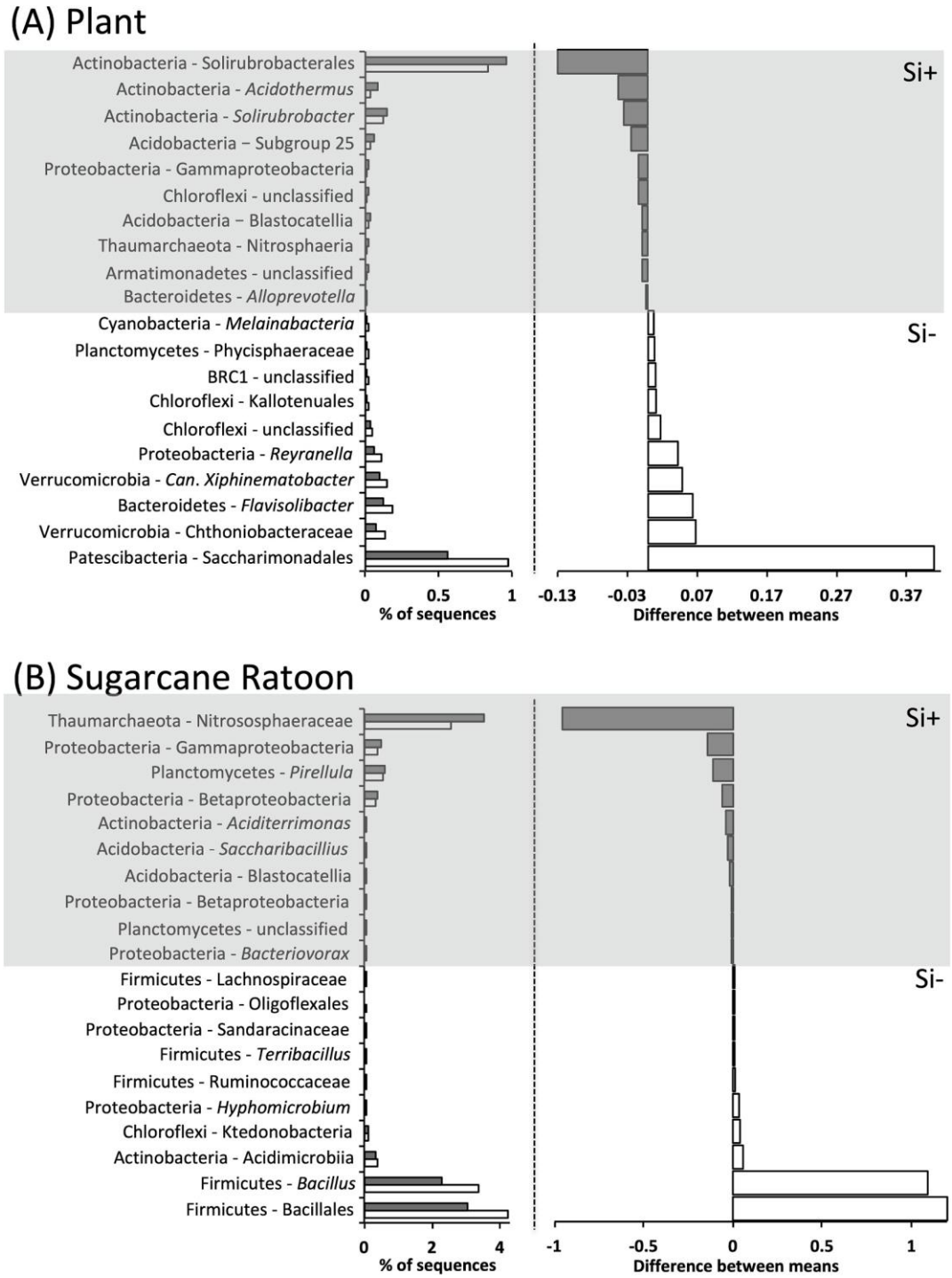


Figure 3. Differential abundance of microbial groups at the genus level comparing the effect of Si application in (A) plant cane and (B) ratoon cane. The affiliation showed is

the lowest classified using the SILVA database at 97 % of similarity. The graphs show the top ten groups with the highest significance. P-values were calculated using a two-sided Tukey-Kramer test, and corrections were made using Benjamini-Hochberg FDR. Si- =no Si application; Si+=100 % of Si application.

3.4 Correlation between prokaryotic communities and sugarcane traits

To analyze the correlation between microbial phyla and some sugarcane traits, Spearman's rank correlation was used and showed positive and negative correlations between phyla and the sugarcane traits (Figure 4A). Proteobacteria and Cyanobacteria showed a more positive correlation with sugarcane traits, mainly TRS and SY. On the other hand, Firmicutes, Hydrogenedentes, and Armatimonadetes presented only negative correlations with some sugarcane traits.

We then compared the correlations between microbial genera and Si (Figure 4B). Our analysis showed that 27 microbial genera correlated positively with Si application while 28 presented a negative correlation. We highlighted here *Catenulispora, Sphingoaurantiacus, Luteitalea*, and *Flavisolbacter* that presented a positive correlation with Si application, while *Alloprevotella, Gaiella, Sporomomusa*, and *Tahibacter* presented negative correlation with Si (Figure 4B).

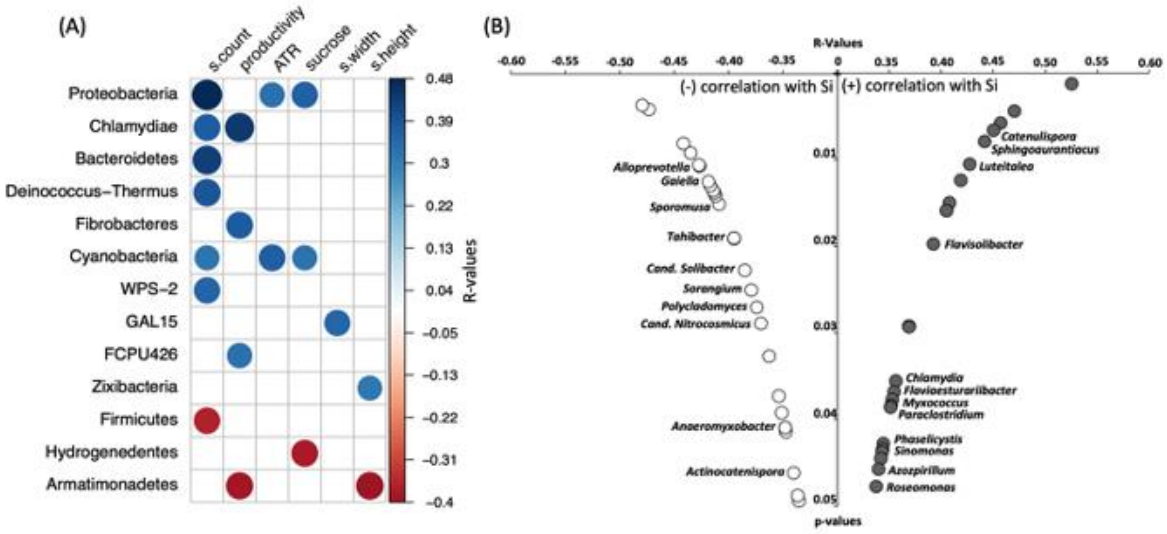


Figure 4. (A) Heatmap of the Spearman's rank correlation coefficients between microbial phyla and plant performance parameters factors. Only significant correlations

(P < 0.05) are shown. The color grading and the circle size are proportional to the weight of the correlation. Positive correlations are shown in blue and negative in red. (B) Correlation between microbial genera and Si application. Only significant (P < 0.05) positive and negative correlations are shown.

3.5 Putative potential functions prediction

We used the FAPROTAX database to predict putative potential functions related to the metabolism of nitrogen and carbon in our samples. Firstly, we compared the rhizosphere community of plant and ratoon cane and showed that sugarcane plant-enriched microbial groups affiliated to nitrogen fixation, aerobic nitrite oxidation, and methanogenesis (Figure 5A). On the other hand, the ratoon cane presented a higher abundance of microbial groups related to denitrification and methylotrophy. We then compared the putative predicted functions within each group to assess the effect of Si application (Figure 5B). For the sugarcane plant, there were no differences, while for the ratoon cane, the Si application increased ammonia oxidation and decreased nitrate reduction and nitrogen fixation.

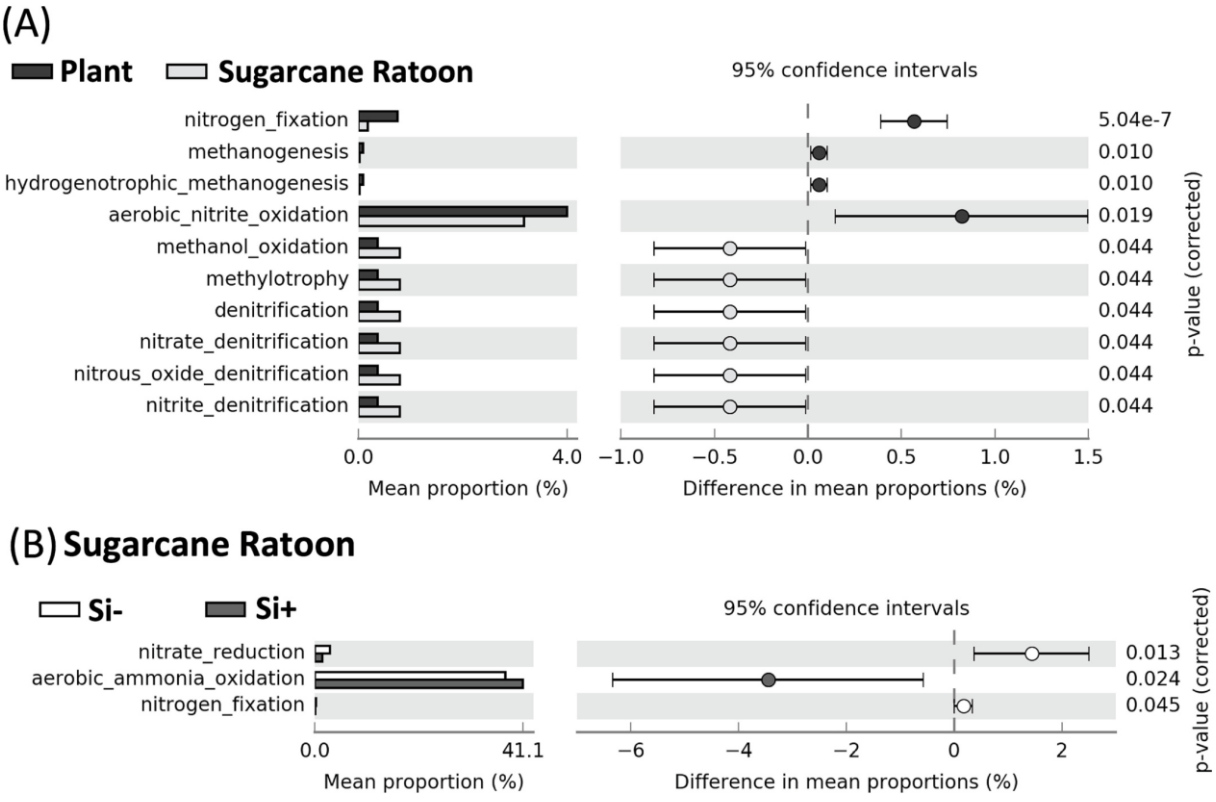


Figure 5. Distribution of the predicted putative microbial functions related to nitrogen, carbon, and sulfur in the rhizosphere of plant cane and ratoon cane. (A) Comparison between plant cane and ratoon cane. (B) Effect of Si application on the putative functions in the ratoon cane rhizosphere. The functional prediction was based on the 16S rRNA genes using the FAPROTAX database. Si- =no Si application; Si+=100 % of Si application.

4 Discussion

4.1 Prokaryotic community structure and diversity

In this study, the effect of two-year Si application on the microbial communities in the rhizosphere of sugarcane genotypes was assessed in the plant cane (first year) and ratoon (second year). Initially, the microbial communities found in the rhizosphere were different from those found in bulk soil, between the sugarcane stages (plant and ratoon), and genotypes. This pattern is already expected when comparing the rhizosphere with bulk soil, where there are differences between the microbial communities in both pools (Araujo et al., 2019; Sousa et al., 2020; Lopes et al., 2021). It is well known that microbial communities in the rhizosphere are stimulated by the intense exudation by the roots and it contributed to recruiting specific microbial groups (Hu et al., 2018). Also, several studies have shown that different plant genotypes and plant development stages recruit a distinct microbial community in the rhizosphere (Bulgarelli et al., 2015; Mendes et al., 2019; Rossmann et al., 2020; Sousa et al., 2020; Moroenyane et al., 2021; Xiong et al., 2021). This rhizosphere effect was also observed for the richness and diversity of the microbial communities, which increased in the rhizosphere as compared to bulk soil. It corroborates previous studies that showed a higher abundance and diversity of microbial communities in the rhizosphere than in bulk soil (Yang et al., 2017; Kokalis-Burelle et al., 2017; Mendes et al., 2014; Sousa et al., 2020).

Then, we compared the effect of Si application between different genotypes and the majority of the community did not present profound differences as shown in PCA analysis. Interestingly, we found differences between genotypes in the responses of microbial richness and diversity to Si application, where the genotype RB021754 with

Si application presented a less diverse microbial community, while the genotype RB92579 presented a richer community than unfertilized sugarcane. Thus, these results are partly in line with the hypothesis that the fertilization of Si influences the microbial communities associated with different genotypes of sugarcane. In general, different genotypes present specific root exudation, which contributed to shaping the microbial communities in the rhizosphere (Zhao et al., 2020; Tayyab et al., 2021). The application of Si could affect the exudation profile with consequences on the community assembly. In a previous study, Yeoh et al. (2015) showed that the sugarcane genotype had a subtle effect on community composition, revealing that, although small, there is still a genotype effect on community assembly. We then assessed the community composition to better understand the effect of genotype and Si application on the abundance of specific microbial groups.

4.2 Prokaryotic community composition

Although the general community structure did not present clear changes, some specific microbial groups were affected by Si application. In general, Actinobacteria and Proteobacteria were the most abundant phyla found in the rhizosphere and it agrees with previous studies assessing microbial communities in the rhizosphere of sugarcane (Val-Moraes et al., 2016; Gao et al., 2019; Solanki et al., 2020; Liu et al., 2021; Pang et al., 2021). Actinobacteria and Proteobacteria are usually considered as copiotroph (Semenov et al., 2020; Li et al. 2021), so their higher abundances can be explained by the large amounts of available organic substrates from root exudates in the rhizosphere (Gao et al., 2019). Interestingly, the higher abundance of these two phyla is important since they act on several positive functions for plants, such as decomposition of organic residues, production of antibiotics, and plant growth-promotion, mainly by the biological N fixation (Doumbou et al., 2001; Zhao et al., 2017**;** Val-Moraes 2011).

The results showed changes in the abundance of specific phyla according to genotypes and Si-fertilization. Proteobacteria increased in the rhizosphere of Si-fertilized genotype RB92579 suggesting possible benefits of Si in increasing this phylum in this specific genotype, which is important since some Proteobacteria are well-known plant growth-promoters, such as *Enterobacter* (Figueiredo et al., 2016). According to Zhao et al. (2020), distinct genotypes of sugarcane present differences in

the quality and quantity of exudates that influence differently the microbial groups in the rhizosphere. In addition, the fertilization with Si promoted shifts in the microbial groups in the rhizosphere. Indeed, Acidobacteria, Thaumarchaeota, and Planctomycetes increased in the rhizosphere of genotypes RB021754, RB036066, and RB92579, respectively, with Si application.

We also observed differences in the abundance of phyla comparing plant cane and ratoon showing that different stages of sugarcane contribute to changing the microbial communities. Indeed, a previous study assessing ratoon cane as compared to new plants has found differences in the abundance of microbial phyla, such as Acidobacteria, Actinobacteria, and Firmicutes (Gao et al., 2019). Interestingly, Gao et al. (2019) observed that the proportion of Actinobacteria in the rhizosphere of ratoon cane was 41.4% lower than that of newly planted sugarcane. It agrees with our study where Actinobacteria decreased in the rhizosphere of ratoon cane. It is unclear how is the influence of sugarcane ratooning on the microbial community in the rhizosphere. One possible explanation would be that sugarcane plant (new plants) presents fine roots transporting more nutrients as compared to roots of ratoon cane (Gaiero et al., 2013), and it contributes to a higher abundance of Actinobacteria, as copiotrophic bacteria, found in the rhizosphere of sugarcane plant (Khan et al., 2022a, b).

In the rhizosphere of sugarcane plant, Si application promoted an increased abundance of the *Acidothermus* and *Solirubrobacter*. These microbial groups belong to Actinobacteria, which are microbes associated with soil organic matter decomposition and nutrient cycling. Indeed, these genera are known bacteria acting on C and N cycling and degrading C sources (Liu et al., 2020; Dobrovolskaya et al., 2020; Ogola et al., 2021). Thus, this result agrees with Samaddar et al., (2019) who reported a higher abundance of Actinobacteria in the rhizosphere of Si-fertilized rice. It is interesting since C and N are important nutrients to sugarcane growth, showing that Si application is stimulating the growth of beneficial microbes. Also, bacteria of the Acidothermus genus were able to grow under acidic conditions and degrade plant tissues, which may increase organic matter and magnesium content, as well as available nitrogen and calcium in soils (Ren et al., 2021). Interestingly, this genus was also positively correlated with sugarcane yield (Khan et al., 2022a, b).

On the other hand, in the ratoon cane, Si promoted the enrichment of the microbial groups Nitrososphaeraceae (Thaumarcheota), Gammaproteobacteria, Betaproteobacteria (Proteobacteria), and *Pirellula* (Planctomycetes). *Nitrososphaeraceae* is an archaeal family involved in the nitrification process, being important to plant growth (Stahl and de La Torre, 2012). Members of the classes Gamma and Betaproteobacteria are well-known bacteria involved in plant-growth promotion (Zuluaga et al., 2020). Bacteria belonging to Planctomycetes groups, such as *Pirellula,* are known as symbionts and act on the plant growth, being important to sugarcane, mainly during the ratoon period (Mori et al., 2014). Although previous studies have reported synergy between *Bacillus* and Si (Rezakhani et al., 2019; Kaloterakis et al., 2021), the sugarcane without Si showed a high abundance of *Bacillus* in the rhizosphere and it is interesting due to the potential of these bacteria in promoting plant growth.

4.3 Correlation between prokaryotic communities and sugarcane traits

The Spearman correlation analysis has shown that Proteobacteria and Cyanobacteria presented a positive correlation with sugarcane traits, mainly those related to technological characteristics, such as TRS and SY. TRS and SY are related to sucrose production, which represents sugar and alcohol quality and indicates the high ability of sugarcane in accumulating carbohydrates from photosynthesis. The features related to photosynthesis may have a direct relation to the efficiency of some bacteria, such as Proteobacteria and Cyanobacteria, in fixing N to Poaceae species, such as sugarcane, since these bacteria can increase the photosynthetic capacity of plants (Figueiredo et al., 2016; Priya et al., 2015). For instance, Priya et al. (2015), have reported the use of Cyanobacteria as biofertilizer applied in Poaceae species, such as rice, increasing the potential in performing photosynthesis. Recently, Li et al., (2020a, b) have reported the functional role of Proteobacteria in increasing the biosynthesis of polysaccharides and C metabolism.

Our analysis also showed some specific microbial genera presenting positive or negative correlation with Si application. Thus, *Catenulispora, Sphingoaurantiacus, Luteitalea*, and *Flavisolibacter* presented more positive correlations. The genus *Catenulispora* are beneficial acidophilic bacteria, present in rice paddies or forest soils,

that have important functions such as transport of phosphate and amino acids, associated with plant growth (Chen et al., 2020). *Sphingoaurantiacus* belongs to Proteobacteria and is known to be tolerant to metals (Jiao et al., 2022), while *Luteitalea* is a chemoorganotrophic bacterium capable of growing in a wide range of pH and temperature (Whitman et al., 2015). Also, the bacterial genus *Flavisolibacter* has been associated with plant growth in several plant species (Xiao et al., 2017; Liu et al., 2020; Lin et al., 2021).

On the other hand, the genera *Alloprevotella, Gaiella, Sporomusa*, and *Tahibacter* showed a more negative correlation with Si. Bacteria from the genus *Alloprevotella* are found in both terrestrial and aquatic environments and participate in the cycles of carbon, nitrogen, phosphorus, and sulfur (Wang et al., 2019). The genus *Gaiella* can be considered a potential resource for the bioremediation of contaminated soils, due to its ability to degrade various organic pollutants (Chen et al., 2019). The genus *Sporomusa* is described as able to contribute to the initial phase of anaerobic degradation of lignin-derived aromatics (Kato et al., 2015). Also, the *Tahibacter* group is composed of bacteria that have the degrading action of various pollutants, such as wastewater, antibiotics, and organic dyes (Bai et al., 2020). Although some of these groups have beneficial traits, they decreased in the presence of the Si. If these functions are being performed by other microbes (functional redundancy) or if they are being lost, further analyses are needed.

4.4 Functional profile prediction

Finally, we predicted potential functions using the FAPROTAX database to assess the effect of plant stage and Si application on the metabolism of nitrogen and carbon in our samples. Although this methodology is based on a potential prediction, it is useful to understand the relationship between microbial groups and their potential predicted functions (Louca et al., 2016; Merloti et al., 2019). The plant cane presented a higher abundance of sequences affiliated to nitrification, while the ratoon cane showed enrichment of the denitrification process. Together, these results indicate that sugarcane at the plant stage depends more on the biological nitrogen fixation than in the ratoon stage, where the nitrite or nitrate generated during the nitrification is reduced by denitrifiers releasing gaseous nitrogen. This potential emission in the ratoon cane

may contribute to environmental problems and is responsible for the nitrogen loss in the system (Li et al., 2016). Regarding carbon metabolism, our results showed a change in the carbon source used by the microbes, with possible consequences on methane flux. The rhizosphere community of the sugarcane plant presented a higher abundance of hydrogenotrophs (microbes that use hydrogen as a source of energy), while the ratoon community presented a higher abundance of methylotrophs (microbes that use methanol as an energy source). Microbial methanogenesis is a major source of methane in soil, consisting in the final step in the anaerobic degradation of organic matter when inorganic electron acceptors have been depleted (Conrad, 2020). Thus, further analyses are needed to better disentangle the dynamics of nitrogen and carbon in this system since more information about these pathways is important to predict greenhouse gas emissions and develop mitigation strategies.

5 Conclusion

In this study, we evaluated the impact of silicon application on the diversity and composition of the prokaryotic community in the rhizosphere of different sugarcane genotypes. Although the overall structure showed minimal variations among treatments, silicon application had an impact on the prokaryotic diversity and changed the abundance of specific microbial groups that varied according to the genotype. Interestingly, the application of silicon led to an enrichment of specific and important microbial groups in the rhizosphere, particularly those known as N fixers and plant growth promoters. This suggests that silicon application can improve sugarcane growth through both chemical and biological mechanisms. Therefore, further studies are recommended to assess the impact of these plant growth-promoters and N-fixers on sugarcane growth and production. Another interesting field of study is the synergism between fungal communities, including mycorrhiza, and the application of silicon in the rhizosphere, as evidenced by previous research (Hajiboland et al., 2018; Semenov et al., 2022). Further studies should be conducted to assess the mycobiome of the sugarcane rhizosphere. Finally, due to the regular removal of straw and the high need for Si uptake, the most weathered soils occur in the tropics where desilicated soils make up roughly 2385 Mha, of which approximately 1890 Mha are cultivated in input-free cropping systems, especially with high Si-accumulator sugarcane. This highlights the

need for silicon fertilizer, which, therefore, reduces soil desilication, in particular, in cultivated cropland with highly or less weathered soils (Li et al., 2020a, b; Li and Delvaux, 2019).

References

ANDERSON, M. J. A new method for non parametric multivariate analysis of variance. **Austral Ecology**, v. 26, p. 32–46, 2001.

ARAUJO, S. F. A.; MIRANDA, A. R. L.; SOUSA, R. S.; MENDES, L. W.; ANTUNES, J. E. L.; OLIVEIRA, L. M. S.; ARAUJO, F. F.; MELO, V. M. M.; FIGUEIREDO, M. V. B. Bacterial community associated with rhizosphere of maize and cowpea in a subsequent cultivation. **Applied Soil Ecology**, v. 143, p. 26–34, 2019.

BAI, N.; LI, S.; ZHANG, J.; ZHANG, H.; ZHANG, H.; ZHENG, X.; NV, W. Efficient biodegradation of DEHP by CM9 consortium and shifts in the bacterial community structure during bioremediation of contaminated soil. **Environmental Pollution**, v. 266, 115112, 2020.

BENJAMINI, Y.; HOCHBERG, Y. Controling the false discovery rate - a practical and powerful approach to multiple testing. **Journal of the Royal Statistical Society**, v. 57, n. 1, p. 289-300, 1995.

BULGARELLI D.; GARRIDO-OTER R.; MÜNCH P.C.; WEIMAN A.; DRÖGE J.; PAN Y.; MCHARDY, A.C.; SCHULZE-LEFERT, P. Structure and function of the bacterial root microbiota in wild and domesticated barley. **Cell Host Microbe**, v. 17, p. 392–403, 2015.

CALLAHAN, B. J.; MCMURDIE, P. J.; HOLMES, S. P. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. **The ISME Journal**, v. 11, p. 2639–2643, 2017.

CAPORASO, J. G.; LAUBER, C. L.; WALTERS, W. A.; DONNA BERG-LYONS, D.; LOZUPONE, C. A.; TURNBAUGH, P. J.; NOAH FIERER, N.; KNIGHT, R. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. **PNAS**, v. 108, p. 4516-4522, 2011.

CHEN, J.; JIANG, X.; TONG, T.; MIAO, S.; HUANG, J.; XIE, S. Sulfadiazine degradation in soils: Dynamics, functional gene, antibiotic resistance genes and microbial community. **Science of The Total Environment**, v. 691, p. 1072-1081, 2019.

CHEN, Y.; BONKOWSKI, M.; SHEN, Y.; GRIFFITHS, B. S.; JIANG, Y.; WANG, X.; SUN, B. Root ethylene mediates rhizosphere microbial community reconstruction when chemically detecting cyanide produced by neighbouring plants. **Microbiome**, v.8, 2020.

CONRAD, R. Importance of hydrogenotrophic, aceticlastic and methylotrophic methanogenesis from methane production in terrestrial, aquatic and other anoxic environments: A mini review. **Pedosphere**, v. 30, p. 25-39, 2020.

CRUSCIOL, C.; CAMPOS, M.; MARTELLO, J. M.; ALVES, C. J.; NASCIMENTO, C.; PEREIRA, J.; CANTARELLA, H. Organomineral fertilizer as source of P and K for sugarcane. **Scientific Reports**, v. 10, n.5398, 2020.

DAS, S.; KIM, G. W.; HWANG, H. Y.; VERMA, P. P.; KIM; P. J. Cropping with slag to address soil, environment, and food security. **Frontiers in Microbiology**, v.10, p.1320, 2019.

DENG, Q.; YU, T.; ZENG, Z.; ASHRAF, U.; SHI, Q.; HUANG, S.; LIAN, T.; CHEN, J.; MUZAFFAR, W.; SHEN, W. Silicon application modulates the growth, rhizosphere soil characteristics, and bacterial community structure in sugarcane. **Frontiers in Plant Science**, v.12, n.710139, 2021.

DOBROVOLSKAYA, T. G.; GOLOVCHENKO, A. V.; LYSAK, L. V.; YURCHENKO, P. T. Taxonomic structure of bacterial communities of rhizospheric soil under Bogs' Plants. **Moscow University Soil Science Bulletin**, v. 75, p. 93–100, 2020.

DORIGUEL, F.; CRUSCIOL, C.A.C.; FLORENTINO, H.O., 2018. Mathematical optimization models in the sugarcane harvestins process, in: Oliveira, A.B. (Ed.), **Sugarcane: Technology and Research**. London: IntechOpen. 2018. p. 207-221.

DOUMBOU, C. L.; SALOVE, M. K. H.; CRAWFORD, D.L.; BEAULIEU, C. Actinomycetes, promising tools to control plant diseases and to promote plant growth. **Phytoprotection** v.82, p.85–102, 2001.

FIGUEIREDO, M. V. B.; BONIFACIO, A.; RODRIGUES, A. C.; ARAUJO, F. F., 2016. Plant Growth-Promoting Rhizobacteria: Key Mechanisms of Action, in: Choudhary, DK, Varma, A. (Eds.) **Microbial-mediated induced systemic resistance in plants**. Singapore: Springer. 2016. p. 23-37.

FRAZÃO, J. J.; PRADO, R. M.; SOUZA JÚNIOR, J. P.; ROSSATTO, D. R. Silicon changes C:N:P stoichiometry of sugarcane and its consequences for photosynthesis, biomass partitioning and plant growth. **Scientific Reports**, v.10, n.1, p. 1-10, 2020.

GAIERO, J. R.; McCALL, C. A.; THOMPSON, K. A.; DAY, N. J.; BEST, A. S.; DUNFIELD, K. E. Inside the root microbiome: bacterial root endophytes and plant growth promotion. **American Journal of Botany**, v.100, n. 9, p. 1738–1750, 2013.

GAO, X.; WU, Z.; LIU, R.; WU, J.; ZENG, Q.; QI, Y. Rhizosphere bacterial community characteristics over different years of sugarcane ratooning in consecutive monoculture. **BioMed Research International**, v. 2019, p.1-10, 2019.

GUERRIERO, G.; HAUSMAN, J.F.; LEGAY, S. Silicon and the Plant Extracellular Matrix. **Frontiers in Plant** Science, v.7, n.463, p. 1-8, 2016.

HAJIBOLAND, R.; MORADTALAB, N.; ALIASGHARZAD, N.; ESHAGHI, Z.; FEIZY, J. Silicon influences growth and mycorrhizal responsiveness in strawberry plants. **Physiology and Molecular Biology of Plants**, v. 24, n. 6, p. 1103–1115, 2018.

HAMMER, O. et al. Past: paleontological statistics software package for education and data analysis. **Palaeontologia Electronica**, v. 4, n. 1, p. 1-9, 2001.

HEINRICHS, R.; OTTO, R.; MAGALHÃES, A.; MEIRELLES, G.C. Importance of Sugarcane in Brazilian and World Bioeconomy, in: Dabbert S., Lewandowski I., Weiss J., Pyka A. (Eds.) **Knowledge-Driven Developments in the Bioeconomy. Economic Complexity and Evolution.** Cham: Springer. 2017. p. 205-217.

HU, H-W.; WANG, J-T.; SINGH, B. K.; LIU, Y-R.; CHEN, Y-L.; ZHANG, Y-J.; HE, J-Z. Diversity of herbaceous plants and bacterial communities regulates soil resistome across forest biomes. **Environmental Microbiology**, v.20, p.3186–3200, 2018.

JIAO, K.; YANG, B.; WANG, H.; XU, W.; ZHANG, C.; GAO, Y.; SUN, W.; LI, F.; JI, D. The individual and combined effects of polystyrene and silver nanoparticles on nitrogen transformation and bacterial communities in an agricultural soil. **Science of The Total Environment**, v. 820, 153358, 2022.

KALOTERAKIS, N.; DELDEN, S. H.; HARTLEY, S.; DEYN, G. B. Silicon application and plant growth promoting rhizobacteria consisting of six pure bacillus species alleviate salinity stress in cucumber (*Cucumis sativus* L). **Scientia Horticulturae**, v. 288, 110383, 2021.

KATO, S.; CHINO, K.; KAMIMURA, N.; MASAI, E.; YUMOTO, I.; KAMAGATA, Y. Methanogenic degradation of lignin-derived monoaromatic compounds by microbial enrichments from rice paddy field soil. **Scientific Reports**, v. 5, 14295, 2015.

KHAN, A.; JIANG, H.; BU, J.; ADNAN, M.; GILLANI, S. W.; ZHANG, M. An insight to rhizosphere bacterial community composition and structure of consecutive winter-initiated sugarcane ratoon crop in Southern China. **BMC Plant Biology**, v. 22, n. 74, 2022.

KOKALIS-BURELLE, N.; McSORLEY, R.; WANG, K. H.; SAHA, S. K.; McGOVERN, R. J. Rhizosphere microorganisms affected by soil solarization and cover cropping in *Capsicum annuum* and *Phaseolus lunatus* agroecosystems. **Applied Soil Ecology**, v. 119, p. 64–71, 2017.

KORNDÖRFER, G. H.; PEREIRA, H. S. NOLLA, A. **Análise de silício: solo, planta e fertilizante**. Uberlândia: Boletim Técnico 2, GPSi-ICIAG-UFU, 2004. 34 p.

KRASKA, J. E.; BREITENBECK G. A. Simple, robust method for quantifying silicon in plant tissue. **Communications in Soil Science and Plant Analysis**, v.41, n.17, p. 2075-2085, 2010.

LI, Q.; GUO, X.; LU, Y.; SHAN, G.; HUANG, J. Impacts of adding FGDG on the abundance of nitrification and denitrification functional genes during dairy manure and sugarcane pressmud co-composting. **Waste Management**, v. 56, p. 63–70, 2016.

LI, W.; ZHANG, Y.; MAO, W.; WANG, C.; YIN, S. Functional potential differences between Firmicutes and Proteobacteria in response to manure amendment in a reclaimed soil. **Canadian Journal of Microbiology**, v. 66, n. 12, p.689-697, 2020a.

LI, Z.; CORNELIS, J.T.; VANDER LINDEN, C.; VAN RANST, E.; DELVAUX, B. Neoformed aluminosilicate and phytogenic silica are competitive sinks in the silicon soil-plant cycle. **Geoderma**, v. 368, 114308, 2020.

LIN H.; LIU C.; LI B.; DONG Y. Trifolium repens L. regulated phytoremediation of heavy metal contaminated soil by promoting soil enzyme activities and beneficial rhizosphere associated microorganisms. **Journal of Hazardous Materials**, v. 402:123829, 2021.

LIN, W.; JIANG, N.; PENG, L.; FAN, X.; GAO, Y.; WANG, G.; CAI, K. Silicon impacts on soil microflora under *Ralstonia Solanacearum* inoculation. **Journal of Integrative Agriculture**, v.19, n.1, p. 251–264, 2020.

LIU, C.; LIN, H.; LI, B.; DONG, Y.; YIN, T. Responses of microbial communities and metabolic activities in the rhizosphere during phytoremediation of Cd-contaminated soil. **Ecotoxicology and Environmental Safety**, v. 202:110958, 2020.

LIU, J.; BASNAYAKE, J.; JACKSON, P. A.; CHEN, X.; ZHAO, J.; ZHAO, P.; YANG, L.; BAI, Y.; XIA, H.; ZAN, F.; QIN, W.; YANG, K.; YAO, L.; ZHAO, L.; ZHU, J.; LAKSHMANAN, P.; ZHAO, X.; FAN, Y. Growth and yield of sugarcane genotypes are strongly correlated across irrigated and rainfed environments. **Field Crops Research**, v. 196, p. 418-425, 2016.

LIU, Y.; YANG, H.; LIU, Q.; ZHAO, X.; XIE, S.; WANG, Z.; WEN, R.; ZHANG, M.; CHEN, B. Effect of two different sugarcane cultivars on rhizosphere bacterial communities of sugarcane and soybean upon intercropping. **Frontiers in Microbiology**, v.11:596472, 2021.

LOPES, L. S.; MENDES, L. W.; ANTUNES, J. E. L.; OLIVEIRA, L. M. S.; MELO, V. M. M.; PEREIRAS, A. P. A.; COSTA, A. F.; OLIVEIRA, J. P.; MARTINEZ, C. R.; FIGUEIREDO, M. V. B.; ARAUJO, A. S. F. Distinct bacterial community structure and composition along different cowpea producing ecoregions in Northeastern Brazil. **Scientific Reports**, v. 11, n. 831, 2021.

LOUCA, S.; PARFREY, L. W.; DOEBELI, M. Decoupling function and taxonomy in the global ocean microbiome. **Science**, v. 353, n. 6305, p. 1272-1277, 2016.

MARTINEZ, H. E. P.; CARVALHO, J. G.; SOUZA, R. B. Diagnose foliar. In: RIBEIRO, A. C.; GUIMARÃES, P. T. G.; ALVAREZ, V. V. H. (Ed.). **Recomendações para o uso de corretivos e fertilizantes em Minas Gerais**: 5ª aproximação. Viçosa, MG: Comissão de Fertilidade do Solo do Estado de Minas Gerais, 1999. p. 143-168.

MARTÍNI, A. F.; VALANI, G. P.; BOSCHI, R. S.; BOVI, R. C.; SIMÕES DA SILVA, L. F.; COOPER, M. Is soil quality a concern in sugarcane cultivation? A bibliometric review. **Soil and Tillage Research**, v. 204, p. 1-8, 2020.

MCCRAY, J. M. and SHANGNING, J. I. Sugarcane yield response to calcium silicate on Florida mineral soils. **Journal of Plant Nutrition**, v.41, p1–12, 2018.

MENDES, L. W.; CHAVES, M. G.; FONSECA, M. C.; MENDES, R.; RAAIJMAKERS, J. M., TSAI, S. M. Resistance breeding of common bean shapes the physiology of the rhizosphere microbiome. **Frontiers in Microbiology**, v.10:3389, 2019.

MENDES, L. W.; KURAMAE, E.E.; NAVARRETE, A. A.; VAN VEEN, J. A.; TSAI, S. M. Taxonomical and functional microbial community selection in soybean rhizosphere. **The ISME Journal**, v. 8, p. 1577–1587, 2014.

MENDES, L. W.; MENDES, R.; RAAIJMAKERS, J. M.; TSAI, S. M. Breeding for soil-borne pathogen resistance impacts active rhizosphere microbiome of common bean. **ISME Journal**, v.12, p. 3038–3042, 2018.

MERLOTI, L. F.; MENDES, L. W.; PEDRINHO, A.; SOUZA, L. F.; FERRARI, B. M.; TSAI, S. M. Forest-to-agriculture conversion in Amazon drives soil microbial communities and N-cycle. **Soil Biology and Biochemistry**, v.137:107567, 2019.

MHETE, M.; EZE, P. N.; RAHUBE, T. O.; AKINYEMI, F. O. Soil properties influence bacterial abundance and diversity under different land-use regimes in semi-arid environments. **Scientific African**, v.7:e00246, 2020.

MORI, H.; MARUYAMA F.; KATO, H.; TOYODA, A.; DOZONO, A.; OHTSUBO, Y.; NAGATA, Y.; FUJIYAMA, A.; TSUDA, M.; KUROKAWA, K. Design and experimental application of a novel non-degenerate universal primer set that amplifies prokaryotic 16S rRNA genes with a low possibility to amplify eukaryotic rRNA genes. **DNA Research**, v.21, n.2, p.217-227, 2014.

MOROENYANE, I.; MENDES, L. W.; TREMBLAY, J.; TRIPATHI, B.; YERGEAU, E. Plant compartments and developmental stages modulate the balance between niche-based and neutral processes in soybean microbiome. **Microbial Ecology**, v. 82, p. 416-428, 2021.

NAEEM, A.; SAIFULLAH, ZIA-UR-REHMAN, M.; AKHTAR, T.; ZIA, M. H.; ASLAM, M. Silicon nutrition lowers cadmium content of wheat cultivars by regulating transpiration rate and activity of antioxidant enzymes. **Environmental Pollution**, v.242, p. 126–135, 2018.

OGOLA, H. J. O.; SELVARAJAN, R.; TEKERE, M. Local geomorphological gradients and land use patterns play key role on the soil bacterial community diversity and dynamics in the highly endemic indigenous afrotemperate coastal scarp forest biome. **Frontiers in Microbiology**, v.12:592725, 2021.

OLIVEIRA, B. G.; MENDES, L. W.; SMYTH, E. M.; TSAI, S. M.; FEIGL, B. J.; MACKIE, R. I. Assessment of microbial diversity associated with CH4 emission from sugarcane vinasse storage and transportation systems. **Journal of Environmental Management**, v.269:110748, 2020.

OLIVEIRA, M. G., MACEDO, G. A. R., MARTINS, J. A., SILVA, V. S. G. Mineral Nutrition and Fertilization of Sugarcane, in: Oliveira, AB (Ed.), **Sugarcane: Technology and Research**. London: IntechOpen. 2017. p. 169-191.

OLIVEIRA, R.L.L., PRADO, R. M., FELISBERTO, G.; CHECCHIO, M. V.; GRATÃO, P. L. Silicon mitigates manganese deficiency stress by regulating the physiology and activity of antioxidant enzymes in sorghum plants. **Journal of Soil Science and Plant Nutrition**, v.19**,** p. 524–534, 2019.

PANG, Z.; DONG, F.; LIU, Q.; LIN, W.; HU, C.; YUAN, Z. Soil Metagenomics Reveals Effects of Continuous Sugarcane Cropping on the Structure and Functional Pathway of Rhizospheric Microbial Community. **Frontiers in Microbiology**, v.12, 2021.

PARKS, D. H.; BEIKO, R. G. Identifying biologically relevant differences between metagenomic communities. **Bioinformatics**, v. 26, n. 6, p. 715-721, 2010.

PRIYA, H.; PRASANNA, R.; RAMAKRISHNAN, B.; BIDYARANI, N.; BABU, S.; THAPA, S.; RENUKA, N. Influence of cyanobacterial inoculation on the culturable microbiome and growth of rice. **Microbiological Research**, v.171, p.78-89, 2015.

QUAST, C. et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. **Nucleic Acids Research**, v. 23, p. 590-596, 2013.

R Development Core Team (2007). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Com- puting, Vienna. Disponivel em: http://www.R-pr oject.org.

RAIJ, B. V.; ANDRADE, J.C.; CANTARELLA, H.; QUAGGIO, J.A. **Análise química para avaliação da fertilidade de solos tropicais**. Campinas: Instituto Agronômico, 2001. 285 p.

RANJAN, A.; SINHA, R.; BALA, M.; PAREEK, A.; SINGLA-PAREEK, S. L.; SINGH, A. K. Silicon-mediated abiotic and biotic stress mitigation in plants: Underlying mechanisms and potential for stress resilient agriculture. **Plant Physiology and Biochemistry**, v. 163, p. 15–25, 2021.

REN, H.; WANG, H.; QI, X.; YU, Z.; ZHENG, X.; ZHANG, S.; WANG, Z.; ZHANG, M.; AHMED, T.; LI, B. The damage caused by decline disease in bayberry plants through changes in soil properties, rhizosphere microbial community structure and metabolites. **Plants**, v. 10, n. 2083, 2021.

REZAKHANI, L.; MOTESHAREZADEH, B.; TEHRANI, M. M.; ETESAMI, H.; HOSSEINI, H. M. Phosphate–solubilizing bacteria and silicon synergistically augment phosphorus (P) uptake by wheat (*Triticum aestivum* L.) plant fertilized with soluble or insoluble P source. **Ecotoxicology and Environmental Safety**, v. 30, n. 173, p. 504-513, 2019.

ROSSMANN, M.; PÉREZ-JARAMILLO, J. E.; KAVAMURA, V. N.; CHIARAMONTE, J. B.; DUMACK, K., FIORE-DONNO, A. M.; MENDES, L. W.; FERREIRA, M. M. C.; BONKOWSKI, M.; RAAIJMAKERS, J. M.; MAUCHLINE, T. H.; MENDES, R.

Multitrophic interactions in the rhizosphere microbiome of wheat: from bacteria and fungi to protists. **FEMS Microbiology Ecology**, v. 96, n. 4, 2020.

SAMADDAR S.; TRUU, J.; CHATTERJEE, P.; TRUU, M.; KIM, K.; KIM S.; SESHADRI S.; AS, T. Long-term silicate fertilization increases the abundance of Actinobacterial population in paddy soils. **Biology and Fertility of Soils**, v.55, n.2, p.109-120, 2019.

SEMENOV, M. V.; KRASNOV, G. S.; SEMENOV, V. M.; BRUGGEN, A. H. Long-term fertilization rather than plant species shapes rhizosphere and bulk soil prokaryotic communities in agroecosystems. **Applied Soil Ecology**, v. 154, 103641, 2020.

SEMENOV, M. V.; KRASNOV, G. S.; SEMENOV, V. M.; BRUGGEN, A. Mineral and organic fertilizers distinctly affect fungal communities in the crop rhizosphere. **Journal of Fungi**, v. 8, n. 3, 2022.

SOLANKI, M. K.; WANG, Z.; WANG, F.-Y.; LI, C.-N.; GUPTA, C. L.; SINGH, R. K.; LI, Y.-R. Assessment of diazotrophic proteobacteria in sugarcane rhizosphere when intercropped with legumes (peanut and soybean) in the field. **Frontiers in Microbiology**, v.11:1814, p.1-12, 2020.

SONG, A.; LI, Z.; LIAO, Y.; LIANG, Y.; WANG, E.; WANG, S.; LI, X.; BI, J.; SI, Z.; LU, Y.; NIE, J.; FAN, F. Soil bacterial communities interact with silicon fraction transformation and promote rice yield after long-term straw return. **Soil Ecology Letters**, v. 3, p. 395–408, 2021.

SOUSA, R. M. S.; MENDES, L. W.; ANTUNES, J. E. L.; OLIVEIRA, L. M. S.; SOUSA, A. M. C. B.; GOMES, R. L. F.; LOPES, A. C. A.; ARAÚJO, F. F.; MELO, V. M. M.; ARAUJO, A. S. F. Diversity and structure of bacterial community in rhizosphere of lima bean. **Applied Soil Ecology**, v.150:103490, 2020.

SOZINHO, D. W. F.; GALLARDO, A. L. C. F.; DUARTE, C. G.; RAMOS, H. R., RUIZ, M. S. Towards strengthening sustainability instruments in the Brazilian sugarcane ethanol sector. **Journal of Cleaner Production**, v. 182, p. 437-454, 2018.

STAHL, D. A.; DE LA TORRE, J. R. Physiology and diversity of Ammonia-Oxidizing Archaea. **Annual Review of Microbiology**, v.66, n.1, p.83-101, 2012.

TAYYAB, M.; FALLAH, N.; ZHANG, C.; PANG, Z.; ISLAM, W.; LIN, S.; LIN, W.; ZHANG, H. Sugarcane cultivar-dependent changes in assemblage of soil rhizosphere fungal communities in subtropical ecosystem. **Environmental Science and Pollution Research**, v. 26, n. 6, 2021.

TRIVELIN, P. C. O.; FRANCO, H. C. J.; OTTO, R.; FERREIRA, D. A.; VITTI, A. C.; FORTES, C.; FARONI, C. E.; OLIVEIRA, E. C. A.; CANTARELLA, H.; Impact of sugarcane trash on fertilizer requirements for São Paulo, **Brazil. Sci. Agric**. v. 70, p. 345–352, 2013.

VAL-MORAES, S.P.; MACEDO, H.S.; KISHI, L.T.; PEREIRA, R. M.; NAVARRETE, A. A.; MENDES, L. W.; FIGUEIREDO, E. B.; LA SCALA, N. JR., TSAI, S. M.; MACEDO, L. E. G., ALVES, L. M. Liming in the sugarcane burnt system and the green harvest

practice affect soil bacterial community in northeastern São Paulo, Brazil. **Antonie van Leeuwenhoek**, v. 109, n. 12, p. 1643-1654, 2016.

VAL-MORAES, S.P.; MARCONDES, J.; CARARETO ALVES, L.M. et al. Impact of sewage sludge on the soil bacterial communities by DNA microarray analysis. **World Journal of Microbiology and Biotechnology**, v.27, p.1997–2003, 2011.

VERMA, K. K.; LIU, X. H.; WU, K. C.; SINGH, R. K.; SONG, Q-Q.; MALVIYA, M. K.; SONG, X-P.; SINGH, P.; VERMA, C. L.; LI, Y. R. The impact of silicon on photosynthetic and biochemical responses of sugarcane under different soil moisture levels. **Silicon**, v. 12, p. 1355-1367, 2020.

WANG, B.; CHU, C.; WEI, H.; ZHANG, L.; AHMAD, Z.; WU, S.; XIE, B. Ameliorative effects of silicon fertilizer on soil bacterial community and pakchoi (*Brassica chinensis L*.) grown on soil contaminated with multiple heavy metals. **Environmental Pollution**, v. 267, p. 1-10, 2020.

WANG, P.; ELDON R. R.; YAN, Y.; MA, W., XIANG, Y. Spatiotemporal evolvement and factors influencing natural and synthetic EDCs and the microbial community at different groundwater depths in the Chaobai watershed: A long-term field study on a river receiving reclaimed water. **Journal of Environmental Management**, v. 246, p. 647-657, 2019.

WHITMAN, W. B.; RAINEY, F.; KÄMPFER, P.; TRUJILLO, M.; CHUN, J.; DEVOS, P.; HEDLUND, B.; DEDYSH, S. **Bergey's Manual of Systematics of Archaea and Bacteria**. Hoboken, New Jersey: Wiley, 2015.

XIAO, X.; FAN, M.; WANG, E.; CHEN, W.; WEI, G. Interactions of plant growth-promoting rhizobacteria and soil factors in two leguminous plants. **Applied Microbiology and Biotechnology**, v. 101, p. 8485–8497, 2017.

XIONG, C.; SINGH, B. K.; HE, J. Z.; HAN, Y. L.; LI, P. P.; WAN, L. H.; MENG, G. Z.; LIU, S. Y.; WANG, J. T.; WU, C. F.; GE, A. H.; ZHANG, L. M. Plant developmental stage drives the differentiation in ecological role of the maize microbiome. **Microbiome**, v. 9, n. 171, 2021.

YANG, Y.; DONG, M.; CAO, Y.; WANG, J.; TANG, M.; BAN, Y. Comparisons of soil properties, enzyme activities and microbial communities in heavy metal con- taminated bulk and rhizosphere soils of *Robinia pseudoacacia L*. in the northern foot of Qinling Mountain. **Forests**, v. 8, n. 430, 2017.

YEOH, Y. K., PAUNGFOO-LONHIENNE, C., DENNIS, P. G., ROBINSON, N., RAGAN, M. A., SCHMIDT, S., HUGENHOLTZ, P. The core root microbiome of sugarcanes cultivated under varying nitrogen fertilizer application. **Environmental Microbiology**, v. 18, p. 1338-1351, 2015.

ZHANG L. and GUAN A. Y. Consistent responses of soil bacterial communities to bioavailable silicon deficiency in croplands. **Geoderma**, v.408, n.115587, 2022.

ZHAO, X.; JIANG, Y.; LIU, Q.; YANG, H.; WANG, Z.; ZHANG, M. Effects of Drought-Tolerant Ea-DREB2B Transgenic Sugarcane on Bacterial Communities in Soil. **Frontiers in Microbiology**, v.11, n.704, p 1-11, 2020.

ZHAO, Y.; ZHAO, Y.; ZHANG, Z.; WEI, Y.; WANG, H.; LU, Q.; LI, Y.; WEI, Z. Effect of thermo-tolerant actinomycetes inoculation on cellulose degradation and the formation of humic substances during composting. **Waste Management**, v.68, p.64-73, 2017.

ZULUAGA, M. Y. A.; MILANI, K. M. L.; GONÇALVES, L. S. A.; OLIVEIRA, A. L. M. Diversity and plant growth-promoting functions of diazotrophic/N-scavenging bacteria isolated from the soils and rhizospheres of two species of *Solanum*. **PLoS ONE**, v. 15, n. e0227422, 2020.

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CAPÍTULO II - Distinct sources of silicon shape differently the prokaryotic microbial community in the rhizosphere of sugarcane

Abstract

The rhizosphere microbial communities play a crucial role in enhancing plant growth, productivity, and nutrient uptake, and the application of fertilizers can influence these communities, leading to significant implications for plant performance. Silicon (Si) is known to have a positive impact on sugarcane production, and this study aims to investigate the effect of two different sources of silicon, K- and Na-silicate, on the structure and composition of prokaryotic communities in both the bulk soil and the rhizosphere of sugarcane. Firstly, our results showed a different response of the microbial community between bulk soil and rhizosphere, where the application of Si resulted in a shift in the prokaryotic community in the rhizosphere compared to the control without Si. Interestingly, the application of Na-silicate had a more pronounced effect on the prokaryotic community than K-silicate, resulting in a shift in the community structure compared to K-silicate. Moreover, the application of Na-silicate led to an increase in the relative abundance of Proteobacteria and Bacteriodetes, while K-silicate promoted an enrichment of Chloroflexi and Acidobacteriota. Also, Si application affects the niche occupancy, increasing the proportion of generalist microbes, with consequences on putative predict functions. Together, the results indicated that different Si sources could promote distinct responses of the microbial community in the rhizosphere of sugarcane, which could be attributed to the composition of the fertilizer. This study highlights the importance of understanding the effect of agricultural practices on the rhizosphere microbial communities to improve plant growth and productivity.

Keywords: *Saccharum* spp., plant-microbe interaction, microbial ecology

1. Introduction

The rhizosphere, i.e., the narrow region of soil under influence of root exudates, represents a dynamic and complex microbial hotspot (Kuzyakov and Blagodatskaya, 2015). The distinct chemical composition of root exudates released in the rhizosphere plays a significant role in shaping the behavior of the microbial community inhabiting this region (Pérez-Jaramillo et al., 2019). As a result, the microbial community in the rhizosphere plays a crucial role in plant growth and development, both directly and indirectly. Through the production and release of plant hormones (Silveira et al., 2019) and cycling nutrients (Pattnaik et al., 2021), the microbial community impacts plant health and productivity. Therefore, there has been extensive research on the microbial community associated with the rhizosphere, with a focus on promoting plant growth and performance (Mendes et al., 2018). Also, the application of herbicides and fertilizers to crops in agricultural fields with varying soil inputs can significantly impact the microbial communities in the rhizosphere, as highlighted in recent studies (Leite et al., 2023; Pertile et al., 2021).

Chemical fertilizers, which are among the most widely used soil inputs, have been found to cause significant shifts in the microbial community within the rhizosphere, particularly nitrogen (N), phosphorus (P), and potassium (K) (Liu et al., 2020; Wang et al., 2018). However, there is an increased interest in studying the effect of silicon (Si) in plants and the rhizosphere, since this element has been recommended to be applied in plants to confer higher vigor and resistance to (a)biotic stresses (Luyckx et al., 2017). In sugarcane, Si acts on the cell wall, providing greater structural stability, increasing the photosynthetic efficiency, and protecting plants (Bakhat et al., 2018; Oliveira Filho et al., 2021; Frazão et al., 2020; Verma et al., 2020).

Regarding the rhizosphere, recent studies have shown that application of Si promotes changes in the microbial community in the rhizosphere (Leite et al., 2023), increasing the abundance and diversity of plant growth-promoting bacteria (Wang et al., 2020). An example of this is the observed increase in the abundance of Proteobacteria in the rhizosphere of sugarcane upon the application of Si, which is significant as this phylum includes several important plant growth promoters (Deng et al., 2021). Recently, Leite et al. (2023) found a decrease in the relative abundance of

Planctomycetes and Gemmatimonadetes, while Proteobacteria increased in sugarcane rhizosphere fertilized with Si.

In agriculture, the main source of Si to plants is through the application of calcium silicate (Gascho, 2001). However, alternative sources of Si, such as sodium (Na) and potassium (K) silicates have been recommended as they present higher efficacy in supplying Si to plants. For instance, K-silicate (K_2SiO_3) has been used as a plant stimulator, being a source of highly soluble Si (Rodrigues et al., 2009). On the other hand, Na-silicate (Na_2SiO_3) has been reported to confer plant resistance to pathogens (Safari et al., 2012) and some sources can present amino acids and peptides. Thus, both types of fertilizers are known to increase plant performance and maintain the biological status of the soil (Zhang et al., 2018; Oliveira et al., 2021; Rocha et al., 2022). Interestingly, the high content of soluble Si present in these sources can increase the initial growth of sugarcane, favoring crop development (Santos, et al., 2020), with greater structural stability, increased photosynthetic rate, greater efficiency of use of nutrients and biomass production (Camargo et al., 2017).

However, while several studies have demonstrated the benefits of using Na- and K-silicates on sugarcane performance, the impact of these various Si sources on the microbial communities within the sugarcane rhizosphere remains unclear. In addition, as reported above, K-silicate has higher Si solubility, while Na-silicate presents amino acids and peptides. This study hypothesized that these different characteristics observed in these two Si sources (Na- and K-silicate) could promote distinct responses of the microbial community in the rhizosphere of sugarcane.

2. Material and methods

2.1 Greenhouse experiment

This study was carried out in a Greenhouse belonging to the Sugarcane Genetic Breeding Program (PMGCA) from the Federal University of Piauí, Teresina, PI, Brazil (5º02'34" S, 42º47'01" W, 72 m). The climate is tropical dry (~1000 mm $year^{-1}$ and ~30 ºC of precipitation and temperature, respectively). The experimental design was completely randomized with three treatments and three replications: a) K-silicate; b) Na-silicate; and c) Si-unfertilized soil. The soil used in the experiment was classified

as Fluvisol, collected in a sugarcane field, at a 0-20 cm depth. The chemical analysis (Table S1) was performed according to the method described by Raij et al. (2001) and the Si content was according to the methodology of Korndörfer et al. (1999).

2.2 Experimental conduction

The soil was placed in plastic pots (7.5 L) and the sugarcane RB021754 was sown in each pot. Fifteen days after emergence, plants were thinned, leaving one plant per pot. Irrigation was performed daily using the gravimetric method based on the soil water retention capacity (WHC), maintaining soil moisture at 70% of the field capacity. The Si sources were applied fifteen days after plant emergence. The K-silicate was supplied by Sifol® (density = 1.41, Si = 168 g L^{-1}, K_2O = 211.5 g L^{-1}); while Na-silicate was supplied by Armurox® (density = 1.27, Si = 46.99 g L^{-1}; amino acids = 3% w/v; peptides = 5.1% w/v). Both sources were balanced using potassium chloride (KCl) solution at a concentration of 1.0 mol L^{-1} supplied via soil.

2.3 Sampling of bulk soil and rhizosphere

At 60th days after emergence, bulk soil and rhizosphere were collected in each pot. For the rhizospheric sampling, the soil adhering to the roots was collected and stored in Eppendorf tubes at -80 ^{o}C. Bulk soil was collected, sieved (2 mm mesh), and stored at -80 °C before analysis.

2.4 DNA extraction and sequencing

DNA samples from bulk soil and rhizosphere were extracted from 0.5 g (total humid weight) using the Powerlyzer Power Soil DNA Isolation Kit. The quality and concentration of DNA were performed by spectrophotometer. The V4 region of the 16S rRNA gene was amplified, by polymerase chain reaction (PCR), with region-specific primers (515F/806R) (Caporaso et al., 2011). This PCR step included Illumina sequencing adapters and dual-index barcodes using the Nextera XT indices to discriminate each sample in the pool. After indexing, the PCR products were cleaned up using Agencourt AMPure XP – PCR purification beads and quantified using the dsDNA BR assay Kit on a Qubit 2.0 fluorometer. The sequencing was done using the Illumina MiSeq sequencing machine (Illumina, San Diego, CA, USA). Sequence data were processed using QIIME 2 version 2023.2. The taxonomic affiliation was performed

using a pre-trained Naïve Bayes classifier with the Python sklearn code, using the Silva database v. 138 at 97% similarity (Quast et al., 2013), and the generated matrix was further used for statistical analyses.

2.5 Data Analysis

For statistical analysis, the homogeneity-normality of variances of the data was verified by Levene and Shapiro–Wilk's tests. Then, we assessed the prokaryotic community structure of bulk soil and rhizosphere using the Principal Component Analysis (PCA). The differential abundance of microbial groups at the phylum and family level was compared using the Statistical Analysis of Metagenomic Profile (STAMP) software (Parks et al., 2014; Parks and Beiko, 2010). P-values were calculated using a two-sided Tukey-Kramer test, and corrections were made using Benjamini-Hochberg's false discovery rate (Benjamini and Hochberg, 1995). Then, the dynamics of the PGPR community were analyzed by comparing the percentage of generalists, specialists, and rare groups using the multinomial classification approach through the 'clamtest' package in R (Pedrinho et al., 2020). Finally, we accessed the putative microbial functions using the FAPROTAX approach v. 1.2.3 including all detected genera (Louca et al., 2016).

3. Results

The principal component analysis explained the data variation distributed in three axes which explained approximately 55% of the total variation (Figure 1). The structure of the prokaryotic community in the rhizosphere and bulk soil differed significantly. Additionally, our analysis revealed a clear separation between the prokaryotic community structure following the application of Na-silicate and K-silicate. Particularly, the structure of the prokaryotic community was similar between the K-silicate and unfertilized treatments in both the bulk soil and rhizosphere. In contrast, the application of Na-silicate resulted in a significant change in the prokaryotic community in both the bulk soil and rhizosphere of sugarcane. These findings suggest that Na-silicate application has a greater impact on the prokaryotic community structure in sugarcane environments compared to K-silicate.

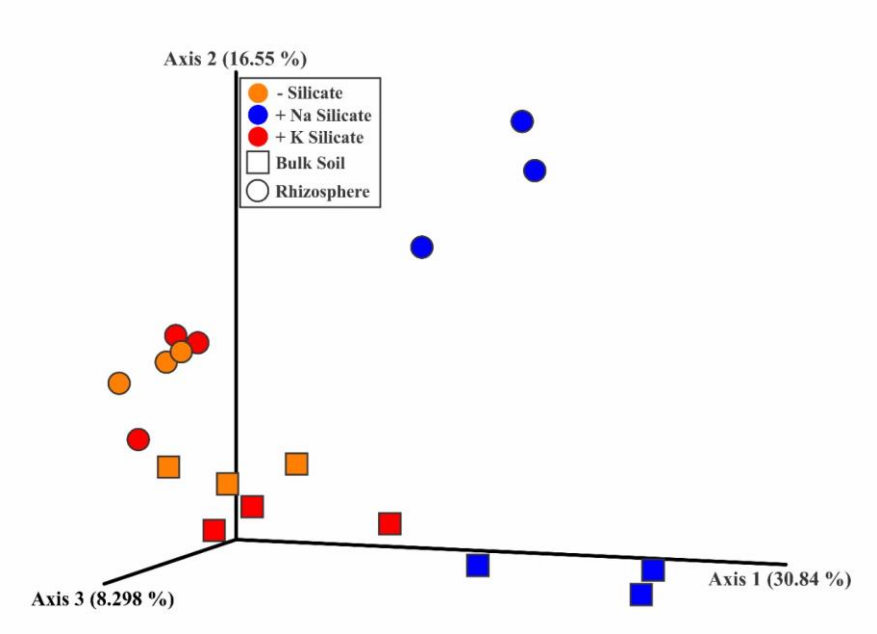


Figure 1. Principal component analysis (PCA) showing the prokaryotic community (bacteria and archaea) structure in bulk soil and rhizosphere of sugarcane with application of two different sources of silicon (K- and Na-silicate). The taxonomic profiling was based on the 16S rRNA gene affiliated to the Silva database at 97 % of similarity.

The composition of the prokaryotic community showed a high relative abundance of Actinobacteria (approximately 23% of the total sequences), Proteobacteria (20%), Firmicutes (12%), Chloroflexi (10%), Acidobacteria (9%), and Bacteroidota (8%) (Figure 2). Despite their high abundance, Actinobacteria and Firmicutes did not exhibit significant variation across treatments in either the bulk soil or rhizosphere. In contrast, the relative abundance of Proteobacteria and Bacteroidota increased significantly with the application of Na-silicate in both the bulk soil and rhizosphere. Conversely, the unfertilized and K-silicate treatments promoted an increase in the abundance of Choroflexi and Acidobacteria. These findings suggest that different bacterial phyla respond differently to soil amendments.

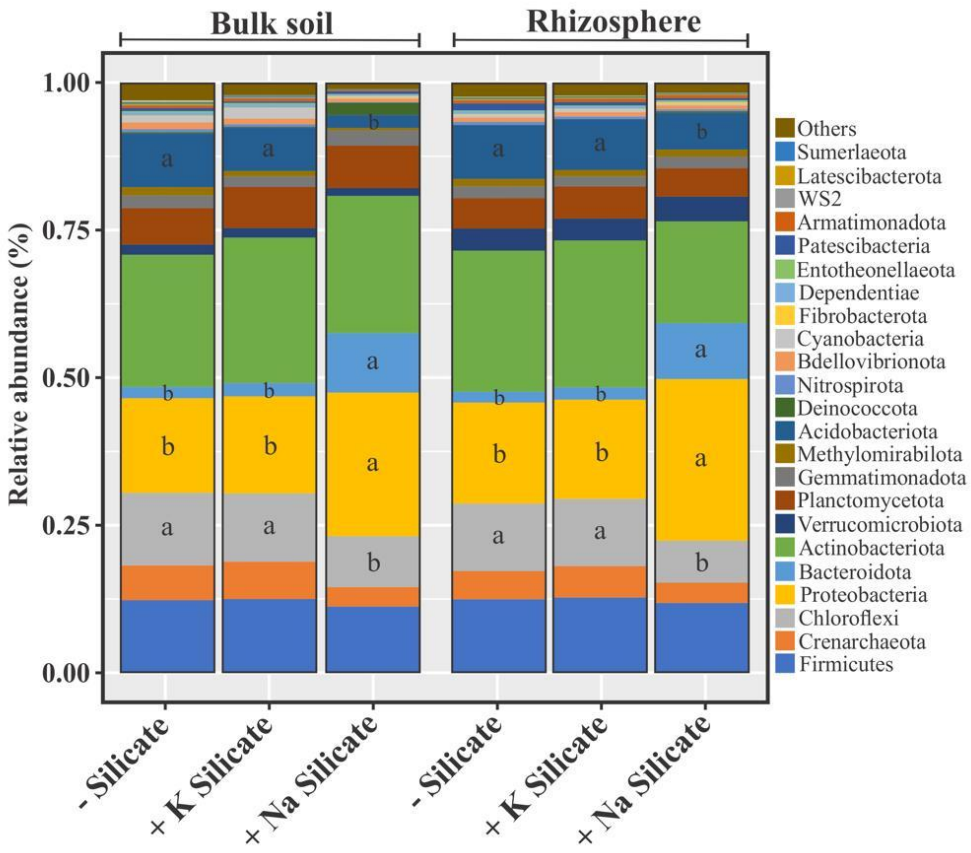


Figure 2. Distribution of the most abundant phyla in the bulk soil and rhizosphere of sugarcane with application of two different sources of silicon (K- and Na-silicate). The taxonomic profiling was based on the 16S rRNA gene affiliated to the Silva database at 97% of similarity. Different lower-case letters refer to significative differential abundance. P-values were calculated using a two-sided Tukey- Kramer test, and corrections were made using Benjamini-Hochberg FDR.

On a deeper taxonomical level, we compared the enrichment of bacterial and archaeal families in both bulk soil and rhizosphere following the application of both silicate sources. Our results showed that both silicate sources promoted different enrichment patterns of microbial families, in both the bulk soil and rhizosphere, compared to the unfertilized treatment (Figures S1 and S2). Moreover, the application of Na-silicate enriched families such as Cyclobacteriaceae, Devosiaceae, Halomonadaceae, Salisediminibacteriaceae, Pirellulaceae, Beijerinckiaceae, and Xanthomonadaceae, while the application of K-silicate enriched Nocardiaceae, KD4-

96, Nitrososphaeraceae, and Bacillaceae (Figure 3). These findings provide a deeper understanding of the specific microbial families that respond to different silicate treatments, highlighting the potential for targeted manipulation of microbial communities in agricultural systems.

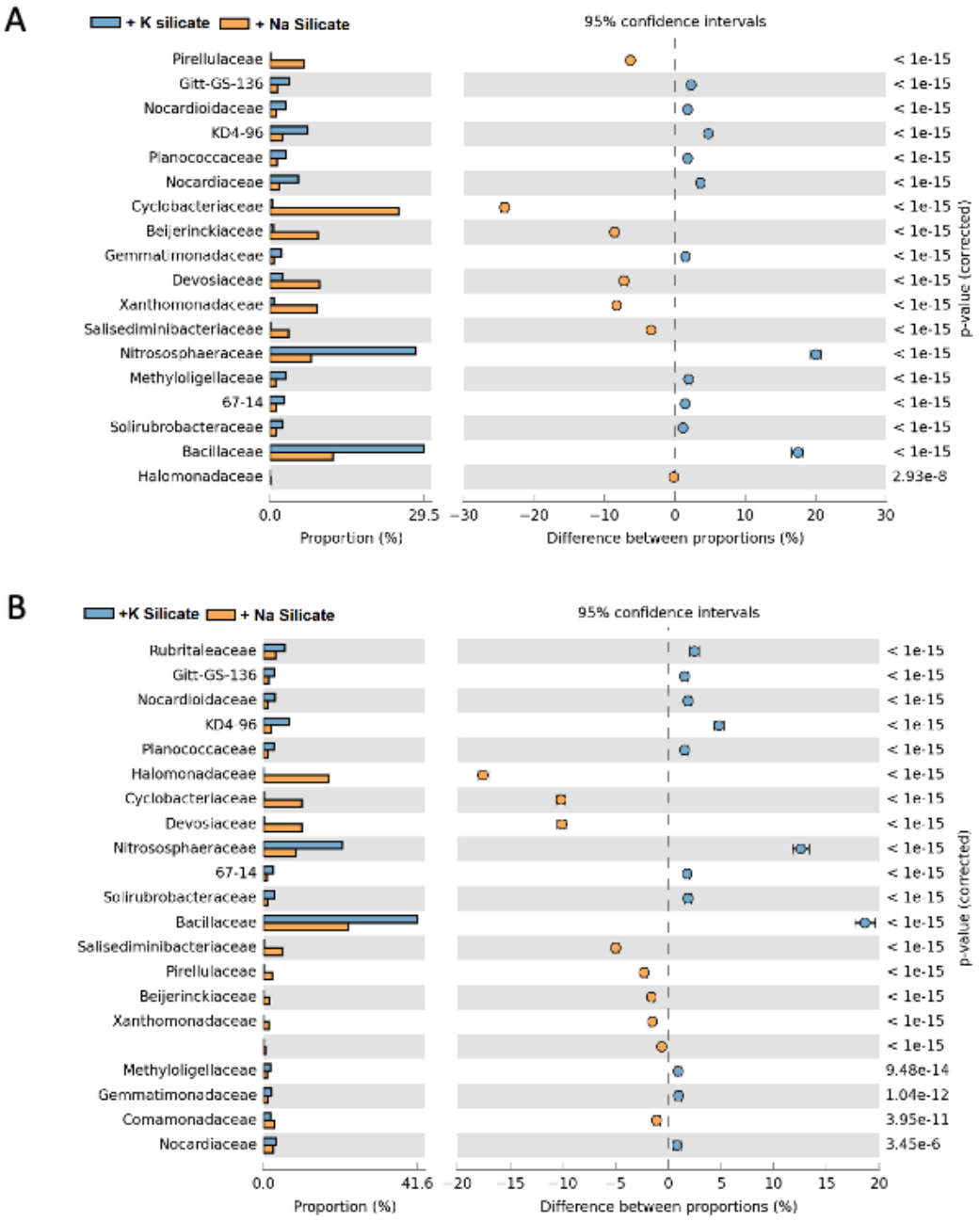


Figure 3. Scatter plots showing the differential abundance of microbial families between sources of silicon (K- and Na-silicate) in both bulk soil (A) and rhizosphere

(B). P-values were calculated using a two-sided Tukey- Kramer test, and corrections were made using Benjamini-Hochberg FDR.

To gain a better understanding of the impact of silicate application on the prokaryotic community, we classified the OTUs into specialists and generalists and analyzed their niche occupancy across treatments. Our results revealed significant variations in the proportion of generalist and specialist microbes among the different treatments, with generalists outnumbering specialist microbes in the rhizosphere of sugarcane (Figure 4).

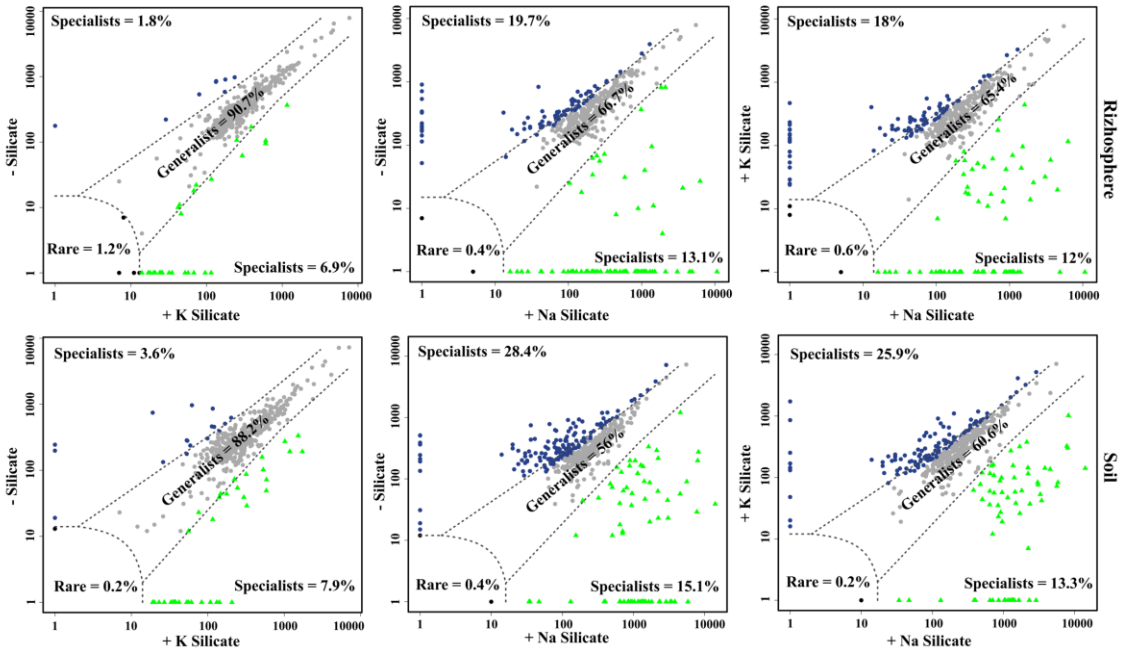


Figure 4. Multinomial species classification method (CLAM) for the species' niche occupancy test in the bulk soil and rhizosphere of sugarcane with application of two different sources of silicon (K- and Na-silicate). The pairwise treatment comparison were with and without silicon application and between the two sources of silicon for rhizosphere and bulk soil. The generalists (gray), specialists (green and blue) and rare (black) are indicated with their respective percentages.

Specifically, the use of K-silicate resulted in an increased proportion of generalist microbes (~90%), while ~7% were classified as specialist microbes in the

rhizosphere of sugarcane compared to the unfertilized treatment. Similarly, the use of Na-silicate also led to an increase in the proportion of generalist microbes (~67%), while ~13% were specialist microbes. Comparing the two silicate sources, generalist microbes accounted for ~65% of the total prokaryotic community in the rhizosphere of sugarcane, with 18% and 12% observed following the application of K- and Na-silicate, respectively.

Finally, we used FAPROTAX to predict potential functions in the rhizosphere and classified all bacterial and archaeal OTUs into 19 functional groups to assess the impact of both silicon sources (Figure 5). Our analysis revealed that the application of Na-silicate led to changes in several potential predicted functions in the rhizosphere, including an increase in microbial groups related to hydrocarbon degradation, respiration of sulfur compounds, nitrate reduction, and chemoheterotrophy. In contrast, the application of K-silicate increased microbial groups related to dark hydrogen oxidation.

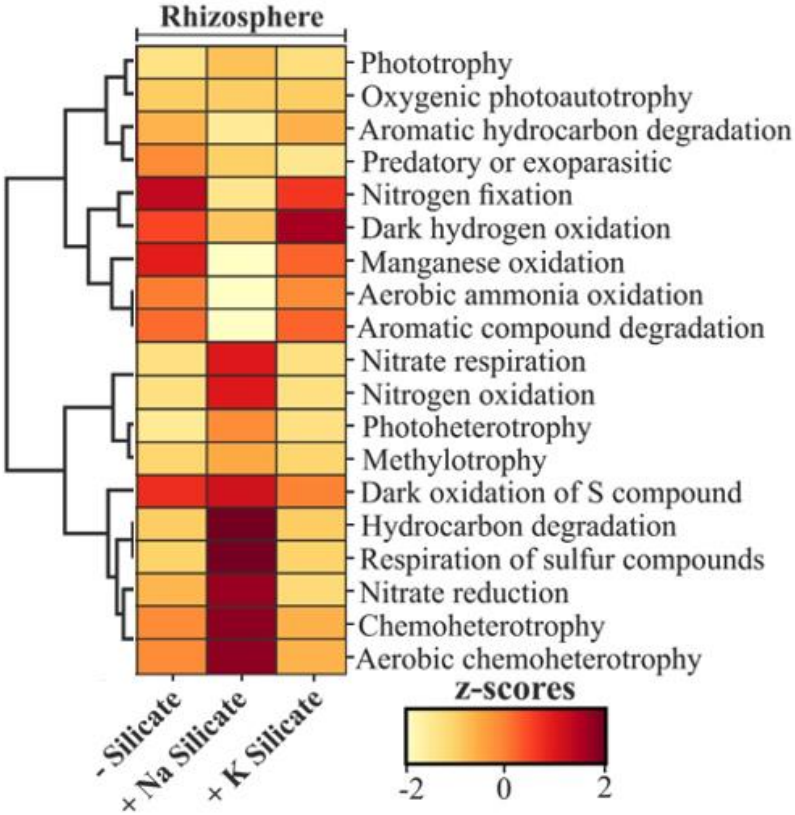


Figure 5. Abundance of the predicted putative microbial functions in the rhizosphere of sugarcane with the application of two different sources of silicon (K- and Na-silicate).

The functional prediction was based on the 16S rRNA genes using the FAPROTAX database. The color key relates the heatmap colors to the standard score (z-score), i.e., the deviation from row mean in units of standard deviation above or below the mean.

4. Discussion

The rhizosphere microbial communities are key points to improving plant growth and productivity, and the plant-microbes interaction is extremely sensitive to changes promoted by agricultural practices, such as the application of chemical fertilizers (Wang et al., 2018; Liu et al., 2020; Leite et al., 2023). Our study aimed to assess the impact of two different sources of silicon, K- and Na-silicate, on the prokaryotic community in both the bulk soil and rhizosphere of sugarcane. As anticipated, the comparison between the two environments revealed significant differences. This can be attributed to the influence of root exudation in the rhizosphere, which affects the recruitment and composition of specific microbial groups (Vieira et al., 2019; Mendes et al., 2019; Zhao et al., 2020).

Previous studies have reported that Si can modulate the root exudation profile which contributes to driving the prokaryotic microbial community (Lin et al., 2020; Song et al., 2021). In the rhizosphere, the application of Si resulted in a shift in the prokaryotic community compared to the control without Si. This observation agrees with A recent study by Leite et al. (2023), which reported a similar effect on the bacterial community in the rhizosphere of sugarcane after the application of Si. Interestingly, the application of different Si sources resulted in a significant shift in the structure and composition of the prokaryotic community in both bulk soil and rhizosphere. This confirms our initial hypothesis that different Si sources (Na- and K-silicate) could promote distinct responses of the microbial community in the rhizosphere of sugarcane.

The application of Na-silicate had a more pronounced effect on the prokaryotic community than K-silicate. Specifically, Na-silicate caused a significant shift in the community structure compared to K-silicate. In addition, the prokaryotic community in the rhizosphere exhibited a different structure following the application of Na-silicate. This could be attributed to the composition of this fertilizer, which contains peptides and amino acids that facilitate Si absorption (Botta et al., 2011). These compounds

also serve as biological sources of carbon and nitrogen for microbes (Broughton et al., 2015) and are known to influence root growth, microbial colonization, symbiotic interactions, and pathogenesis in the rhizosphere (Moe, 2013). Therefore, the presence of peptides and amino acids in Na-silicate likely contributed to the change in the prokaryotic community structure, particularly in the rhizosphere.

In general, the prokaryotic community in both bulk soil and rhizosphere was predominantly composed of Proteobacteria, Actinobacteria, and Firmicutes, which are commonly found in soil and rhizosphere of sugarcane (Chandra et al., 2021; Deng et al., 2021; Val-Moraes et al., 2016). For instance, Chandra et al. (2021) observed a higher abundance of Actinobacteria and Proteobacteria in the rhizosphere of newly planted sugarcane compared to samples from ratoon. Our study did not detect any significant differences in the relative abundance of the Actinobacteria and Firmicutes, indicating that these phyla are widely distributed and adaptable to different conditions. This finding is particularly important since Actinobacteria and Firmicutes are known to act as plant growth promoters and nitrogen fixers, suggesting their positive role in promoting plant health (Yadav et al., 2018; Yurgel et al., 2022). However, the composition of the prokaryotic microbial community changed according to the different sources of Si. Specifically, the application of Na-silicate led to an increase in the relative abundance of Proteobacteria and Bacteriodetes, both of which are associated with plant growth promotion in sugarcane (Deng et al., 2021; Teheran-Sierra et al., 2021). This finding is consistent with previous research by Tian et al. (2014), who reported a higher predominance of Proteobacteria in bulk soil and rhizosphere of cucumber after the application of Na-silicate. As reported above, the presence of amino acids and peptides in the Na-silicate may have influenced the higher relative abundance of Proteobacteria and Bacteroidetes. Indeed, recent studies have reported that the addition of amino acids increased the relative abundance of Proteobacteria and Bacteroidetes, mainly in the rhizosphere (Tan et al., 2023; Wen et al., 2022). In contrast, the application of K-silicate promoted an enrichment of Chloroflexi and Acidobacteriota. This could be attributed to the fact that K, a nutrient that favors the growth of Chloroflexi (Jin et al., 2022), was present in the K-silicate. This feature is important since Chloroflexi bring positive benefits to sugarcane in tropical soils and can grow in drought conditions, triggering physiological functions and conferring

greater tolerance to water stress (DeBruyn et al., 2011). Therefore, the enrichment of Chloroflexi due to the application of K-silicate is an important feature, as it can potentially enhance the productivity and resilience of sugarcane crops.

Regardless of the source of Si, the application of Si resulted in the enrichment of specific microbial families that differed from those found in Si-unfertilized bulk soil and rhizosphere. This is consistent with previous studies that have demonstrated distinct microbial enrichment with Si application (Deng et al., 2021; Leite et al., 2023). Notably, the application of different Si sources resulted in the enrichment of different microbial groups, with Na-silicate exhibiting the highest level of enrichment. Once again, the composition of Na-silicate containing peptides and amino acids can contribute to enriching different microbial groups, mainly in the rhizosphere (Knief et al., 2012; Wang et al., 2021). These findings suggest that the choice of Si source can have a significant impact on the composition of microbial communities in the soil, which may have important implications for plant growth and health.

To assess the impact of Si sources on microbial niche occupancy, we analyzed the distribution of generalist and specialist species. In general, the proportion of generalists was found to be higher than specialists, indicating that a greater percentage of microbial species can adapt to different habitats or conditions (Xu et al., 2020). Interestingly, the highest proportion of generalists was observed following the application of K-silicate, which suggests a greater similarity in chemical properties between environments, i.e., this specific source of exogenous Si did not influence major changes in soil and rhizosphere. A comparison of Si-unfertilized treatments with Na-silicate revealed a greater differentiation in both soil and rhizosphere environments, as evidenced by the relatively smaller proportion of generalist microbes. This result suggests that Na-silicate, which contains peptides and amino acids, provides a distinct condition that favors the growth of specialist microorganisms. Our niche analysis also revealed that when different exogenous silicon sources were compared, there was a higher proportion of generalist microbes in both reservoirs (65.4% - rhizosphere; 60.6% - bulk soil), indicating that these sources share a majority of bacterial communities. These results suggest that the choice of Si source can have a significant impact on microbial community composition, with Na-silicate promoting the growth of specialist microbes and other sources promoting more generalist communities. Further research

is needed to fully understand the implications of these findings for soil health and plant growth.

Finally, we used the FAPROTAX database to predict relevant potential functions of the microbial communities in our treatments. The information gathered from functional annotations of cultivated representatives is utilized to map prokaryotic taxa to potential functions in this database (Louca et al., 2016; Merloti et al., 2019). The results from our FAPROTAX analysis suggest that the choice of Si source can have a significant impact on potential microbial functions in the rhizosphere. Specifically, our analysis revealed that the application of Na-silicate led to changes in several predicted functions, including an increase in microbial groups related to hydrocarbon degradation, respiration of sulfur compounds, nitrate reduction, and chemoheterotrophy. These changes suggest that Na-silicate may promote the growth of microbes with important roles in nutrient cycling and environmental remediation. On the other hand, the application of K-silicate increased microbial groups related to dark hydrogen oxidation, which may have implications for the efficiency of carbon cycling and energy transfer in the soil ecosystem (Yan et al., 2018). Overall, these findings suggest that the choice of Si source can have important implications for soil health and ecosystem functioning, and further research is needed to fully understand the mechanisms underlying these effects.

5. Conclusion

In this study, we aimed to assess the impact of K- and Na-silicate on the prokaryotic community in the bulk soil and rhizosphere of sugarcane. Our results demonstrated that Si sources induced significant shifts in the prokaryotic community in both environments. The application of Na-silicate had a more significant effect on the prokaryotic community structure than K-silicate, increasing the relative abundance of Proteobacteria and Bacteroidetes in the rhizosphere, while the application of K-silicate enriched the relative abundance of Chloroflexi and Acidobacteriota in the bulk soil. Also, the silicon application affected the niche occupancy, increasing the proportion of generalist microbes, with consequences on the potential functions performed by these microorganisms. Our findings highlight the importance of understanding the impact of different agricultural practices on the microbial community

structure in the rhizosphere, which is essential for improving plant growth and productivity. Further studies are necessary to investigate the potential of different Si sources to modulate the microbial community's composition and function, leading to an improved understanding of their contribution to plant health and productivity. In conclusion, our study indicates that the application of Na- and K-silicate in the rhizosphere of sugarcane can promote distinct responses of the microbial community, which can have positive effects on plant growth and productivity.

References

BAKHAT, H. F.; BIBI, N.; ZIA, Z.; ABBAS, S.; HAMMAD, H. M.; FAHAD, S.; ASHRAFC M. R.; SHAHA, G. M.; RABBANI, F.; SAEED, S. Silicon mitigates biotic stresses in crop plants: A review. **Crop Protection**, v.104, p. 21–34, 2018.

BENJAMINI, Y.; HOCHBERG, Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. **Journal of the Royal Statistical Society: Series B (Methodological)**, v.57, n.1, p. 289–300, 1995.

BOTTA, A.; SIERRAS, N.; MARÍN, C.; CARRIÓN, M.; PIÑOL, R. Powdery mildew protection with Armurox: an improved strategy for silicon application. **Journal of Agricultural Science and Technology**, v.11, p. 1032-1039, 2011.

BROUGHTON, R.C.I.; NEWSHAM, K.K.; HILL, P.W.; STOTT, A.; JONES, D.L. Differential acquisition of amino acid and peptide enantiomers within the soil microbial community and its implications for carbon and nitrogen cycling in soil. **Soil Biology and Biochemistry**, v.88, p. 83-89, 2015.

CAMARGO, M.S.; BEZERRA, B. K. L.; VITTI, A.C.; SILVA, M. A.; OLIVEIRA, A.L. Silicon fertilization reduces the deleterious effects of water deficit in sugarcane. **Journal of Soil Science and Plant Nutrition**, v.17, n.1, p. 99-111, 2017.

CAPORASO, J. G.; LAUBER, C. L.; WALTERS, W. A.; BERG-LYONS, D.; LOZUPONE, C. A.; TURNBAUGH, P. J.; FIERER, N.; KNIGHT, R. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. **Proceedings of the National Academy of Sciences**, v. 108, p. 4516 – 4522, 2011.

CHANDRA, A.; GAUR, V.; TRIPATHI, P. Microbiome analysis of rhizospheres of plant and winter-initiated ratoon crops of sugarcane grown in sub-tropical India: utility to improve ratoon crop productivity. **3 Biotech**, v. 11, n. 1, 2021.

DEBRUYN J. M.; NIXON L. T.; FAWAZ M. N.; JOHNSON A. M.; Radosevich, M. Global biogeography and quantitative seasonal dynamics of Gemmatimonadetes in soil. Appl. **Environmental Microbiology**, v. 77, p. 6295–6300, 2011.

DENG, Q.; YU, T.; ZENG, Z.; ASHRAF, U.; SHI, Q.; HUANG, S.; LIAN, T.; CHEN, J.; MUZAFFAR, W.; SHEN, W. Silicon application modulates the growth, rhizosphere soil

characteristics, and bacterial community structure in sugarcane. **Frontiers in Plant Science**, v.12, n.710139, 2021.

FRAZÃO, J. J.; PRADO, R. M.; SOUZA JÚNIOR, J. P.; ROSSATTO, D. R. Silicon changes C:N:P stoichiometry of sugarcane and its consequences for photosynthesis, biomass partitioning and plant growth. **Scientific Reports**, v.10, n.1, p. 1-10, 2020.

GASCHO, G. J. Chapter 12 Silicon sources for agriculture. **Studies in Plant Science**, v. 8, 2001, p. 197-207, 2001.

JIN, Q.; ZHANG, Y.; WANG, Q.; LI, M.; SUN, H.; LIU, N.; ZHANG, L.; ZHANG, Y.; LIU, Z. Effects of potassium fulvic acid and potassium humate on microbial biodiversity in bulk soil and rhizosphere soil of Panax ginseng. **Microbiological Research**, v. 254, 126914, 2022.

KNIEF, C.; DELMOTTE, N.; CHAFFRON, S.; STARK, M.; INNEREBNER, G.; WASSMANN, R.; VON MERING, C.; VORHOLT, J. A. Metaproteogenomic analysis of microbial communities in the phyllosphere and rhizosphere of rice. **The ISME Journal**, v. 6, p. 1378–1390, 2012.

KORNDÖRFER, G. H.; COELHO, N. M.; SNYDER, G. H.; MIZUTANII, C. T. Avaliação de métodos de extração de silício para solos cultivados com arroz de sequeiro. **Revista Brasileira de Ciência do Solo**, v. 23, n. 1, p.101-106, 1999.

KUZYAKOV, Y.; BLAGODATSKAYA, E. Microbial hotspots and hot moments in soil: Concept & review. **Soil Biology and Biochemistry**, v. 83, p. 184-199, 2015.

LEITE, M. R. L.; ALCÂNTARA NETO, F.; DUTRA, A. F.; MENDES, L. W.; ANTUNES, J. E. L.; MELO, V. M. M.; OLIVEIRA, F. A. S.; ROCHA, S. M. B.; PEREIRA, A. P. A.; PRADO, R. M.; ARAUJO, A. S. F. Silicon application influences the prokaryotic communities in the rhizosphere of sugarcane genotypes. **Applied Soil Ecology**, v. 187, 104818, 2023.

LIU, J.; MA, Q.; HUI, X.; RAN, J.; MA, Q.; WANG, X.; WANG, Z. Long-term high-P fertilizer input decreased the total bacterial diversity but not *phoD*-harboring bacteria in wheat rhizosphere soil with available-P deficiency. **Soil Biology and Biochemistry**, v. 149, 107918, 2020.

LOUCA, S.; PARFREY, L. W.; DOEBELI, M. Decoupling function and taxonomy in the global ocean microbiome. **Science**, v. 353, n. 6305, p. 1272-1277, 2016.

LUYCKX, M.; HAUSMAN, J. F.; LUTTS, S.; GUERRIERO, G. Silicon and plants: Current knowledge and technological perspectives. **Frontiers in Plant Science**, v. 8, 2017.

MENDES, L. W.; CHAVES, M. G.; FONSECA, M. C.; MENDES, R.; RAAIJMAKERS, J. M., TSAI, S. M. Resistance breeding of common bean shapes the physiology of the rhizosphere microbiome. **Frontiers in Microbiology**, v.10:3389, 2019

MENDES, L. W.; MENDES, R.; RAAIJMAKERS, J. M.; TSAI, S. M. Breeding for soil-borne pathogen resistance impacts active rhizosphere microbiome of common bean. **ISME Journal**, v.12, p. 3038–3042, 2018.

MERLOTI, L. F.; MENDES, L. W.; PEDRINHO, A.; SOUZA, L. F.; FERRARI, B. M.; TSAI, S. M. Forest-to-agriculture conversion in Amazon drives soil microbial communities and N-cycle. **Soil Biology and Biochemistry**, v.137:107567, 2019.

MOE, L. A. Amino acids in the rhizosphere: From plants to microbes. **American Journal of Botany** v. 100, n. 9, 1692-705, 2013.

OLIVEIRA FILHO, A. S. B.; PRADO, R. M.; TEIXEIRA, G. C. M.; ROCHA, A. M. S.; SOUZA JUNIOR, J. P.; CÁSSIA PICCOLO, M.; ROCHA, J. R. Silicon attenuates the effects of water deficit in sugarcane by modifying physiological aspects and C:N:P stoichiometry and its use efficiency. **Agricultural Water Management**, v. 255, 107006, 2021.

PARKS, D. H.; BEIKO, R. G. Identifying biologically relevant differences between metagenomic communities. **Bioinformatics**, v. 26, p. 715–721, 2010.

PARKS, D. H.; TYSON, G. W.; HUGENHOLTZ, P.; BEIKO, R. G. STAMP: Statistical analysis of taxonomic and functional profiles. **Bioinformatics**, v. 30, p. 3123–3124, 2014.

PATTNAIK, S.; MOHAPATRA, B.; GUPTA, A. Plant growth-promoting microbe mediated uptake of essential nutrients (Fe, P, K) for crop stress management: microbe–soil–plant continuum. **Frontiers in Agronomy**, v. 3, 689972, 2021.

PEDRINHO, A.; MENDES, L. W.; MERLOTI, L. F.; ANDREOTE, F. D.; TSAI, S. M. The natural recovery of soil microbial community and nitrogen functions after pasture abandonment in the Amazon region. **FEMS Microbiology Ecology**, v. 96, p. 1–12, 2020.

PÉREZ-JARAMILLO, J. E.; HOLLANDER, M.; RAMÍREZ, C. A.; MENDES, R.; RAAIJMAKERS, J. M.; CARRIÓN, V. J. Deciphering rhizosphere microbiome assembly of wild and modern common bean (*Phaseolus vulgaris*) in native and agricultural soils from Colombia. **Microbiome**, v. 7, p. 1–16, 2019.

PERTILE, M.; SOUSA, R. M. S.; MENDES, L. W.; ANTUNES, J. E. L.; OLIVEIRA, L. M. S.; ARAUJO, F. F.; MELO, V. M. M.; ARAUJO, A. S. F. Response of soil bacterial communities to the application of the herbicides imazethapyr and flumyzin. **European Journal of Soil Biology**, v. 102, 103252, 2021.

QUAST, C.; PRUESSE, E.; YILMAZ, P.; GERKEN, J.; SCHWEER, T.; YARZA, P.; PEPLIES, J.; GLÖCKNER, F.O. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. **Nucleic Acids Research**, v. 41, p. 590–596, 2013.

RAIJ, B.; ANDRADE, J. C.; CANTARELLA, H.; QUAGIO, J. A. 2001. Análise química para avaliação da fertilidade de solos tropicais. Campinas, Instituto Agronômico de Campinas, 285p.

ROCHA, J. R.; PRADO, R. M.; PICCOLO, M. C. New outcomes on how silicon enables the cultivation of Panicum maximum in soil with water restriction. **Scientific Reports**, v. 12, n. 1897, 2022.

RODRIGUES, F. A.; DUARTE, H. S. S.; DOMICIANO, G. P.; SOUZA, C. A.; KORNDRFER, G. H.; ZAMBOLIM, L. Foliar application of potassium silicate reduces the intensity of soybean rust. **Australasian Plant Pathology**, v. 38, p. 366–372, 2009.

SAFARI, S.; SOLEIMANI, M. J.; ZAFARI, D. Effects of silicon pretreatment on the activities of defense-related enzymes in cucumber inoculated with *Fusarium oxysporum*. **Advances in Environmental Biology**, v. 6, n. 12, p. 4001-4007, 2012.

SANTOS, L. C. N.; TEIXEIRA, G. C. M.; PRADO, R. M.; ROCHA, A. M. S.; PINTO, R. C. S. Response of pre-sprouted sugarcane seedlings to foliar spraying of potassium silicate, sodium and potassium silicate, nanosilica and monosilicic acid. **Sugar Tech**, v. 22, p. 733-781, 2020.

SILVEIRA SARTORI SILVA, M.R., PEREIRA DE CASTRO, A., KRÜGER, R.H., BUSTAMANTE, M. Soil bacterial communities in the Brazilian Cerrado: Response to vegetation type and management. **Acta Oecologica**, v. 100, 103463, 2019.

SONG, A.; LI, Z.; WANG, E.; XU, D.; WANG, S.; BI, J.; FAN, F. Supplying silicon alters microbial community and reduces soil cadmium bioavailability to promote health wheat growth and yield. **Science Total Environment**, v. 796, 148797, 2021.

TAN, Y.; WANG, J.; HE, Y.; YU, X.; CHEN, S.; PENTTINEN, P.; LIU, S.; YANG, Y.; ZHAO, K.; ZOU, L. Organic Fertilizers Shape Soil Microbial Communities and Increase Soil Amino Acid Metabolites Content in a Blueberry Orchard. **Microbial Ecology**, v. 85, n. 1, p. 232-246, 2023.

TEHERAN-SIERRA, L. G.; FUNNICELLI, M. I. G.; CARVALHO, L. A. L.; FERRO, M. I. T.; SOARES, M. A.; PINHEIRO, D. G. Bacterial communities associated with sugarcane under different agricultural management exhibit a diversity of plant growth-promoting traits and evidence of synergistic effect. **Microbiological Research**, v. 247, 126729, 2021.

TIAN, Y.; GAO, L. Bacterial diversity in the rhizosphere of cucumbers grown in soils covering a wide range of cucumber cropping histories and environmental conditions. **Microbial Ecology**, v. 68, p. 794–806, 2014.

VAL-MORAES, S.P.; MACEDO, H.S.; KISHI, L.T.; PEREIRA, R. M.; NAVARRETE, A. A.; MENDES, L. W.; FIGUEIREDO, E. B.; LA SCALA, N. JR., TSAI, S. M.; MACEDO, L. E. G., ALVES, L. M. Liming in the sugarcane burnt system and the green harvest

practice affect soil bacterial community in northeastern São Paulo, Brazil. **Antonie van Leeuwenhoek**, v. 109, n. 12, p. 1643-1654, 2016.

VERMA, K. K.; LI, D. M.; SINGH, M.; RAJPUT, V. D.; MALVIYA, M. K.; MINKINA, T.; SINGH, R. K.; SINGH, P.; SONG, X. P.; LI, Y. R. Interactive role of silicon and plant–rhizobacteria mitigating abiotic stresses: A new approach for sustainable agriculture and climate change. **Plants**, v. 9, n. 9:1055, 2020.

VIEIRA, S., SIKORSKI, J., DIETZ, S.; HERZ, K.; SCHRUMPF, M.; BRUELHEIDE, H.; SCHEEL, D.; FRIEDRICH, M.; OVERMANN, J. Drivers of the composition of active rhizosphere bacterial communities in temperate grasslands. **The ISME Journal**, v. 14, n. 10, p. 463–475, 2019.

WANG, B.; CHU, C.; WEI, H.; ZHANG, L.; AHMAD, Z.; WU, S.; XIE, B. Ameliorative effects of silicon fertilizer on soil bacterial community and pakchoi (*Brassica chinensis L*.) grown on soil contaminated with multiple heavy metals. **Environmental Pollution**, v. 267, p. 1-10, 2020.

WANG, J.; LIU, L.; GAO, X.; HAO, J.; WANG, M. Elucidating the effect of biofertilizers on bacterial diversity in maize rhizosphere soil. **PLoS One**, v.16, n. 4, e0249834, 2021.

WANG, Q.; JIANG, X.; GUAN, D.; WEI, D.; ZHAO, B.; MA, M.; CHEN, S.; LI, L.; CAO, F.; LI, J. Long-term fertilization changes bacterial diversity and bacterial communities in the maize rhizosphere of Chinese Mollisols. **Applied Soil Ecology**, v. 125, p. 88-96, 2018.

WEN, T.; YU, G. H.; HONG, W. D.; YUAN, J.; NIU, G. Q.; XIE, P. H.; SUN, F. S.; GUO, L. D.; KUZYAKOV, Y.; SHEN, Q. R. Root exudate chemistry affects soil carbon mobilization via microbial community reassembly. **Fundamental Research**, v. 2, n. 5, p. 697-707, 2022.

XU, Q.; LING, N.; CHEN, H.; DUAN, Y.; WANG, S.; SHEN, Q.; VANDENKOORNHUYSE P. Long-term chemical-only fertilization induces a diversity decline and deep selection on the soil bacteria. **Msystems**, v.5, n.4, 2020.

YADAV, A. N.; VERMA, P.; KUMAR, S.; KUMAR, V.; KUMAR, M.; SUGITHA, T. C. K.; SINGH, B. P.; SAXENA, A. K.; DHALIWAL, H. S. Actinobacteria from rhizosphere: molecular diversity, distributions, and potential biotechnological applications. In: Singh, B. P., Gupta, V. K., Pasari, A. K. **(Ed.)** New and Future Developments in Microbial Biotechnology and Bioengineering. Amsterdam: Elsevier (2018). p. 13–41.

YAN, Z.; JOSHI, P.; GORSKI, C. A.; FERRY, J. G. A biochemical framework for anaerobic oxidation of methane driven by Fe (III)-dependent respiration. **Nature Communications,** v.9, n.1642, 2018.

YURGEL, S. N.; AJEETHAN, N.; SMERTENKO, A. Response of plant-associated microbiome to plant root colonization by exogenous bacterial endophyte in perennial crops. **Frontiers in Microbiology**, v.13, 863946, 2022.

ZHANG, W., YU, X., LI, M., LANG, D., ZHANG, X., XIE, Z. Silicon promotes growth and root yield of Glycyrrhiza uralensis under salt and drought stresses through enhancing osmotic adjustment and regulating antioxidant metabolism, **Crop Protection**, v. 107, p. 1-11, 2018.

ZHAO, M.; ZHAO, J.; YUAN, J.; HALE, L.; WEN, T.; HUANG, Q.; VIVANCO, J. M.; JIZHONG ZHOU, J.; KOWALCHUK, G. A.; SHEN, Q. Root exudates drive soil-microbe-nutrient feedbacks in response to plant growth. **Plant, Cell & Environment**, v.44, p. 613–628, 2020.

Anexos

(CAPÍTULO I)

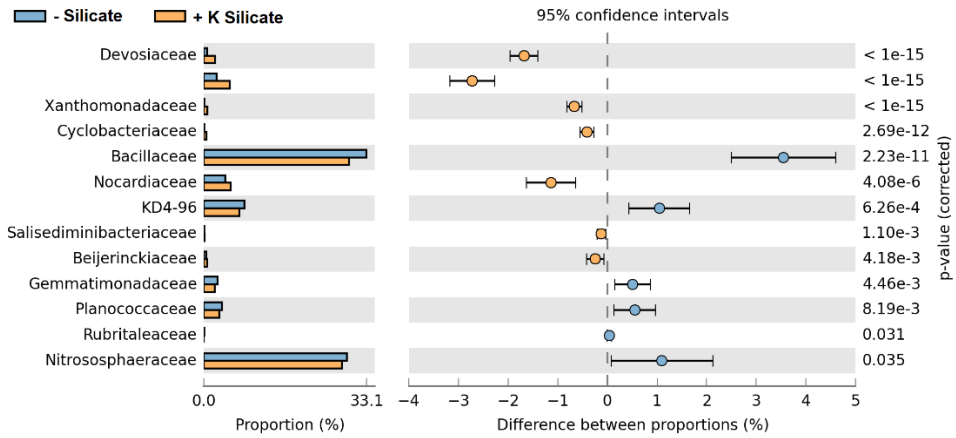
Table 3. Soil chemical properties (0-20 cm) at 120 days (Supplementary Table S1)

	Sugarcane plant	Sugarcane ratoon
	Depth (m) 0 – 0.20	
Soil pH	6.7	6.3
OM (g Kg^{-1})	11.0	14.0
P (mg dm^{-3})	57.0	32.0
S (mg dm^{-3})	10.0	8.0
Si (mg dm^{-3})	6.0	10.0
Ca (mg dm^{-3})	2.7	1.8
Mg (mg dm^{-3})	1.0	0.8
K (mg dm^{-3})	0.2	0.4
Al^{3+} ($cmol_c$ dm^{-3})	0.02	0.0
H^+ ($cmol_c$ dm^{-3})	1.5	0.9
SB ($cmol_c$ dm^{-3})	4.0	3.0
CEC ($cmol_c$ dm^{-3})	5.5	3.8
V (%)	73.2	77.1
m (%)	0.5	0.0

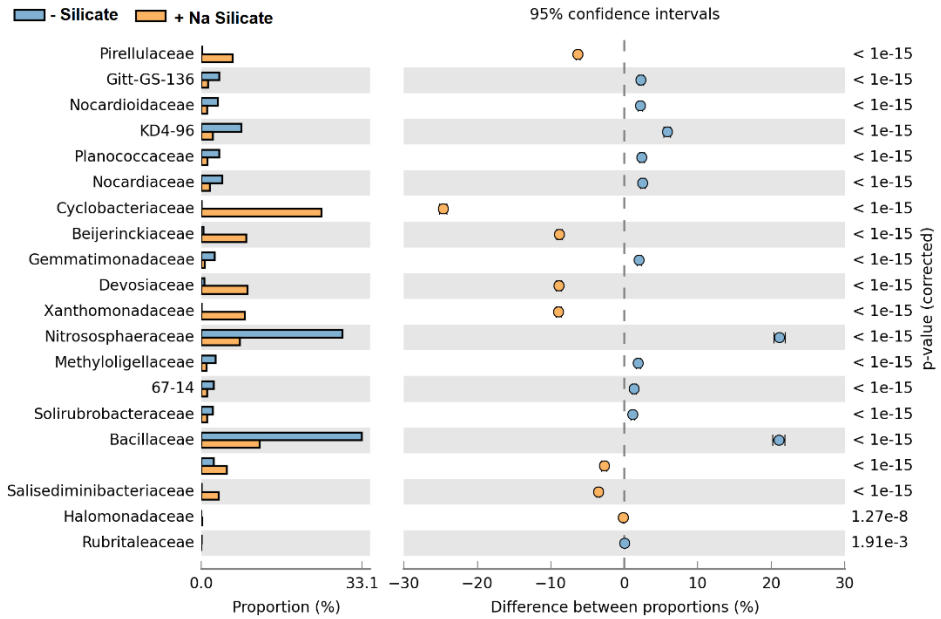
OM: organic matter, SB: sum of basis, CEC: cation exchange capacity, V (%): basis saturation, m (%): Al^{3+} saturation

(CAPÍTULO II)

(A)

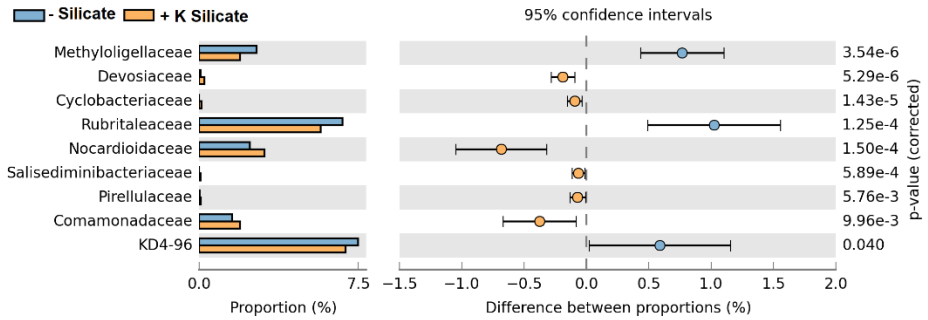


(B)

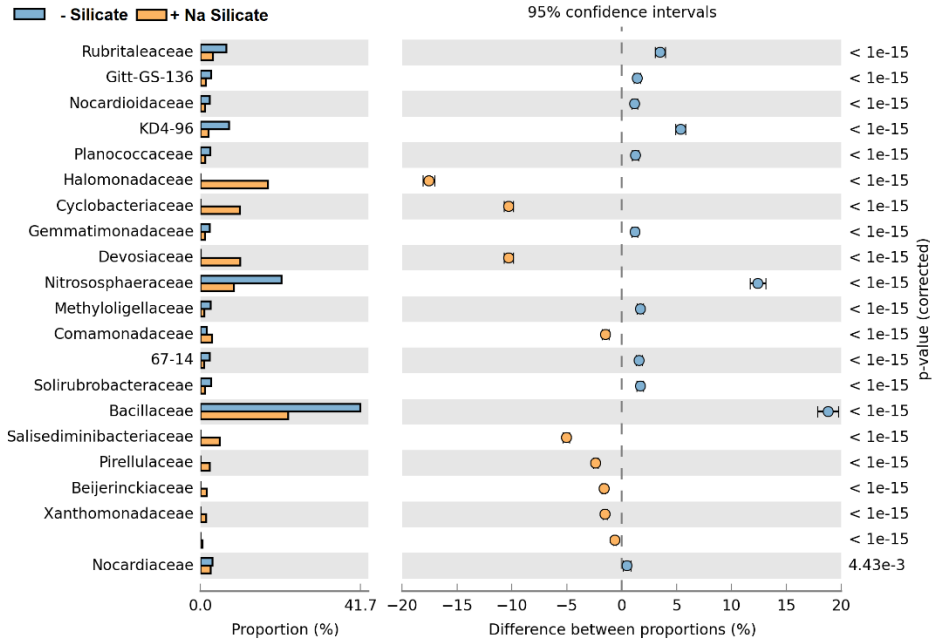


Supplementary Figure S1. Scatter plots showing the differential abundance of microbial families between no Si application and sources of K-silicate (A) and Na-silicate (B) in bulksoil. P-values were calculated using a two-sided Tukey- Kramer test, and corrections were made using Benjamini-Hochberg FDR.

(A)



(B)



Supplementary Figure S2. Scatter plots showing the differential abundance of microbial families between no Si application and sources of K-silicate (A) and Na-silicate (B) in rhizosphere. P-values were calculated using a two-sided Tukey- Kramer test, and corrections were made using Benjamini-Hochberg FDR.