University of São Paulo
"Luiz de Queiroz" College of Agriculture

Arbuscular mycorrhizal fungi and rhizobacteria attenuating drought stress in maize plants

Antonio Marcos Miranda Silva

Thesis presented to obtain the degree of Doctor in Science. Area: Soil and Plant Nutrition

Piracicaba
2023

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Dados Internacionais de Catalogação na Publicação
DIVISÃO DE BIBLIOTECA – DIBD/ESALQ/USP

Silva, Antonio Marcos Miranda

Arbuscular mycorrhizal fungi and rhizobacteria attenuating drought stress in maize plants / Antonio Marcos Miranda Silva. - - Piracicaba, 2023.

107 p.

Tese (Doutorado) - - USP / Escola Superior de Agricultura “Luiz de Queiroz”.

1. Simbiose micorrízica 2. Inoculação bacteriana 3. Déficit hídrico 4. Interações solo-planta 5. Caroá 6. Planta da ressurreição 7. *Tripogon spicatus* I. Título

I dedicate my thesis to my beloved grandfather, **Francisco Gabriel de Miranda**, affectionately known as "Pai Chico" (*in memoriam*), whose gentle teachings on the virtues of kindness and perseverance have left an indelible mark on my character and life aspirations.

ACKNOWLEDGEMENTS

I would like to express that this thesis would not have been possible without the support of a large number of people and institutions. My profound gratitude goes out to all those directly and indirectly involved in my educational journey thus far.

Firstly, I would like to thank God for guiding me and allowing me to be here participating in different human experiences. This life is so short, and being kind and wise is the main virtue anyone can have.

Subsequently, I would like to express my gratitude to the following institutions:

- The Luiz de Queiroz College of Agriculture, University of São Paulo (Esalq/USP).
- The Soil Science Department (Esalq/USP) and the Postgraduate Program in Soil and Plant Nutrition
- The São Paulo Research Foundation (FAPESP) for financially supporting this research through #2019/13436-8 and #2020/12751-4 projects.
- The Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for providing the inicial financial support (Finance Code 001).
- The Embrapa Semiárido (Petrolina, Pernambuco, Brazil) and Embrapa Meio Ambiente (Jaguariúna, São Paulo, Brazil).
- Bangor University, School of Natural Sciences, Environment Centre of Wales, (ECW).

I owe enormous thanks and gratitude to my advisor Elke Cardoso, who, in addition to guiding me professionally over the last seven years, has given me emotional support as a friend. You have inspired and presented me with fantastic opportunities, thank you for helping me grow and develop as a researcher.

Likewise, I would like to thank Davey Jones and Dave Chadwick for all their support and guidance during my internship at Bangor University, Wales – UK. It was a pleasant experience. In addition, it is worth mentioning the support of the technicians, Sarah and Jonathan Roberts.

I would like to thank Gileno, Henrique Feiler, Paulo Ivan and Saulo Aidar for the sampling support in Petrolina, Pernambuco.

I would like to thank my friends from the Soil Microbiology Laboratory, Maiele, Bruna Bacco, Henrique, German, Marcos, Arthur, Mauricio, Tâmara, Carol Rosa, Carol Medrado, Cátia Simon, Victor Lucas, Filipe, Yasmin, Izabelly, Danilo, Hiroko, Caio, Aline, Felipe, Nariane, Alice,

Vinicius and Rafael. Special thanks to Denise Mescolotti and Fernando Baldesin for all technical support during the laboratory analyses.

Friends from my internship at Bangor University in the UK, Xue, Hima, Lis, Gisella, Usman, Alejandra, Jaqueline Goés, and the special Samuel Jones, who has captured my heart and committed to a lifetime of loving care.

I would like to thank Simone Cotta and Itamar Mello for all suggestion raised during my qualification exam.

I would like to thank my friends from Piracicaba, Allanna, Bruna Arruda, Fabio, Aline, Lina, Melina, Luciane, Rony, Andrés, Ana Luisa, Carla, Paulo Pedro, Tamires, Thuanne, Sônia. Your support and friendship are very important to me.

Special thanks to my two mums (Gilvanete e Clarice), father (Lourivaldo, in memoriam) godfather (Osvaldo), brother (João Miranda), sisters (Marizélia e Maise), nephews and niece (Enzo, Ana Laura, João, Guilherme e Anthony) for your ceaseless and unconditional support and love in every aspect of life.

Finally, I would like to express my profound gratitude for the privilege of having each one of you as an integral part of my life's journey. I am truly blessed to have absorbed the collective wisdom of your experiences and perspectives, which have undoubtedly contributed to my growth and evolution as a person.

Thanks!

The role of the infinitely small is infinitely large

Louis Pasteur

CONTENTS

RESUMO

Fungos micorrízicos arbusculares e rizobactérias atenuando o estresse hídrico em plantas de milho

Os fungos micorrízicos arbusculares (FMA) e as rizobactérias promotoras de crescimento vegetal (RPCV) são essenciais para a saúde das plantas, aumentando sua tolerância a estresses bióticos e abióticos. Este estudo teve como objetivo investigar o papel de FMA e RPCV nativos, selecionados do Bioma Caatinga, um ambiente extremo, na mitigação dos efeitos da seca e na promoção do crescimento do milho (*Zea mays* L.). Três hipóteses foram testadas: (i) plantas adaptadas à Caatinga abrigam FMA e RPCV com potencial para serem usados como inoculantes na cultura do milho. (ii) a eficácia de um inóculo de FMA nativo obtido de um ambiente hostil varia de acordo com os níveis de seca, levando a mudanças nos parâmetros microbiológicos do solo e da planta relacionados ao ciclo de nutrientes e ao sistema antioxidante da planta. (iii) uma combinação de FMA e RPCV pode aumentar a absorção de ^{33}P em plantas de milho sob estresse hídrico do solo. Para testar a primeira hipótese, amostras de solo rizosférico de *Neoglaziovia variegata* e *Tripogonella spicata* foram coletadas no Bioma Caatinga, e a comunidade micorrízica foi caracterizada usando sequenciamento de alto rendimento do gene parcial 18S rRNA. Os resultados revelaram que a comunidade de fungos micorrízicos arbusculares na rizosfera de cada planta abrange uma composição, estrutura e modularidade únicas, que podem ajudá-las diferencialmente no ambiente hostil. Para a segunda hipótese, um experimento em casa de vegetação foi realizado, usando tratamentos que simularam seca severa (30% da capacidade de retenção de água [CRA]), seca moderada (50% da CRA) e nenhuma seca (80% da CRA). Os resultados mostraram que o melhor uso do inóculo de AMF variou de acordo com os níveis de seca, com melhor desempenho observado sob seca moderada devido ao aumento da biomassa da planta. Por fim, para testar a terceira hipótese, foi realizado um experimento de microcosmo para investigar o efeito da combinação de FMA (*Rhizophagus clarus*) e RPCV (*Bacillus* sp.) na absorção de ^{33}P em plantas de milho sob estresse hídrico do solo. Em geral, a facilitação do ^{33}P foi modulada pelo teor de água do solo, com a dupla inoculação e as rizobactérias sozinhas sendo mais eficientes sob seca severa, enquanto sob condições de seca moderada, as micorrizas sozinhas foram mais eficazes na promoção da absorção de ^{33}P pela planta. Em conclusão, este estudo destaca o potencial de usar FMA e RPCV nativos como uma estratégia combinada para promover o crescimento e desenvolvimento de plantas sob condições de seca. No entanto, mais pesquisas são necessárias para explorar seu potencial na melhoria do rendimento das culturas em condições não controladas

Palavras-chave: Simbiose micorrízica, Inoculação bacteriana, Déficit hídrico, Interações solo-planta, Caroá, Planta da ressurreição, *Tripogon spicatus*

ABSTRACT

Arbuscular mycorrhizal fungi and rhizobacteria attenuating drought stress in maize plants

Arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) are essential for enhancing plant health by increasing their tolerance to biotic and abiotic stresses. This study aimed to investigate the role of native AMF and PGPR screened from the Caatinga Biome, an extreme environment, in mitigating drought effects and promoting maize (*Zea mays* L.) growth. Three hypotheses were tested: (i) Caatinga-adapted plants harbour potential AMF and PGPR that can be screened and used as inoculants in maize crops; (ii) the effectiveness of a native AMF inoculum obtained from a harsh environment varies with drought levels, leading to changes in soil and plant microbiological parameters related to nutrient cycling and the plant antioxidant system; (iii) a combination of AMF and PGPR can enhance ^{33}P uptake in maize plants under soil water stress. To test the first hypothesis, soil rhizosphere samples from *Neoglaziovia variegata* and *Tripogonella spicata* were sampled in Caatinga Biome, and the mycorrhizal community was characterized using high-throughput sequencing of the partial 18S rRNA gene. The results revealed that the community of arbuscular mycorrhizal fungi in the rhizosphere of each plant encompasses a unique composition, structure, and modularity, which can differentially assist them in the hostile environment. For the second hypothesis, a greenhouse experiment was carried out, using treatments simulating severe drought (30 % of water-holding capacity [WHC]), moderate drought (50 % of WHC), and no drought (80 % of WHC). The results showed that the better use of the AMF inoculum varied according to drought levels, with better performance observed under moderate drought due to an increase in plant biomass. Finally, to test the third hypothesis, a microcosm experiment was conducted to investigate the effect of a combination of AMF (*Rhizophagus clarus*) and PGPR (*Bacillus* sp.) on ^{33}P uptake in maize plants under soil water stress. Overall, ^{33}P facilitation was modulated by soil water content, with dual-inoculation and rhizobacteria alone being more efficient under severe drought, whereas under moderate drought conditions, mycorrhizae alone were more effective in promoting plant ^{33}P uptake. In conclusion, this study highlights the potential of using AMF and PGPR as a combined strategy to promote plant growth and development under drought conditions. However, further research is needed to explore their potential for improving crop yields under non-controlled conditions.

Keywords: Mycorrhizal symbiosis, Bacterial inoculation, Water deficit, Soil-plant interactions, Caroá, Resurrection plant, *Tripogon spicatus*

1. INTRODUCTION

Amidst the growing effect of climate change, such as more frequent and severe droughts, the development of cutting-edge projects on how to overcome or mitigate these issues based on eco-friendly strategies is becoming increasingly critical (TWI2050, 2018; FAO, 2021). This is particularly significant since droughts have knock-on effects, for example on human welfare, as water deficits in the soil engender declines in crop yields, leading to impacts in soil health, food production, the plant nutritional status, and consequently, substantial economic losses and social threats (Stanke et al., 2013). The effects of drought on agriculture and human well-being may be worse in naturally water-deficient nations, as highlighted by Orimoloye et al. (2022).

On this basis, the use of microbial inoculants in agriculture has been a prominent strategy to assist plants during these abiotic stresses. While microbial assistance in overcoming biotic and abiotic stresses is well-established, the potential of indigenous soil microbes that have adapted to extreme conditions for this purpose remains largely unexplored, primarily because most of them are uncultured (Fierer, 2017; Steen et al., 2019). This is one of the reasons why the soil microbial community is often referred to as the "black box". Nevertheless, new ways to open up this box are emerging with the need to restore soil biodiversity, improving crop yields and plant nutritional status to reduce hunger and malnutrition (Sachs et al., 2019).

Since maize (*Zea mays* L.) is c recognized globally as one of the most important crops due to its multi-purpose (food and non-food use), it is essential to seek strategies to intensify maize production in a confounding scenario of climatic variability and consequent increased abiotic stresses (Shiferaw et al., 2011; Erenstein et al., 2022). For maize crops, inoculants based on *Azospirillum brasilense* strains have been increasingly used by Brazilian farmers over the last 13 years to increase plant growth and yield due to improved nutrient uptake and nitrogen fixation, resulting in up to 25 % reduction in N-fertiliser usage (Hungria et al., 2010; Marks et al., 2015; Fukami et al., 2016; Santos et al., 2021). Furthermore, co-inoculation of *A. brasilense* with rhizobia has been shown to promote growth parameters in soybean and common bean (Hungria et al., 2013). Nonetheless, there is a lack of scientific evidence concerning the efficacy of utilizing plant-promoting rhizobacteria co-inoculated with arbuscular mycorrhiza fungi (AMF) in different drought levels in soil, particularly when the microbes are derived from arid habitats.

The term arbuscular mycorrhiza refers to a symbiotic association between fungi and plant roots, from the terms "myco" (meaning fungus) and "rrhiza" (meaning root). In this mutualistic association, both partners, the fungus (also referred to as mycobiont) and the plant

(referred to as phytobiont) benefit, where the fungus receives carbon from the plant through photoassimilates, whilst the plant receives water and nutrients (Tedersso and Bahram, 2019). Arbuscular mycorrhiza is the most common type of mycorrhiza and is formed in a wide variety of host plants (more than 80 %) through an obligatory association with the plant, making it difficult to cultivate AMF in laboratories via plate growth. AMF belongs to the phylum Glomeromycota, which includes 4 orders, 11 families, 25 genera, and 334 species (Smith and Read, 2008; Redeck et al., 2013).

Recent findings reported by Santana et al. (2023) have shed light on the physiological response of maize plants when inoculated with AMF (*Rhizophagus clarus*, *Claroideoglomus etunicatum*, and the commercial inoculant ROOTELLA BR), under severe drought conditions (corresponding to 20% of water-holding capacity) and no-drought conditions (corresponding to 80% of water-holding capacity). The authors observed that plants inoculated with AMF exhibited higher water potential values and stomatal conductance, resulting in better growth than plants under no-drought conditions. Similarly, Oliveira et al. (2022) investigated the mechanisms by which AMF alleviates drought stress in soybean and found that the effect varies according to plant genotype, but overall, AMF enhances nutrient uptake, water potential, and water use efficiency in plants.

Furthermore, rhizobacteria (also known as plant growth-promoting rhizobacteria (PGPR)) play a critical role in enhancing plant tolerance to drought through various direct and indirect mechanisms. Direct mechanisms include the production of phytohormones (*e.g.*, abscisic acid (ABA), indole-3-acetic acid (AIA), and ethylene), which regulates stomatal closure, reducing water loss from plant tissues. Additionally, some bacteria are capable of synthesizing osmoprotectants, such as proline and trehalose, which help plants to maintain their cellular turgor pressure and cope with water scarcity. Indirect mechanisms include the promotion of plant growth and development by improving nutrient uptake and assimilation, the production of ACC-deaminase and volatile organic compounds (VOCs), and activation of the antioxidant system (enzymatic and non-enzymatic) (Glick, 2012). Numerous investigations have demonstrated that these mechanisms are responsible for enhancing plant tolerance to drought stress. For instance, inoculation with PGPR has been shown to improve drought tolerance in maize plants by increasing ABA production and activating the antioxidant system (Kuromori et al., 2018). Moreover, Araújo et al. (2023), working with a pool of PGPR from Caatinga Biome, found that the better performance of these bacteria varies depending on soil moisture content. Notably, *Streptomyces* sp. had a positive impact on maize growth under moderate drought conditions, while

Arthrobacter sp. exhibits a negative effect when maize plants are subjected to a drying-wetting cycle in the soil.

Overall, the main mechanisms underlying the beneficial effects of AMF and PGPR on maize plants during periods of drought stress can be broadly classified into four categories: (A) physiological and biochemical, (B) metabolomic, (C) morphological and anatomical, and (D) transcriptomic. **Figure 1** provides an overview of these primary mechanisms.

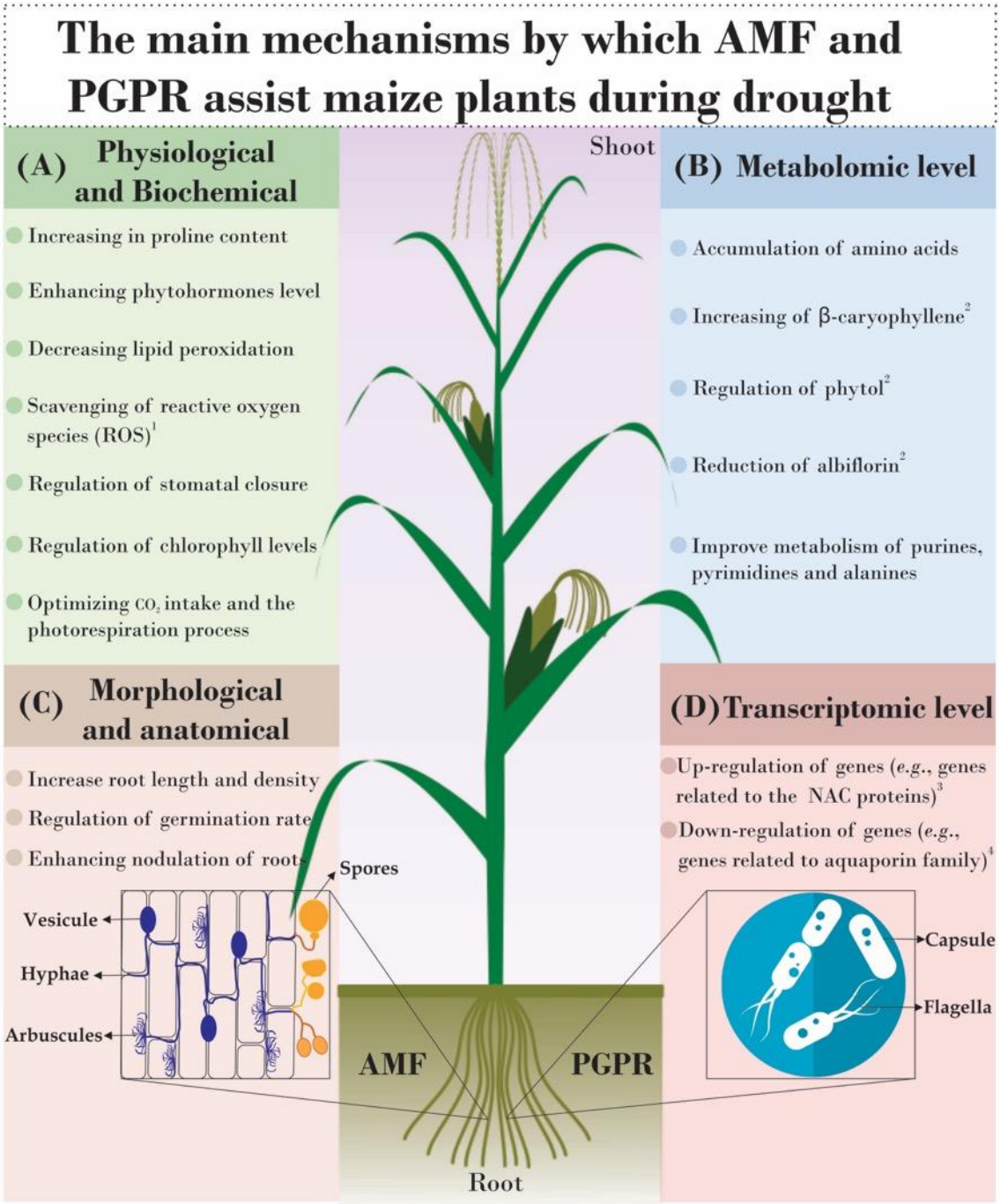

Figure 1. Four categories describe the main mechanisms behind the beneficial effects of arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) on maize (*Zea mays* L.) plants during drought stress. (A) physiological and biochemical, (B) metabolomic, (C) morphological and anatomical, and (D) transcriptomic. These mechanisms involve complex interactions between the plant and the microbial symbionts, resulting in changes at the molecular, cellular, and tissue levels that enhance the plant's ability to cope with water deficit conditions. By enhancing water uptake, regulating hormone levels, modulating gene expression, and promoting root growth, AMF and PGPR can alleviate the negative impact of drought stress on maize plants and promote their growth and productivity. [1]ROS are reactive molecules such as superoxide anion (O2−), hydrogen peroxide (H_2O_2), and hydroxyl radical (HO•) that are formed as natural byproducts of cellular metabolism but their excess can cause damage to cellular components. AMF and PGPR can increase the enzymes and antioxidant systems, which maintain their levels within a range that is optimal for cellular function (Carvalho, 2008; Sies et al., 2022). [2]β-caryophyllene, phytol and albiflorin belong to the family of volatile organic compounds known as terpenes, and their metabolism may be influenced by the specific association between AMF and the plant (Kaur and Suseela, 2020). [3]NAC proteins are one of the largest families of plant-specific transcription factors (TFs) required for drought tolerance mechanism (Zheng et al., 2009). [4]Aquaporins are a diverse and extensive family of proteins that function as membrane transporters (Chaumont et al., 2001; SkZ et al., 2018).

So far, the synergistic application of arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) derived from the Caatinga Biome, an extremely austere and largely neglected Brazilian ecosystem, has not been thoroughly investigated. Therefore, given the dearth of knowledge on this topic and the imperative to discover new reservoirs of these microorganisms, the present study was designed to rigorously address the following hypothesis: i) plants adapted to Caatinga Biome harbour potential AMF and PGPR that can be screened to use as inoculant in maize crop; ii) the effectiveness of a native AMF inoculum obtained from a harsh environment varies according to the drought levels, causing changes in soil and plant microbiological parameters related to nutrient cycling and the plant antioxidant system; iii) a combination of AMF and PGPR could enhance ^{33}P uptake in maize plants under soil water stress.

Therefore, the main aim of this thesis was to evaluate the potential of rhizosphere-dwelling AMF and PGPR, screened from an austere environmental niche, to ameliorate the effects of drought and stimulate maize growth. To achieve this objective, the thesis has been organized into three key chapters and a final remarks section. In the **first chapter**, the composition, structure, and modularity of the mycorrhizal community of the rhizosphere of *Neoglaziovia variegata* (commonly known as 'caroá') and *Tripogonella spicata* (known as the 'resurrection plant') were elucidated through high-throughput sequencing of the partial 18S rRNA gene, revealing valuable insights into the AMF community. In the **second chapter**, it is demonstrated that the use of the AMF inoculum from a harsh environment is able to increase maize biomass and change the response of soil attributes, especially under moderate drought. The **third chapter** demonstrates that, using specific strains of AMF and PGPR, inoculated plants exhibit superior performance in terms of ^{33}P uptake, particularly under drought stress, with dual-inoculation or PGPR inoculation exhibiting higher efficacy under severe drought, in contrast to AMF inoculation, which is more effective under moderate drought. Finally, the **final remarks** provide a summary of the key findings and underscore their significance in formulating potential strategies for mitigating the impacts of drought and enhancing maize yields in the foreseeable future.

In brief, the findings achieved through this thesis have been published and submitted as the following manuscripts:

1. **Silva AMM**, Feiler HP, Lacerda-Júnior GV, Fernandes-Júnior PI, Aidar ST, Araújo VLVP, Matteoli FP, Pereira APA, Melo IS, Cardoso EJBN. Arbuscular mycorrhizal fungi associated with the rhizosphere of an endemic terrestrial bromeliad and a grass in the Brazilian neotropical dry forest. Brazilian Journal of Microbiology (*Under review*).

2. **Silva AMM**, Feiler HP, Qi X, Araújo VLVP, Lacerda-Júnior GV, Fernandes-Júnior PI, Cardoso EJBN. Soil and plant microbiological responses in the presence of a pool of arbuscular mycorrhizal fungi under varying drought levels. Microorganisms (*Under review*).

3. **Silva AMM**, Jones D, Chadwick DR, Qi X, Cotta SR, Araújo VLVP, Matteoli FP, Lacerda-Júnior GV, Pereira APA, Fernandes-Júnior PI, Cardoso EJBN. Can arbuscular mycorrhizal fungi and rhizobacteria facilitate ^{33}P uptake in maize plants under water stress?. Microbiological Research, v. 271, 127350, 2023. https://doi.org/10.1016/j.micres.2023.127350

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2. ARBUSCULAR MYCORRHIZAL FUNGI ASSOCIATED WITH THE RHIZOSPHERE OF AN ENDEMIC TERRESTRIAL BROMELIAD AND A GRASS IN THE BRAZILIAN NEOTROPICAL DRY FOREST [1]

Abstract

Arbuscular mycorrhizal fungi form symbiotic associations with 80-90 % of all known plants, allowing the fungi to acquire plant-synthesized carbon, and confer an increased capacity for nutrient uptake by plants, improving tolerance to abiotic and biotic stresses. We aimed at characterizing the mycorrhizal community in the rhizosphere of *Neoglaziovia variegata* (so-called 'caroa') and *Tripogonella spicata* (so-called resurrection plant), using high-throughput sequencing of the partial 18S rRNA gene. Both plants are currently undergoing a bioprospecting program to find microbes with the potential of helping plants tolerate water stress. Sampling was carried out in the Caatinga biome, a neotropical dry forest, located in northeastern Brazil. Illumina MiSeq sequencing of 37 rhizosphere samples (19 for *N. variegata* and 18 for *T. spicata*) revealed a distinct mycorrhizal community between the studied plants. According to alpha diversity analyses, *T. spicata* showed the highest richness and diversity based on the Observed ASVs and the Shannon index, respectively. On the other hand, *N. variegata* showed higher modularity of the mycorrhizal network compared to *T. spicata*. The four most abundant genera found (higher than 10 %) were *Glomus*, *Gigaspora*, *Acaulospora*, and *Rhizophagus*, with *Glomus* being the most abundant in both plants. Nonetheless, *Gigaspora*, *Diversispora*, and *Ambispora* were found only in the rhizosphere of *N. variegata*, whilst *Rhizophagus*, *Paraglomus*, and *Archaeospora* were exclusive to the rhizosphere of *T. spicata*. Therefore, the community of arbuscular mycorrhizal fungi of the rhizosphere of each plant encompasses a unique composition, structure and modularity, which can differentially assist them in the hostile environment.

Keywords: environmental DNA sequencing, *Tripogon spicatus*, mycorrhizal symbiosis, Glomeromycota, Mucoromycota, Glomeromycotina

2.1. Introduction

Caatinga, the Neotropical dry forests, also referred to as seasonally dry tropical forests (SDTFs), are one of the most threatened tropical forests in the world, with deforestation being the main threat, especially in Brazil, which comprises most of them [1-3]. SDTFs cover extensive areas from Mexico (Central America) to Argentina (South America) and throughout the Caribbean [4].

The Brazilian Caatinga biome harbours the largest SDTFs, composed of a shrubland ecosystem that covers 844,453 km^2 and represents 10.1 % of the Brazilian territory [5]. According to Teixeira et al. [6], only 1.3 % of the Caatinga biome is really protected, and conservation actions are urgently needed, as the Caatinga has unique biodiversity patterns. The evolutionary history confined to this biome converged to its uniqueness, presenting plant species restricted to it [7]. Alongside, it is known that a host microbiome co-evolving with endemic species can help

[1] Manuscript submitted to Brazilian Journal of Microbiology

them survive in a harsh environment [8]. Therefore, the Caatinga biome is a screening hotspot for microbes that may be employed to mitigate abiotic stresses [9-12]. However, little is known about the diversity and community composition of arbuscular mycorrhizal fungi (AMF) associated with plants in dry forests, especially terrestrial bromeliads and resurrection grass, as revealed by our mini-review (Supplementary Note and Tables S1 and S2).

The mycorrhizal symbiosis plays a key role in maintaining plant growth and, compared to other known symbioses (e.g., nitrogen-fixing bacteria), it is the oldest, originating approximately 450 million years ago. AMF colonize about 80-90 % of all plant species, and only very few plant families cannot generate mycorrhiza in symbiosis with AMF, such as *Brassicaceae*, *Chenopodiaceae*, *Cyperaceae* and *Juncaceae* [13]. According to Spatafora et al. [14], AMF have been included in the phylum Mucoromycota and subphylum Glomeromycotina, while, for Tedersoo et al. [15], they have been included in a single phylum, the Glomeromycota, but this has been controversial for years and remains so [16].

In the root system of SDTFs, some investigations have shown the AMF associated with epiphytic bromeliads (belonging to the family *Bromeliaceae*), which is considered one of the most species-rich and ecologically important plant families in the neotropics [17-20]. However, there are no studies on AMF communities associated with the rhizosphere of the terrestrial bromeliad *Neoglaziovia variegata* (Arruda) Mez, endemic to the Caatinga biome. However, there are studies showing its gastroprotective, antibacterial and acaricidal potential [21-23]. Likewise, there are no studies investigating the AMF communities associated with the rhizosphere of *Tripogonella spicata* (Nees) P.M.Peterson & Romasch, the so-called resurrection plant. The term resurrection plant is due to its capacity to survive dehydration to an air-dried state for months, losing most of its cellular water, and quickly resume normal physiological activities after rehydration [24-26]. In addition, other plant species from the families *Myrothamnaceae*, *Selaginellaceae*, *Velloziaceae*, and *Scrophulariaceae* are equally known as resurrection plants [27]. Perhaps, the associated rhizosphere microbiota acts as the downstream agent modulating this upstream response.

Thus, this investigation has pioneered in revealing the arbuscular mycorrhizal fungi composition, structure and modularity of the rhizosphere of *N. variegata* and *T. spicata,* using high-throughput sequencing of the partial 18S rRNA gene. Therefore, our study represents a significant contribution in the mycorrhizal ecology, especially in studies with terrestrial bromeliads and resurrection plants.

2.2. Materials and Methods

2.2.1. Location site and characteristics

The investigation was conducted in the Caatinga biome in the State of Pernambuco located in northeastern Brazil (Fig. 1a and Fig. 1b). Rhizosphere sampling was carried out at the experimental stations of Brazilian Agricultural Research Corporation (Embrapa Semi-arid; 9º 03' 58" S, 40 º 19' 14"W and 8º 48' 11.6"S, 40º 14' 48.4"W), located in the State of Pernambuco, Brazil (Fig. 1c). The climate is BSwh' according to the Köppen–Geiger classification, with an annual average temperature of 26.3 °C and rainfall of 577 mm. The soil is classified as red–yellow Ultisol [28], corresponding to Argissolo Vermelho-Amarelo in the Brazilian Soil Classification System [29] (Fig. 1d). Both experimental stations share the same soil physical and chemical characteristics as shown in Table 1. More information about those sampling areas can be found in [10, 12, 30]. Moro et al. [31] and Moro et al. [32] presented detailed information about the phytogeographical patterns of the Caatinga biome.

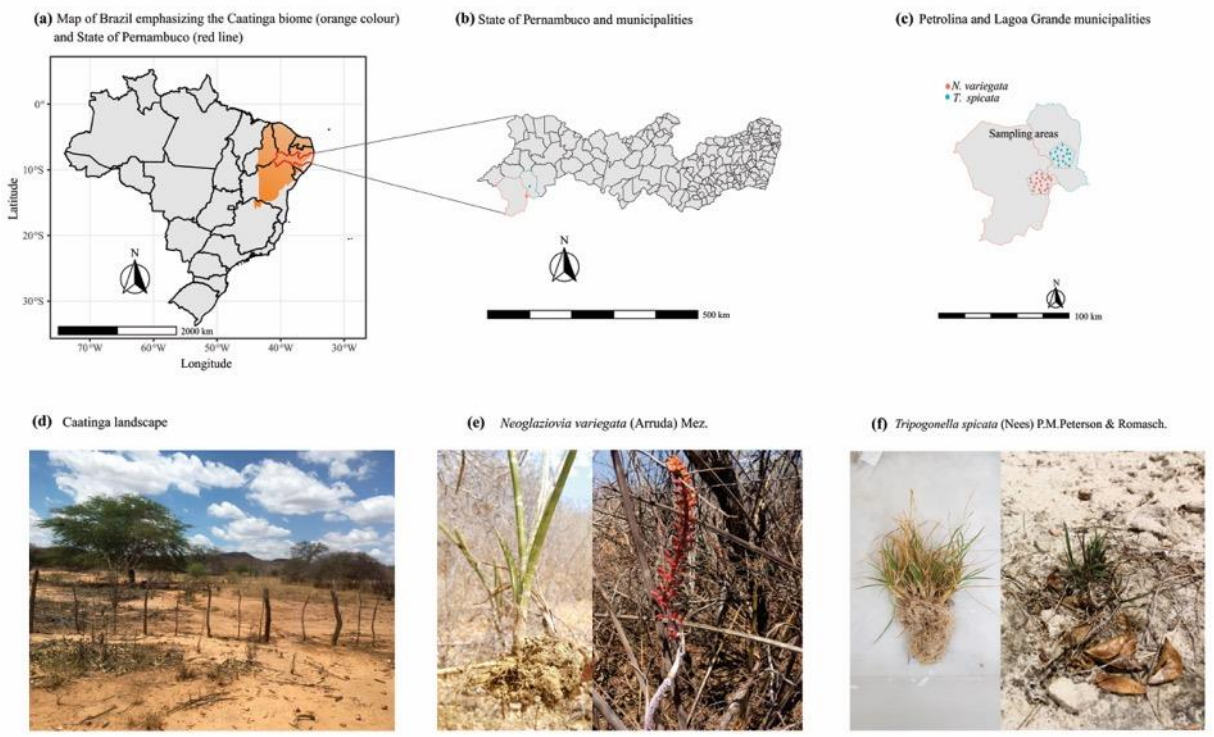

Fig. 1 (a) Location of the sampling area and distribution of the Caatinga biome in Brazil. **(b)** Map of the State of Pernambuco in Brazil, showing its municipalities, highlighting the municipalities where the sampling was carried out. **(c)** Map of Petrolina and Lagoa Grande municipalities and sampling points. **(d)** A common landscape of the Caatinga biome during late dry season showing some *Mimosa tenuiflora* trees. **(e)** Sampled plant *Neoglaziovia variegata* (Arruda) Mez., a bromeliad so-called 'caroá'. **(f)** sampled plant *Tripogonella spicata* (Nees) P.M. Peterson & Romasch., a grass so-called the resurrection plant.

2.2.2. Sampling of the rhizosphere

Forty-eight native plants were studied, among which 24 rhizosphere samples were from *N. variegata* (Fig. 1e) and the other 24 rhizosphere samples from *T. spicata* (Fig. 1f) rhizosphere. Sampling was done in October 2018, in the late dry season. The rhizosphere soil was sampled according to Batista et al. [33]. Briefly, plants were removed from the soil using a shovel, followed by manual agitation, and considering the aggregates adhered to the roots as rhizosphere soil. The samples were stored at the Embrapa Semi-arid until shipment to the University of São Paulo, in the municipality of Piracicaba, in the State of São Paulo, Brazil (22º 42' 35" S, 47º 38' 05" W), where they were stored at −80 °C prior to molecular analysis. We used composite rhizosphere samples for the physicochemical soil characterization (Table 1).

Table 1. Chemical characterization of rhizosphere soil associated with Caatinga plants

Chemical or physical Attribute	Rhizosphere Plant	
	N. variegata	*T. spicata*
pH ($CaCl_2$)	5.1 a	5.4 a
SOM (g kg^{-1})	11.0 a	10.0 a
P (mg kg^{-1})	<3.0 a	5.0 a
S (mg kg^{-1})	<5.0 a	6.0 a
K ($mmol_c$ kg^{-1})	1.2 a	2.5 a
Ca ($mmol_c$ kg^{-1})	11.0 a	6.0 a
Mg ($mmol_c$ kg^{-1})	3.0 a	3.0 a
B (mg kg^{-1})	0.3 a	0.3 a
Cu (mg kg^{-1})	0.5 a	0.3 a
Mn (mg kg^{-1})	10.5 a	4.3 a
Zn (mg kg^{-1})	1.3 a	0.7 a
Na (mg kg^{-1})	9.0 a	15.0 a
Al ($mmol_c$ kg^{-1})	<0.02 a	<0.02 a
H+Al ($mmol_c$ kg^{-1})	15.0 a	12.0 a
SB ($mmol_c$ kg^{-1})	15.2 a	11.5 a
CEC ($mmol_c$ kg^{-1})	30.2 a	23.5 a
Sand (g kg^{-1})	726.0 a	726.0 a
Silt (g kg^{-1})	226.0 a	226.0 a
Clay (g kg^{-1})	49.0 a	49.0 a

pH: active acidity measured in $CaCl_2$. SOM: soil organic matter measured by colorimetric method. P: phosphorus measured with anion exchange resin. S: sulphur measured with 0.01 mol L^{-1} calcium phosphate. K, Ca and Mg: potassium, calcium and magnesium measured in anion exchange resin. B: boron measured with hot water. Cu, Fe, Mn, Zn and Na: copper, iron, manganese, zinc and sodium measured with diethylenetriaminepentacetic acid (DTPA). Al: Aluminum measured in 1 mol KCl L^{-1}. H+Al: potential acidity in SMP buffer. SB: sum of bases (Ca, Mg and K). CEC: cation exchange capacity. $mmol_c$ kg^{-1}: millimoles of charge per kilogram of soil according to SI unit (International Standard of Units). Letters indicate differences between the chemical or physical attributes of plant rhizospheres by Tukey's test at 5% ($p \leq 0.05$).

2.2.3. Soil rhizosphere DNA extraction

Samples of freeze-dried soil (400 mg) were used for DNA extraction with the PowerSoil DNA Isolation kit (QIAGEN Inc., Valencia, CA, USA), according to the manufacturer. DNA concentrations were determined using the Qubit quantification platform with Quant-iT dsDNA BR Assay Kit (Invitrogen, Carlsbad, CA, USA). DNA quality was verified by electrophoresis in 1 % agarose gel using tris-acetate-EDTA buffer (1 x TAE), 5µl extracted DNA and 1µl GelRed™ stained (0.5 µg mL^{-1}), followed by visualization on a UV transilluminator (DNR – Bio Imaging Systems/MiniBis Pro).

2.2.4. Arbuscular mycorrhizal fungi sequencing and data analyses

Only 19 samples of *N. variegata* and 18 samples of *T. spicata* presented enough DNA concentration and quality for sequencing. Sequencing was carried out using the MiSeq platform (250 bp paired-end) provided by the NGS Soluções Genômicas Facility (Piracicaba, São Paulo, Brazil), and libraries built using a 500-cycle V2 Sequencing kit. A nested PCR (polymerase chain reaction) was used to cover part of the 18S rRNA, a small subunit (SSU) ribosomal RNA gene [34]. For the first amplification step, the forward primer NS31 (5'-TTGGAGGGCAAGTCTGGTGCC-3') [35] and the reverse primer AML2 (5'-GAACCCAAACACTTTGGTTTCC-3') [36] were used. Whilst for the second amplification step were used the forward primer AMV4. 5NF (5'- AAGCTCGTAGTTGAATTTCG -3') and the reverse primer AMDGR (5'- CCCAACTATCCCTATTAATCAT -3') [37]. Sequencing data were processed using QIIME2 [38] classify-sklearn command using MaarjAM database [39]. The workflow used in our investigation is depicted in Fig. 2**.**

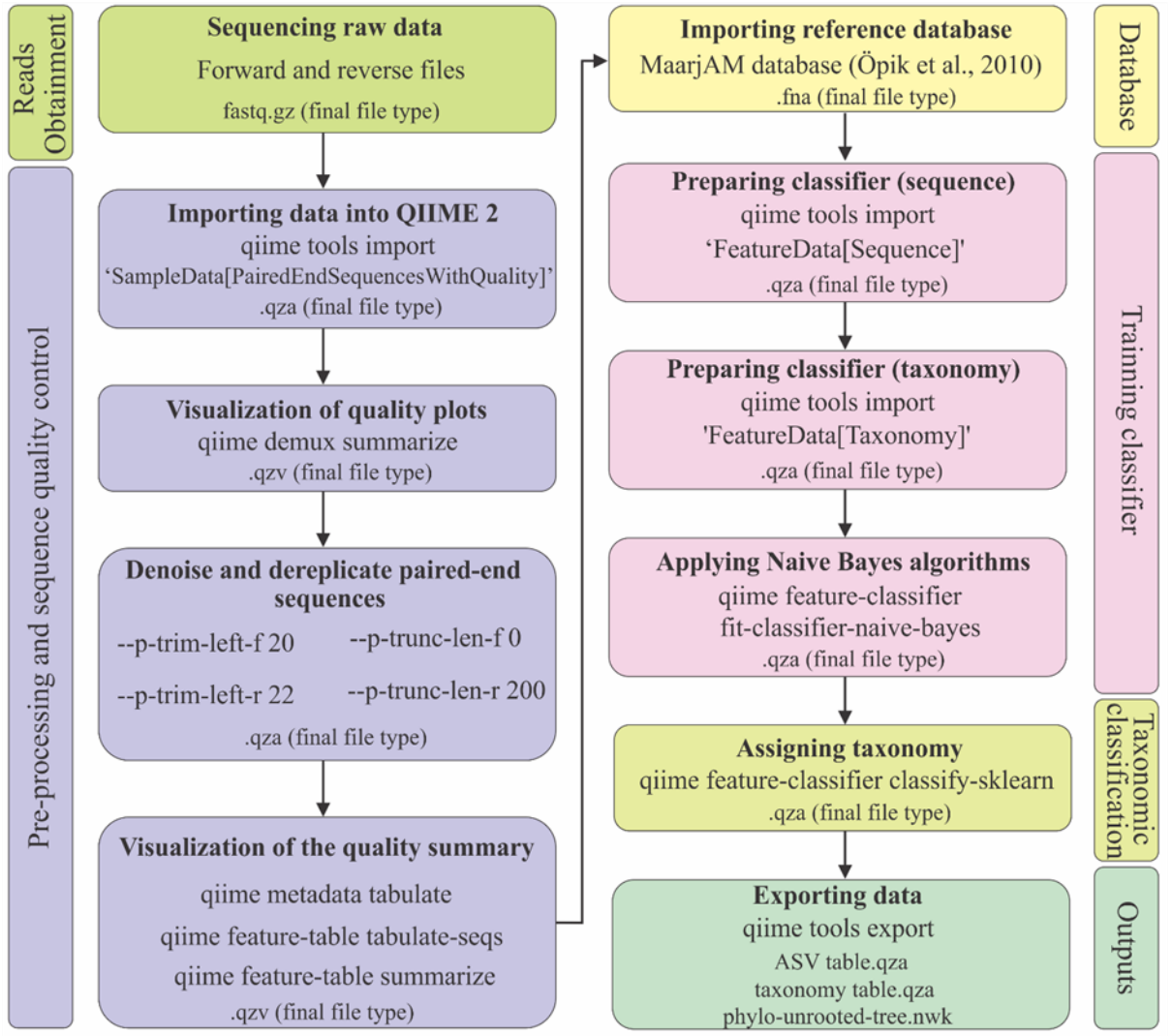

Fig. 2 Workflow of the pipeline used to analyse the AMF amplicon sequencing using nested PCR with NS31and AML2 as the first reaction and AMV4.5NF and AMDGR primers as the second round of PCR reactions.

Briefly, raw reads were demultiplexed, quality-filtered, joined, and grouped within amplicon sequence variants (ASVs) using DADA2 [40]. Subsequently, the taxonomic, diversity, and abundance analyses were performed. Sequences were submitted to the National Centre for Biotechnology Information (NCBI) and Sequence Read Archive (SRA) database with the BioProject PRJNA861682.

Alpha and beta diversity analyses were performed following Silva et al. [41]. The alpha diversity metrics used here were Shannon index (ASV diversity), Observed ASVs, Chao1 (ASV richness), and Faith's phylogenetic diversity (Faith PD). Differences were detected by Wilcoxon signed rank test, a non-parametric test [42]. Changes in beta diversity between the sampled plants were tested using principal coordinate analysis (PCoA) with Bray-Curtis distances coupled with a permutational analysis of variance (PERMANOVA, 999 permutations). We used a network

analysis based on the Newman-Girvan algorithm for determining edge betweenness to detect mycorrhizal communities in the rhizosphere of the sampled plant species. For this method, high-betweenness edges are removed sequentially (recalculating at each step) and the best partitioning of the network is selected [43]. We used RNAseq pipeline edgeR [44] and limma voom [45], available from the Bioconductor project [46], to investigate differential abundances, revealing which mycorrhizal taxonomic groups were enriched or depleted between sampled plants based on the log-fold changes (logFC). For these analyses, we considered the Benjamini–Hochberg false discovery rate correction (FDR < 0.10) [47].

For clarity, relative abundance considered the fraction of the taxa observed in the feature table relative to the sum of all taxa in the sample, and therefore varying between 0 and 100%. Whilst differential abundance considered the abundant taxa between two or more environments (in our case the rhizosphere of *N.variegata* and *T. spicata*) based on the log-fold changes [48]. Relative and differential taxonomic abundance results were presented at the order and genus level due to the wide use of the scientific community that investigates microbial communities with amplicon sequencing [49].

2.3. Results

2.3.1. Overview of amplicon sequencing

The total number of 527,246 high quality mycorrhizal sequences was generated by Illumina Miseq sequencing, with an average of 14,249.89 sequences per sample (Table S3). The rarefaction curves showed an adequate sequencing depth (Fig. S1a). Mycorrhizal sequences were grouped into 175 ASV. *Neoglaziovia variegata* (Arruda) Mez and *Tripogonella spicata* (Nees) P.M.Peterson & Romasch shared only four mycorrhizal ASVs (i.e., about 3 %) (Fig. S1b).

2.3.2. Differential abundance analyses between mycorrhizal communities

Regardless of the taxa level, the mycorrhizal composition and the specific taxa abundance shifted between the two plant species (*N. variegata* and *T. spicata*).

At the order level for *N. variegata,* the mycorrhizal composition was summarized by the predominance of Glomerales (71 %), Diversisporales (21 %), and Archaeosporales (8 %), whilst for *T. spicata* the predominance was based on Glomerales (76 %), Diversisporales (18 %), and Paraglomerales (6 %) (Fig. 3a). At the genus level for *N. variegata*, the mycorrhizal composition

was composed of *Glomus* (68 %), *Gigaspora* (11 %), *Ambispora* (8 %), *Diversispora* (7 %), *Claroideoglomus* (3 %), *Acaulospora* (2 %), and *Scutellospora* (1 %), while for *T. spicata* the mycorrhizal composition was based on the presence of *Glomus* (62 %), *Acaulospora* (16 %), *Rhizophagus* (11 %), *Paraglomus* (6 %), *Claroideoglomus* (3 %), *Scutellospora* (1.5 %), and *Diversispora* (0.5 %) (Fig. 3b).

Overall, at the level of orders, for *N. variegata,* there was an enrichment of Glomerales, Diversisporales, and Archaeosporales, while for *T. spicata* there was an enrichment of Paraglomerales (Fig. 3c), according to the differential abundance analysis. At the genus level, substantial enrichments were detected in *N. variegata* for the genera *Glomus*, *Claroideoglomus*, *Gigaspora*, and *Ambispora*. Equally, substantial enrichments were seen in *T. spicata* for the genera *Acaulospora*, *Rhizophagus, Paraglomus*, and *Archaeospora*. There was depletion of *Diversispora* in *T. spicata*, whereas in *N. variegata* this genus was enriched (Fig. 3d).

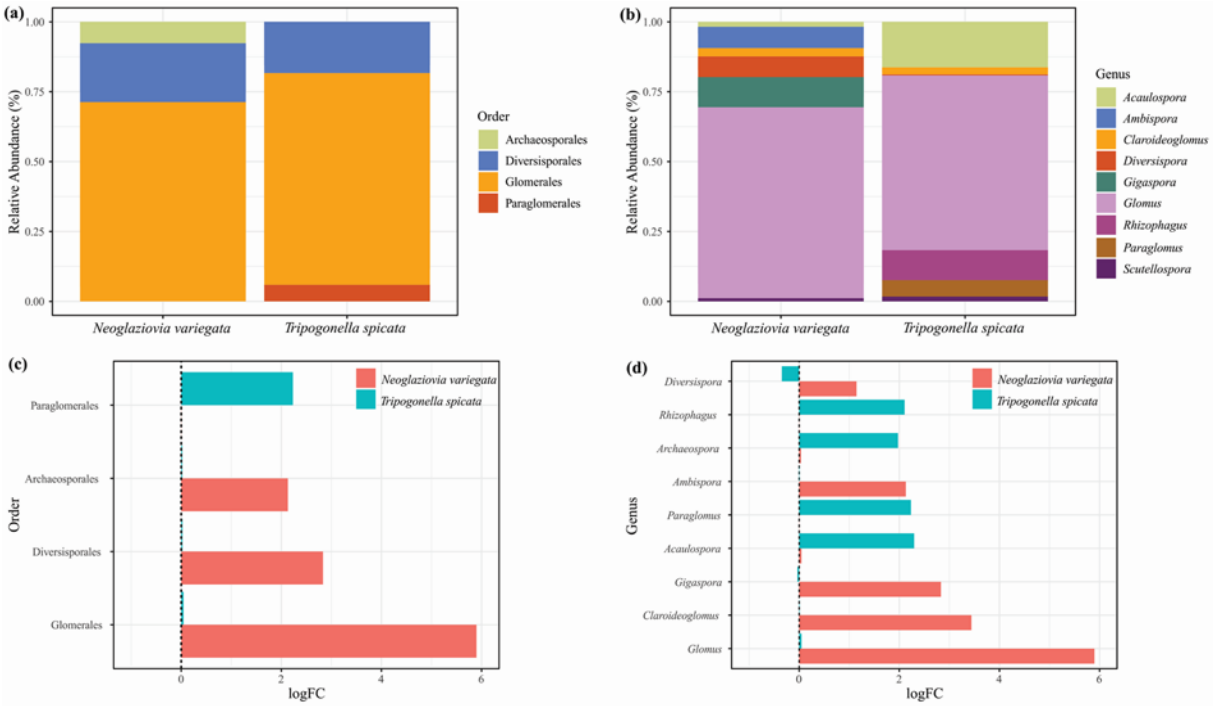

Fig. 3 Community composition of arbuscular mycorrhizal fungi (AMF) in two plants sampled in the Caatinga biome based on the relative abundance of **(a)** order and **(b)** genus. Differential abundance analysis considering **(c)** order and **(d)** genus taxa of the AMF community for the plants with the results expressed by log-fold changes (logFC). logFC was calculated by subtracting the base mean counts of log ratios of each microbial taxa present at *Neoglaziovia variegata* from microbial taxa present at *Tripogonella spicata*. Analysis was performed using the edgeR and limma voom packages available from the Bioconductor project in R environment.

2.3.3. Alpha and beta diversity, and community detection

The alpha-diversity of the mycorrhizal community differed significantly according to the plants, with the highest Shannon diversity ($p < 0.05$), observed ASV ($p < 0.01$), Chao1 ($p < 0.01$), and Faith PD ($p < 0.05$) being found in the rhizosphere of *T. spicata* (Fig. 4).

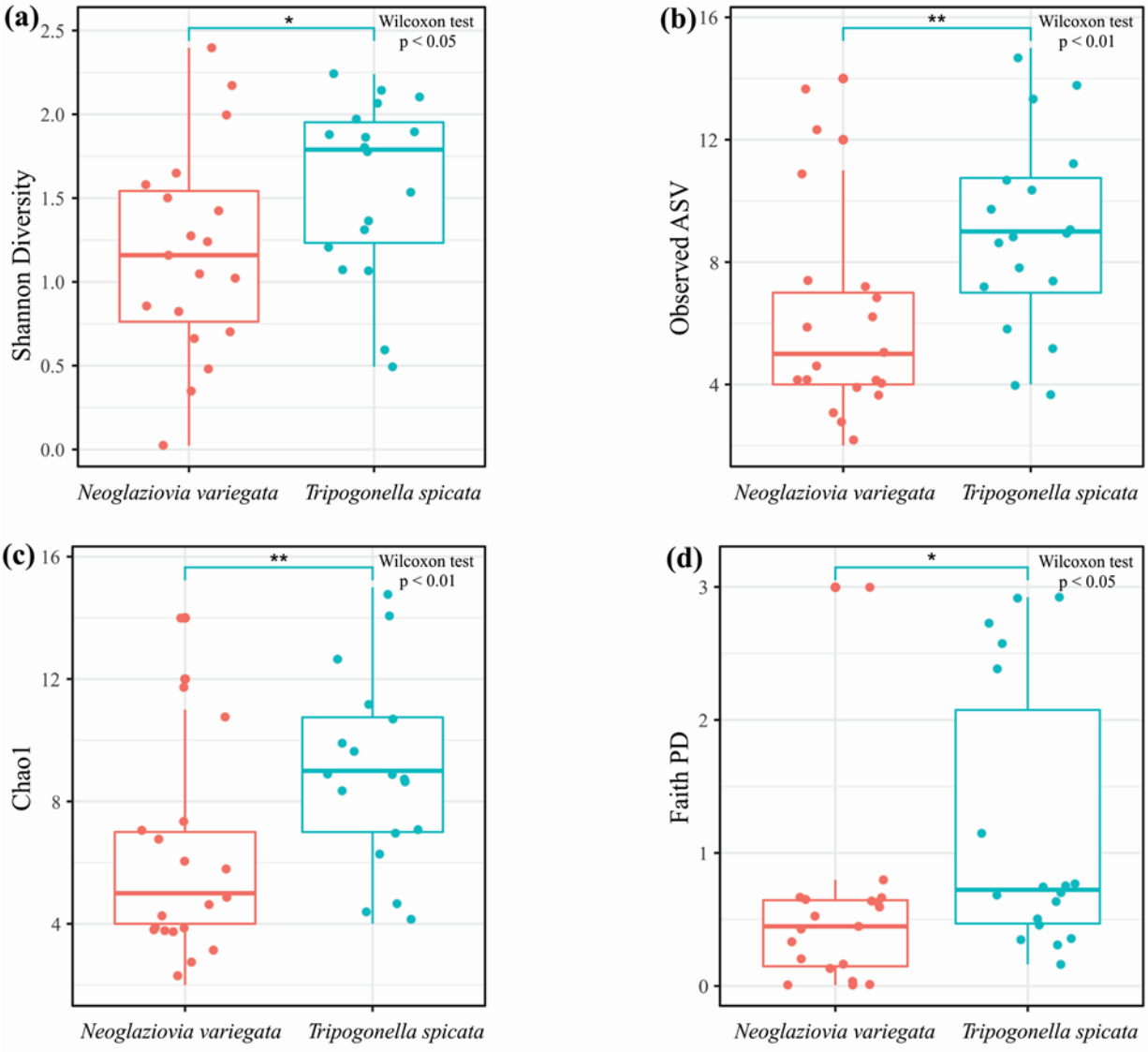

Fig. 4 (a) Alpha diversity indices considering the two plants sampled in the Caatinga biome, expressed by Shannon diversity. **(b)** Observed ASV [amplicon sequence variant]. **(c)** Chao1. **(d)** Faith's phylogenetic diversity. Statistical differences are denoted as *($p < 0.05$) and **($p < 0.01$) by the Wilcoxon test. Heavy horizontal line within a box represents the median, the box represents the interquartile range, and whiskers indicate the variability outside the upper and lower quartiles.

Likewise, beta-diversity showed higher dissimilarities of the mycorrhizal community between the plants based on Bray-Curtis distance, which was confirmed by the PERMANOVA ($p < 0.001$) (Fig. 5a, Table S4).

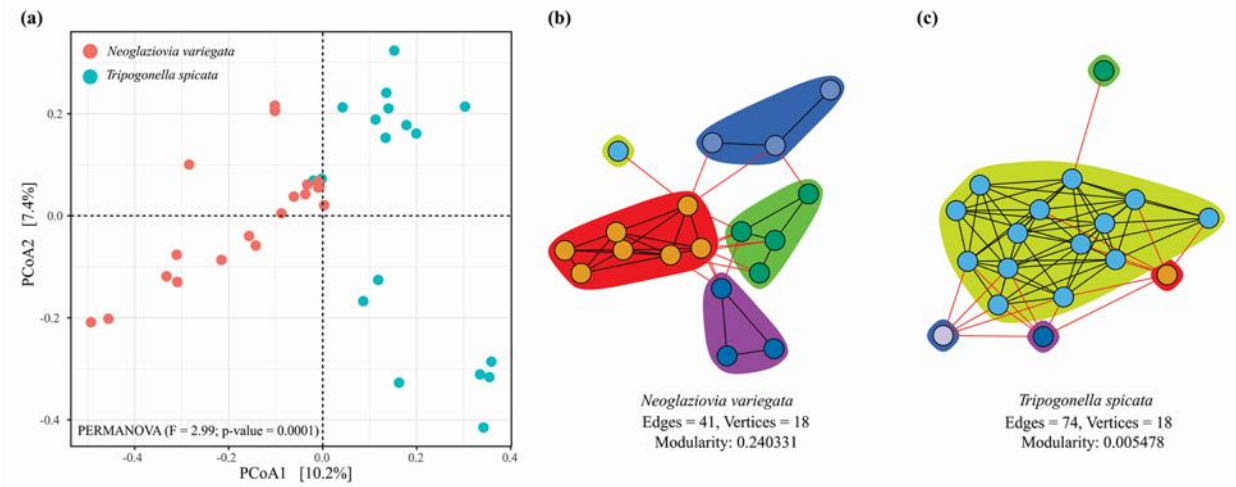

Fig. 5 (a) Beta diversity expressed by principal coordinate analysis (PCoA) using Bray-Curtis distances, depicting mycorrhizal data from two plants sampled in the Caatinga biome. Network analysis for mycorrhizal community detection within **(b)** *N. variegata* and **(c)** *T. spicata* based on edge betweenness (Newman-Girvan). Each node represents the samples and colours represent the different mycorrhizal communities detected. Same colours between panels b and c represent similar mycorrhizal community.

According to the algorithm to perform community detection based on edge betweenness (Newman-Girvan), we noticed a different pattern of sample clustering within the same plant, with the most pronounced differentiation in *N. variegata*. Overall, four different mycorrhizal groups of ASVs were detected within *N. variegata*, in which each one was composed of at least three samples. Higher modularity was found for *N. variegata* (0.240331), reflecting dense connections within groups of ASVs and sparse connections across communities (Fig. 5b). For *T. spicata* we observed a prevalence of only one group of ASV and lower modularity (0.005478) (Fig. 5c). In other words, the mycorrhizal community of the rhizosphere of *T. spicata* has strong similarity, clustering in the same module class, while the rhizosphere of *N. variegata* comprises different mycorrhizal communities strongly dissimilar to each other as evidenced by their distinct module classes.

2.4. Discussion

Unravelling the composition of the mycorrhizal community associated with the rhizosphere of plants of hostile environments can be a step forward in field investigations of mycorrhizal ecology. Our investigation is the beginning of an ongoing project and, therefore, some limitations and future perspectives can be pointed out. Firstly, we must consider that the entire AMF community may not have been assessed due to limitations, when using the molecular approach, i.e., bias from DNA extraction to low accuracy of the DNA reference databases [20,

50]. Secondly, it is important to consider that precipitation and temperature regulate the composition and diversity of the AMF community [51-53]. Considering that our sampling strategy was done at the end of the dry season, we might have different results for the rainy season.

Undoubtedly, these issues do not discredit our investigations or the role of arbuscular mycorrhizal fungi (AMF) in providing essential ecosystem services [54]. Though some authors reject the necessity of considering the mycorrhizal community for the plant health under harsh environments or when managing crops in agriculture [55, 56], we firmly advocate that these ancient symbiotic groups are crucial for soil-plant sustainability. We go further and argue that there are keystone taxa of AMF, which, combined with the physiological plant traits, are essential to help plants overcome drought events. Notwithstanding, it is reassured that AMF is among the most ubiquitous plant mutualists that improve plant growth and yield by facilitating the uptake of phosphorus and water, as well as other nutrients [57, 58].

In our investigation, the lack of information about the mycorrhizal ecology of the sampled plants was detected by our mini-review (supplementary material), especially when they are considered as a host of microbes that can help crop plants to tolerate shortages of water in the soil. Overall, the AMF community found in the rhizosphere of *N. variegata* differs from the rhizosphere of *T. spicata* and this was reassured by the difference in network modularity observed. Briefly, modularity is a measure of network structure, where high modularity indicates that the network has dense connections within certain groups of nodes and sparse connections between the other groups [59].

Although the plants were sampled at two different sites, we observed that the soil chemical and physical characterization of both sites was similar, suggesting that the distinct mycorrhizal composition may be related to the host phylogeny rather than the sampled site. Indeed, the two plants studied are not phylogenetically related and therefore may harbour a different AMF community and exploit their soil resources in different ways [60-62].

Although the AMF community differed between the rhizosphere of the plants species, more than 90 % of the mycorrhizal community for both plants was composed of the order Glomerales and Diversisporales. Likewise, Leroy et al. [20], investigating the taxonomic and functional diversity of root-associated fungi in bromeliads (none of them being *N. variegata*), found the order Glomerales to be dominant, and *Rhizophagus*, *Funneliformis* and *Glomus* to be the main genera, while here for our bromeliad, the main genera found were *Glomus*, *Gigaspora*, *Ambispora* and *Diversispora*. These taxonomic differences may be expected, since life forms,

nutritional modes and environmental traits drive the root fungal community structure in bromeliads.

On the other hand, we can also find similar results with a distinct host, although it is known that the partner specificity in mycorrhizal symbiosis occurs at the level of ecological groups, rather than at the species level [63]. For example, dos Passos et al. [62], evaluating the composition of the AMF community of soil samples from the rhizosphere of *Mimosa tenuiflora* (legume), found the order Glomerales to be dominant and argued that some taxa of this order are recognised for colonising plants first, allowing their establishment in diverse environments. Several studies using native plants of the Caatinga have shown similar results [53, 64-67]. In addition, Davison et al. [68] have documented the worldwide predominance of Glomerales across a range of local environmental conditions and spatial configurations.

We observed that *T. spicata,* besides the highest diversity indices and enrichment of different taxa, presented a well-structured AMF group according to the algorithm for community detection (i.e., lower modularity), and we raised the following questions: (1) would this structuring (presence of only a dense ASV group) translate into benefits for the plant? (2) could the enriched taxa observed here (*Acaulospora*, *Rhizophagus*, *Paraglomus*, and *Archaeospora*) play a crucial role in the desiccation tolerance trait of *T. spicata*?.

Indeed, there are evidences that *Rhizophagus* sp. and *Acaulospora* sp. have a pronounced effect on the response of plants to water shortage in the soil, especially due to their intrinsic character of stress-tolerance and widespread geographical distribution, adapting to adverse environmental conditions [69, 70]. For example, Ortiz et al. [71] showed that inoculation of *Rhizophagus intraradices* significantly enhanced the relative water content in *Trifolium repens*, particularly when associated with bacteria. Also, Chitarra et al. [72] working with *Solanum lycopersicum* demonstrated the efficiency of *R. intraradices* to minimize drought stress-imposed effects, showing that the enhancement of water transport combined with an increase of plant osmolytes, stomatal density and gene expression related to plant hormones are the main altered mechanisms. Furthermore, there are investigations that show the positive effect of *Acaulospora* sp. on promoting the plant-growth response under water stress in soil [73, 74]. Little is known about how *Paraglomus* sp. and *Archaeospora* sp. can overcome the negative effects of drought in plants.

Comparing the plants, we noticed a different mycorrhizal enrichment for *N. variegata* for the genera *Claroideoglomus* (order Glomerales), *Gigaspora* (order Diversisporales), and *Ambispora* (order Archaeosporales). Among these genera, *Claroideoglomus* sp. has been the most studied in the Caatinga and has shown promise to increase shoot dry weight of native plants due to its rapid establishment and symbiotic interactions with the host [51, 69]. Therefore, considering these

results alongside the community detection algorithm for *N. variegata*, the C-R-S framework proposed by Chagnon et al. [69] fits very well. Briefly, it classifies AMF species into three functional groups, namely, competitor (C), ruderal (R), and stress tolerating (S). The aforementioned authors argued that species belonging to the genus *Gigaspora* sp. have competitive traits (higher soil hyphae density and stronger carbon-sink strength), and *Claroideoglomus* sp. have ruderal traits (higher growth rate and more efficient hyphae healing). *Ambispora* sp. appears to exhibit stress tolerating traits, such as low growth rate and long-lived mycelium [69, 75]. However, this was not completely true for *T. spicata*, given the lower modularity and enrichment of other taxa observed. As the first investigation to describe the mycorrhizal community of the rhizosphere of these plants, we argue that studies evaluating the mycorrhizal community in the plant roots are necessary to understand the benefits of the rhizosphere community in the plant performance.

2.5. Conclusions

We concluded that, although arbuscular mycorrhizal communities found in the rhizosphere differ between *N. variegata* and *T. spicata*, they have *Glomus* sp. as the most abundant genus. Furthermore, we argue that the genera *Gigaspora*, *Diversispora*, *Ambispora*, *Rhizophagus*, *Paraglomus*, and *Archaeospora* may be playing a key role for both plant species. Considering that the sampled sites shared the same soil chemical and physical traits, we concluded that the host species was the main driver for mycorrhizal diversity, richness and modularity in the rhizosphere.

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Supplementary Materials

Supplementary Note 1. Mini-review on investigations with *N. variegata* and *T. spicata*

Methods

We performed a mini-review to obtain studies that reported the plant species evaluated in our study, i.e., *Neoglaziovia variegata* (Arruda) Mez and *Tripogonella spicata* (Nees) P.M.Peterson & Romasch. We used Web of Science as our primary database and ScienceDirect to search full texts published in scientific journals and used the search string "Neoglaziovia variegata" OR "caroa" for N. variegata plants, and the search string "Tripogonella spicata" OR "Tripogon spicatus" for *T. spicata* plants. We investigated the search terms in title, abstract, and keywords. We did not use "resurrection plant" as a search string, although resurrection plants are a relatively small group, they exhibit a wide taxonomic diversity, comprising several families of plants as reviewed by Gechev et al. [1]. In addition, we used "Tripogon spicatus" as a search string due to the old classification for this species [2].

Results

The total of 20 articles published in the last 27 years (from 1995 to 2022) were found for *N. variegata*, and most of them revealed chemical molecules acting as acaricidal and antibacterial agents. In addition, we found several studies showing the seed morphometric and physiological characterization of *N. variegata* (Table S1). Whilst for *T. spicata* a total of seven articles published in the last 10 years (from 2012 to 2022) were found, part of them related to the characterization and use of the rhizosphere bacterial community as plant growth-promoting agents. We also observed other studies relating botanical aspects of *T. spicata* (Table S2).

Reference

1. Gechev T, Lyall R, Petrov V, Bartels D (2021) Systems biology of resurrection plants. Cell Mol Life Sci 78:6365–6394. https://doi.org/10.1007/s00018-021-03913-8
2. Royal Botanic Gardens, Kew (2021).The World Checklist of Vascular Plants (WCVP). https://doi.org/10.15468/6h8ucr. Accessed 13 November 2021

Table S1. List of studies found using search terms for the *N.variegata* plant in the Web of Science and ScienceDirect databases

N.	Article Tile	Research area	Journal	Language	Year	DOI
1	Effect of thinning and slashing on forage phytomass from a caatinga-of-Petrolina, Pernambuco, Brazil	Agriculture	Pesquisa Agropecuária Brasileira	English	1995	NA
2	Mechanical, Morphological, and Structural Characteristics of Caroa (Neoglaziovia variegata) Fibres	Polymer Science	Polymers & Polymer Composites	English	2008	10.1177/096739110801600902
3	Soils and vegetation relations in areas under desertification in Jataúba County, Pernambuco State, Brazil	Agriculture	Revista Brasileira de Ciência do Solo	Portuguese	2008	10.1590/S0100-06832008000300036
4	Micropropagation and in vitro conservation of Neoglaziovia variegata (Arr. Cam.) mez, a fiber producing bromeliad from Brazil	Applied Sciences	Brazilian Archives of Biology and Technology	English	2009	10.1590/S1516-89132009000400016
5	Genetic variability estimated among caroá populations through RAPD markers	Agriculture	Pesquisa Agropecuária Brasileira	Portuguese	2009	10.1590/S0100-204X2009000300010
6	Evaluation of the effects of acetylation surface treatments on 'caroá' fiber	Agriculture	Revista Brasileira de Engenharia Agrícola e Ambiental	Portuguese	2011	10.1590/S1415-43662011000100012
7	Germinative response of *Neoglaziovia variegata* (Arruda) Mez seeds	Agriculture	Ciência e Agrotecnologia	Portuguese	2011	10.1590/S1413-70542011000500012
8	Gastroprotective Effect of an Ethanolic Extract from Neoglaziovia variegata (Arruda) Mez (Bromeliaceae) in Rats and Mice	Pharmacology & Pharmacy	Zeitschrift fur Naturforschung section c-a journal of biosciences	English	2013	10.5560/ZNC.2013.68c0097
9	Somatic embryogenesis of *Neoglaziovia variegata* (Arruda) Mez, an important source of fiber from native Brazilian bromeliads	Life Sciences & Biomedicine	Brazilian Archives of Biology and Technology	English	2013	10.1590/S1516-89132013000400004
10	Morpho-physiological aspects of in vitro pre-acclimatization and acclimatization of the caroá plant	Agriculture	Revista Ciência Agronômica	Portuguese	2013	10.1590/S1806-66902013000300017
11	Antimicrobial potential of ethanolic plant extracts against gram negative bacilli isolated from the cervical-vaginal mucosa of sheep raised in the region of Petrolina-PE	Agriculture	Semina-Ciencias Agrarias	Portuguese	2014	10.5433/1679-0359.2014v35n2p883
12	Acaricidal activity of extracts from the leaves and aerial parts of *Neoglaziovia variegata* (Bromeliaceae) on the cattle tick *Rhipicephalus* (Boophilus) *microplus*	Veterinary Sciences	Research in Veterinary Science	English	2015	10.1016/j.rvsc.2015.04.012
13	Antibacterial potential of native plants from the Caatinga biome against *staphylococcus* spp. isolates from small ruminants with mastitis	Veterinary Sciences	Revista Caatinga	English	2016	10.1590/1983-21252016v29n328rc
14	Non-Polar Natural Products from Bromelia laciniosa, *Neoglaziovia variegata* and *Encholirium spectabile* (Bromeliaceae)	Chemistry	Molecules	English	2017	10.3390/molecules22091478
15	Innovation in floriculture with ornamental plants from Caatinga biome	Agriculture	Ornamental Horticulture-Revista Brasileira de Horticultura Ornamental	English	2017	10.14295/oh.v23i3.1081

16	A novel poly-oxygenated flavone glucoside from aerial parts of the Brazilian plant *Neoglaziovia variegata* (Bromeliaceae)	Science & Technology	Heliyon	English	2019	10.1016/j.heliyon.2019.e01369
17	Gastroprotective Activity of *Neoglaziovia variegata* (Arruda) Mez. (Bromeliaceae) in Rats and Mice	Nutrition & Dietetics	Journal of Medicinal Food	English	2021	10.1089/jmf.2020.0182
18	Acaricidal efficacy and chemical study of hexane extracts of the leaves of *Neoglaziovia variegata* (Bromeliaceae) against the tick *Rhipicephalus microplus*	Entomology	Experimental and Applied Acarology	English	2021	10.1007/s10493-021-00611-9
19	Influence of Seasonality on Phytochemical Composition, Phenolic Content and Antioxidant Activity of Neoglaziovia variegata (Bromeliaceae)	Chemistry	Biointerface Research in Applied Chemistry	English	2022	10.33263/BRIAC123.28892904
20	Morphometric characterization and functional traits of fruits and seeds of *Neoglaziovia variegata* (Arruda) Mez.	Agriculture	Journal of Seed Science	English	2022	10.1590/2317-1545v44250044

NA: not available, Source: Web of Science and ScienceDirect

Table S2. List of studies found using search terms for the *T. spicata* plant in the Web of Science and ScienceDirect databases

N.	Article Tile	Research area	Journal	Language	Year	DOI
1	Assessment and quantification of phytoliths present in Chloris elata Desv., Chloris gayana Kunth, *Tripogon spicatus* (Ness) Ekman (Chloridoideae)	Plant Sciences	Iheringia Serie Botanica	Portuguese	2012	NA
2	The resurrection plant *Tripogon spicatus* (poaceae) harbors a diversity of plant growth promoting bacteria in northeastern brazilian caatinga	Agriculture	Revista Brasileira de Ciência do Solo	English	2015	10.1590/01000683rbcs20140646
3	A molecular phylogeny and classification of the Cynodonteae (Poaceae: Chloridoideae) with four new genera: Orthacanthus, Triplasiella, Tripogonella, and Zaqiqah; three new subtribes: Dactylocteniinae, Orininae, and Zaqiqahinae; and a subgeneric classification of Distichlis	Evolutionary Biology	Taxon	English	2016	10.12705/656.4
4	Vegetative desiccation tolerance of *Tripogon spicatus* (Poaceae) from the tropical semiarid region of northeastern Brazil	Plant Sciences	Functional Plant Biology	English	2017	10.1071/FP17066
5	*Tilletia tripogonellae* (Tilletiaceae), a new smut fungus on *Tripogonella spicata* (Poaceae) from Argentina	Plant Sciences	Annales Botanici Fennici	English	2018	10.5735/085.055.0407
6	Inoculation of plant growth-promoting bacteria attenuates the negative effects of drought on sorghum	Microbiology	Archives of Microbiology	English	2020	10.1007/s00203-020-01810-5
7	Beneficial Plant-Associated Microorganisms From Semiarid Regions and Seasonally Dry Environments: A Review	Microbiology	Frontiers in Microbiology	English	2021	10.3389/fmicb.2020.553223

NA: not available, Source: Web of Science and ScienceDirect

Table S3. Reads per sample obtained by sequencing of mycorrhizal (targeted to ITS and 18S rRNA) considering two sampled plants, *Tripogonella spicata* (Nees) (*n* = 18) and *Neoglaziovia variegata* (Arruda) Mez. (*n* = 19)

Sample ID	NS31/AML2 and AMV4.5NF/ AMDGR primers (reads)	Plant	NCBI* BioSample accessions
109	13,108	*Tripogonella spicata* (Nees)	SAMN29923066
110	13,157	*Tripogonella spicata* (Nees)	SAMN29923067
111	13,740	*Tripogonella spicata* (Nees)	SAMN29923068
112	14,896	*Tripogonella spicata* (Nees)	SAMN29923069
113	14,144	*Tripogonella spicata* (Nees)	SAMN29923070
115	16,033	*Tripogonella spicata* (Nees)	SAMN29923071
116	14,195	*Tripogonella spicata* (Nees)	SAMN29923072
117	14,601	*Tripogonella spicata* (Nees)	SAMN29923073
118	14,528	*Tripogonellasspicata* (Nees)	SAMN29923074
119	15,255	*Tripogonella spicata* (Nees)	SAMN29923075
120	17,121	*Tripogonella spicata* (Nees)	SAMN29923076
121	12,608	*Tripogonella spicata* (Nees)	SAMN29923077
122	12,843	*Tripogonella spicata* (Nees)	SAMN29923078
123	13,184	*Tripogonella spicata* (Nees)	SAMN29923079
124	15,850	*Tripogonella spicata* (Nees)	SAMN29923080
125	12,606	*Tripogonella spicata* (Nees)	SAMN29923081
126	10,930	*Tripogonella spicata* (Nees)	SAMN29923082
127	12,415	*Tripogonella spicata* (Nees)	SAMN29923083
128	13,202	*Neoglaziovia variegata* (Arruda) Mez.	SAMN29923084
129	12,804	*Neoglaziovia variegata* (Arruda) Mez.	SAMN29923085
130	15,204	*Neoglaziovia variegata* (Arruda) Mez.	SAMN29923086
131	15,588	*Neoglaziovia variegata* (Arruda) Mez.	SAMN29923087
132	14,513	*Neoglaziovia variegata* (Arruda) Mez.	SAMN29923088
133	20,967	*Neoglaziovia variegata* (Arruda) Mez.	SAMN29923089
134	13,792	*Neoglaziovia variegata* (Arruda) Mez.	SAMN29923090
135	9,803	*Neoglaziovia variegata* (Arruda) Mez.	SAMN29923091
136	14,855	*Neoglaziovia variegata* (Arruda) Mez.	SAMN29923092
137	10,002	*Neoglaziovia variegata* (Arruda) Mez.	SAMN29923093
138	14,077	*Neoglaziovia variegata* (Arruda) Mez.	SAMN29923094
139	11,242	*Neoglaziovia variegata* (Arruda) Mez.	SAMN29923095
140	14,198	*Neoglaziovia variegata* (Arruda) Mez.	SAMN29923096
141	12,826	*Neoglaziovia variegata* (Arruda) Mez.	SAMN29923097
143	16,263	*Neoglaziovia variegata* (Arruda) Mez.	SAMN29923098
144	15,920	*Neoglaziovia variegata* (Arruda) Mez.	SAMN29923099
146	16,450	*Neoglaziovia variegata* (Arruda) Mez.	SAMN29923100
147	15,878	*Neoglaziovia variegata* (Arruda) Mez.	SAMN29923101
149	18,448	*Neoglaziovia variegata* (Arruda) Mez.	SAMN29923102

*NCBI: National Center for Biotechnology Information, available at: https://www.ncbi.nlm.nih.gov/

Table S4. Permutational Multivariate Analysis of Variance of the mycorrhizal community structure using *Adonis* test based on Bray Curtis distance (Permutation = 999)

	DF	**SS**	**MS**	**F Model**	**R^2**	p-**value**
Plant	1	1.343	1.3430	2.9884	0.079	0.001 ***
Residuals	35	15.729	0.4494		0.921	
Total	36	17.072			1.00	

DF: degrees of freedom, SS: sum of squares, MS: Mean of squares, ***: $p \leq 0.001$

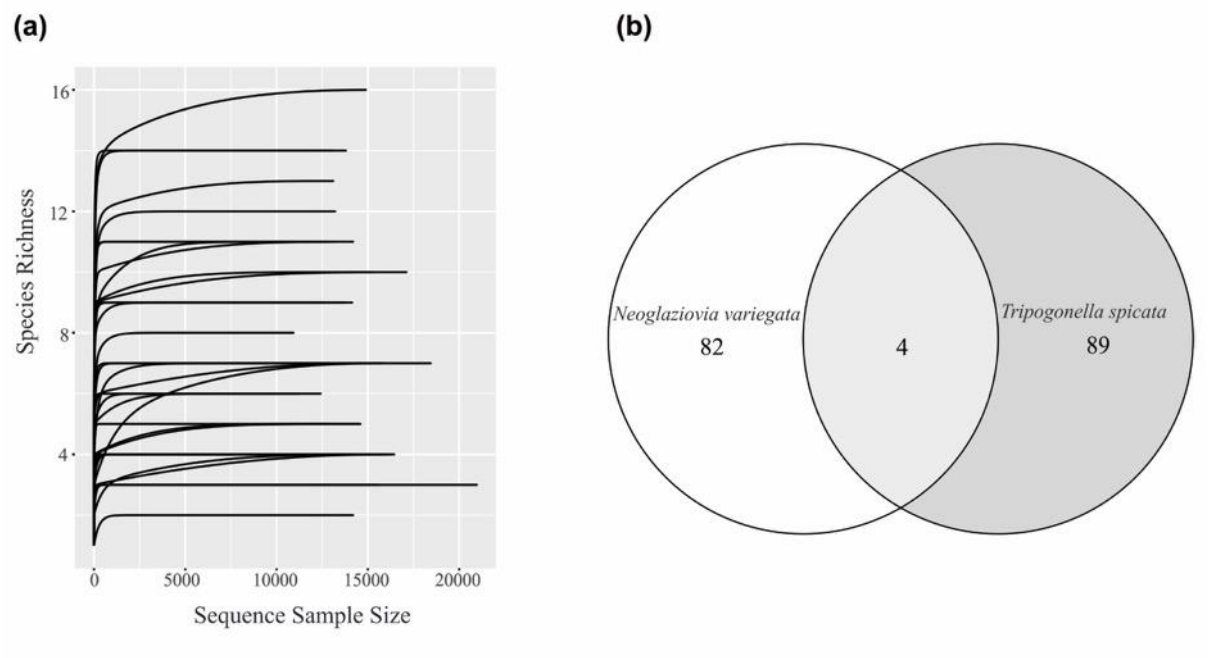

Fig. S1 (a) Sequencing rarefaction curves indicating the sampling depth per sample for mycorrhizal community measured by NS31/AML2 primers targeted to ITS and 18S rRNA amplicon sequencing. **(b)** Venn diagrams of the overall amplicon sequence variants (ASVs) distribution across the sampled plants for mycorrhizal community.

3. SOIL AND PLANT MICROBIOLOGICAL RESPONSES IN THE PRESENCE OF A POOL OF ARBUSCULAR MYCORRHIZAL FUNGI UNDER VARYING DROUGHT LEVELS [2]

Abstract

Arbuscular mycorrhizal fungi (AMF) play a crucial role in plant health due to their ability to improve tolerance to biotic and abiotic stresses. Our aim was to evaluate the effectiveness of a pool of native AMF from a harsh environment on plant performance and changes in soil attributes under levels of drought. An experiment using maize was established, varying the soil water content to simulate severe drought (30 % of the water-holding capacity [WHC]), moderate (50 % of the WHC) and no drought (80 % of the WHC, control treatment). Soil and plant attributes were measured (enzyme activity, microbial biomass, AMF root colonisation and plant biomass and nutrient uptake). There was a 2-fold increase in plant biomass under moderate drought when compared to no drought treatment, but there was no difference in nutrient uptake. Under severe drought, there were the highest enzyme activities related to phosphorus (P) cycling and P microbial biomass, indicating higher P microbial immobilization. Increases in AMF root colonisation were observed in plants under moderate and no drought. Our findings demonstrated that the better use of the AMF inoculum varied according to drought levels, with better performance under moderate drought due to the increase in plant biomass.

Keywords: soil water shortage; mycorrhizal symbiosis; microbial inoculation; water stress alleviation; *Zea mays* L.

3.1. Introduction

Arbuscular mycorrhizal fungi (AMF) belong to the monophyletic phylum Glomeromycota and are symbionts with most vascular plants (more than 80%), spreading worldwide, which is why they are recognized as ubiquitous [1,2]. The first evidence of AMF dates from over 450 million years ago and played a crucial role in facilitating the colonization of land by plants, with both partners (the mycobiont: AMF, and the phytobiont: plants) coevolving over time [3,4]. The established intimate association of AMF with plant roots is called mycorrhizal symbiosis and is often associated with enhanced root growth, nutrient uptake, biotic and abiotic stress tolerance, and crop and forest productivity [5-9].

Due to the crucial role that AMF play in plant health, great efforts have been concentrated in understanding the impact of climate change on mycorrhizal symbiosis [10-12]. However, global change factors are not usually independent of one another, but comprise a complex multifactorial scenario. Therefore, holistic research approaches involving soil-plant-atmosphere systems and various issues resulting from anthropogenic activities (e.g., elevated CO_2, rising temperatures, and drought events) must be prioritized by scientists [13]. In this line, bio-

[2] Manuscript submitted to Microorganisms

prospecting programs have been developed to find native pool of microbes with the potential of helping crop plants to overcome abiotic stresses such as drought stress [14,15].

Undoubtedly, one of the main concerns in agriculture is the increase in drought events that have occurred in recent decades. The foremost impact of drought are reduction in soil water availability and nutrient mobility, thereby reducing crop yields, leading to severe economic losses and potentially disrupting food security [16].

Evidence to date shows that the use of AMF is able to overcome drought in plants, however little is known of how the use of native AMF inoculum from a tropical harsh environment can affect plant performance under drought levels. In light of this, Torres-Arias et al. [14] demonstrated that a native AMF inoculum could bring better development of local plants due to its high adaptability to the edaphoclimatic conditions of the region.

The main mechanisms that AMF can alleviate the drought effects in plants and also increase soil carbon sequestration were well detailed by Augé [17], Chen et al. [18] and Agnihotri et al. [19]. Briefly, they involve: i) morphological adaptability such as improving root architecture and hydraulic conductivity due to the network of mycorrhizal hyphae provided. ii) physiological responses based on increasing enzymatic antioxidant defence system (e.g., synthesis of superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX)) or non-enzymatic (e.g., synthesis of glutathione, cysteine, tachopherols and thioredoxin) to scavenge reactive oxygen species (ROS). iii) molecular responses such as strigolactone synthesis genes expression, up-regulation of antioxidant genes, down-regulation in the relative expression of polyamines catabolic enzyme genes, and increased cell membrane transporters.

Given the outstanding importance of AMF for plant health, our investigation was set out to test the hypothesis that the effectiveness of a native AMF inoculum obtained from a harsh environment varies according to the drought levels, causing changes in soil and plant microbiological parameters related to nutrient cycling and the plant antioxidant system. Therefore, our aim was to evaluate under severe, moderate and no drought conditions the plant performance and soil attributes when a native AMF inoculum was applied.

3.2. Materials and Methods

3.2.1. Experimental design

The experiment was establish in a one-factor completely randomized design with three levels and five replicates. The single factor was the drought levels (30 %, 50 % and 80 % of the

water-holding capacity (WHC), simulating severe drought, moderate drought and no drought, respectively).

Each experimental unit comprised a 3 L pot (20 cm inner diameter and 15 cm high). The soil used for this experiment was obtained from the Ah horizon (0-10 cm deep) of a field site located in Brazil (22°42' S, 47°38' W) and classified as an Arenosol [20], with a texture of sandy clay loam (22.6 % clay, 23.0 % silt, 75.1 % sand). According to the soil chemical characterization [21], soil presented a pH (soil : $CaCl_2$ ratio of 1:2.5) of 6.5, organic matter of 15 g kg^{-1}, low available P content (< 6 mg kg^{-1}), extractable S of 18.0 mg kg^{-1}, and cation-exchange capacity of 35.8 $mmol_c$ kg^{-1}.

Maize seeds (*Zea mays* L. (cv. BRS Gorotuba)) were surface sterilized and germinated in Petri dishes at 25 °C in the dark (obtaining 90 % germination rate) prior to being planted in plastic pots. Initially, pots had $^1/_3$ of their capacity filled with the sterilized soil (at 121 °C for 2 h). Then, 50 g of AMF inoculum was added (see 2.3 section) and the remaining volume of the pot was completed with the sterilized soil.

3.2.2. Water-holding capacity determination and experimental management

Soil WHC was monitored, weighed daily, and distilled water was added when necessary to reach the moisture content of interest. The soil WHC was determined based on the water retention curve (WRC). Briefly, samples were initially saturated by gradually raising a water layer. Then, they were weighed to estimate the water content at saturation (θs). Next, samples were taken to determine the water in equilibrium at potentials (Ψ) at -0 .01, -0.04, -0.06, -0.1 kPa on an automated tension table, and at -0.3, -0.5 and -1.0 kPa in Richards chambers. After reaching water balance at each potential, the samples were weighed. Subsequently, they were dried in an oven at 105 °C to quantify the water content (θ), in cm^3 cm^{-3}, associated with each Ψ.

The WRCs were modelled using van Genuchten's model (Equation 1) which associates the moisture values obtained at equilibrium with the respective Ψ, with Mualem's restriction (Equation 2) [22].

$$\theta = \theta r + \frac{(\theta s - \theta r)}{[1 + (\alpha\, \Psi\, m)^n]^m} \quad (1)$$

$$m = 1 - \left(\frac{1}{n}\right) \quad (2)$$

Where θ is the volumetric soil moisture (cm^3 cm^{-3}). θr is the residual volumetric soil moisture at 1500 kPa tension (cm^3 cm^{-3}). θs is the volumetric moisture of the saturated soil (cm^3 cm^{-3}). Ψ is the soil water potential (kPa). α and n are empirical parameters of the equation, obtained by fitting the model, and m is the empirical parameter of the equation, obtained according to Mualem's restriction. The parameters were obtained using the 'soilphysics' package [23] and curve plotted using 'ggplot 2' package [24] – Appendix A (Figure A1).

Maize plants were cultivated following the same water management described by Silva et al. [9], where they were kept under severe drought (30 % of WHC), moderate drought (50 % of WHC) and no drought (80 % of WHC, control treatment) for 120 days until the harvesting procedure. Hoagland solution was applied to keep nutritional balance of the plants. The final solution of pH 5.5 was composed of 4 mM Ca $(NO_3)_2$, 6 mM KNO_3, 2 mM $MgSO_4$, 1 mM Fe-EDTA, and 1 mM trace elements [25].

Notably, our experiment was specifically designed to investigate the role of the AMF inoculum against drought levels. Therefore, all plants were inoculated, since the aim of our investigation was to evaluate the soil and plant response in the presence of AMF inoculum obtained from a harsh environment, varying only the water soil content. Thus, the control treatment was considered as the optimal water condition (no drought). Xiao et al. [26] have used this approach to assess the effect of drought stress on soil quality parameters in experiments with different plant species. Likewise, Sendek et al. [27] used this approach to explore the effects of plant genotype richness and AMF richness on plant yield under ambient and drought conditions. Still, Torres-Arias et al. [14] used a similar approach aiming to find the most appropriate abiotic conditions for the production of native AMF.

3.2.3. AMF inoculum characterization

The AMF inoculum consisted of the rhizosphere soil of *Tripogonella spicata* (Nees) plants, the so-called resurrection grass, due to their surprising rehydration capacity after a drought period [28,29].

Briefly, AMF inoculum were collected at the Brazilian Agricultural Research Corporation (Embrapa Semi-arid; 8°48'11.6"S, 40°14'48.4"W), in northeastern Brazil, where a bio-prospecting program was developed to find microbes with the potential of helping crop plants to tolerate drought stress [9,30,31].

The AMF inoculum consisted of soil containing mycorrhizal roots (with 12 % of AMF root colonisation, Appendix B, Figure A2), spores and extraradical mycelium. On average, 620

AMF spores composed 50 g of AMF inoculum. These spores belong to the genera *Acaulospora*, *Ambispora*, *Gigaspora*, *Glomus* and *Rhizophagus* and a description of the species is provided in Table 1, while their morphological features are displayed in Appendix C (Figure A3).

Table 1. Species composition of the AMF inoculum (50 g) used in this experiment

AMF species	**Number of spores/ 50 g of inoculum**
Acaulospora morrowiae Spain & N.C. Schenck	94
Ambispora sp.	417
Gigaspora decipiens I.R. Hall & L.K. Abbott	63
Gigaspora gigantea (T.H. Nicholson & Gerd) Gerd. & Trappe	9
Glomus glomerulatum Sieverding	7
Glomus sp.	9
Rhizophagus clarus (T.H. Nicholson & N.C. Schenck) C. Walker & A. Schüßler)	21
Total	620

3.2.4. Soil and plant sampling

Soil and plants were collected 120 days after planting, with soil stored in 50 cm^3 polypropylene tube at 4 °C for biological analysis or 25 °C for chemical analysis. Two fresh diagnostic leaves (newly expanded laminae leave) were collected and immediately wrapped in an aluminium bag, frozen in liquid N and stored at – 80 °C prior to enzyme activities analysis.

3.2.5. Analytical analyses

3.2.5.1. Plant biomass and nutrient content

Plant samples (shoot and root) were dried in a forced-air oven at 65 °C for 72 h to measure dry weight and then ground in Wiley mill prior determination of nutrient content. Nutrient contents (N, P, K, Ca, Mg, and S) were analysed by acid digestion, followed by steam distillation [32].

3.2.5.2. Soil and plant enzyme activities

In soil, inorganic pyrophosphatase (EC 3.6.1.1), acid (EC 3.1.3.2) and alkaline phosphatases (EC 3.1.3.1) activities were evaluated due to their direct role in phosphorus (P) cycling in the soil and were determined following the methodology proposed by Dick and Tabatabai [33] and Tabatabai [34]. Briefly, inorganic pyrophosphatase was determined using an extraction and colorimetric determination of the orthophosphate (Pi) released when 1 g soil was incubated with buffered (pH 8) pyrophosphate (PPi) solution at 37 °C for 5h. The 1N H_2SO_4 was used to extract Pi and the calorimetric method used to determine the Pi extracted in the presence of PPi [33]. Whilst phosphatases were determined using incubation with a buffer solution (at pH 6.5 for acid phosphatase or at pH 11 for alkaline phosphatase) and a soil-buffer mixture of *p*-nitrophenyl-phosphate (PNF) [34].

In plant, ascorbate peroxidase APX (EC 1.11.1.11), Catalase CAT (EC 1.11.1.6), and superoxide dismutase SOD (EC 1.15.1.1) activities were evaluated due to their role in the plant antioxidant system, i.e., responsible for the detoxification of reactive oxygen species (ROS). Firstly, plant material was macerated in the presence of liquid nitrogen prior to extraction buffer addition according to the extraction procedure of Azevedo et al. [35]. The extract was used to determine: i) APX activity according to Moldes et al. [36] in a spectrophotometer at 290 nm. ii) CAT activity according to Kraus et al. [37] modified by Azevedo et al. [35] in a spectrophotometer at 240 nm. iii) SOD activity according to Ciannopolitis and Ries [38] and Cembrowska-Lech et al. [39] in a spectrophotometer at 560 nm.

3.2.5.3. Soil microbial biomass (carbon and phosphorus)

Microbial biomass carbon (MBC) and phosphorus (MBP) were extracted using the fumigation-extraction method proposed by Brookes et al. [40.41]. MBC was determined by titration according to Vance et al. [42], whilst MBP was determined by spectrophotometry (882 nm) according to Murphy and Riley [43].

3.2.5.4. Soil Glomalin and AMF root colonisation

Glomalin, a glycoprotein synthesized majority by mycorrhizal fungi, was obtained from autoclave extraction (at 121 °C for 30 min), using 1g of soil and 8 mL of 20 mM sodium citrate solution (pH 7.4). Then, samples were centrifuged at 5000 g for 20 min and the supernatant was

removed for quantification according to a method proposed by Bradford [44] and Wright and Upadhyaya [45].

For mycorrhizal root colonisation, roots (< 2 mm in diameter) were washed with tap water, and immersed in 10 % potassium hydroxide (KOH), for 24 hours, at room temperature (25 °C). Then, the tubes with root segments were transferred to a 90 °C water bath for 30 minutes with the addition of 10 % hydrogen peroxide (H_2O_2) to completely bleach the segments [46]. The roots were washed in tap water and then received a staining solution with blue ink (PakerQuink®) and 5 % acetic acid. Finally, they were immersed in a 90 °C water bath for 15 seconds [47]. The roots were washed in tap water and were preserved in a lactoglycerol solution (1:1:1, lactic acid, glycerol, and water). The evaluation of the percentage of mycorrhizal colonization was performed using the intersect method for a minimum of 100 intersects [48] using a stereoscopic microscope (Leica MZ12.5). AMF colonisation represents the proportion (percentage) of the root occupied by any AMF structure (arbuscules, hyphae, vesicles, or spore).

3.2.6. Data analyses

As a univariate approach, we used the one-way analysis of variance (ANOVA) to identify significant differences between drought levels in plant dry biomass, nutrient uptake, soil and plant enzyme activities, soil microbiological parameters (microbial biomass carbon and phosphorus, and glomalin content), and AMF root colonisation. Previously, data were tested for normal distribution using the Shapiro-Wilk test, followed by the homogeneity of variances tests, using the Bartlett test. Tukey's test was used as a posthoc pairwise comparison ($p < 0.05$) using the 'ExpDes' package [49]. We used the 'ggplot2' package [24] to create the stacked bar graph and boxplot graphs.

Principal component analysis (PCA) was performed as a multivariate approach to integrate all obtained data, correlating variables with the drought levels. Firstly, data were transformed into log (x + 1) to meet the multivariate normality, and the attributes subject to collinearity were removed [50]. For the PCA, we used the statistical packages 'FactoMineR' [51] and 'factoextra' [52]. All statistical analyses were performed in the R® program [53].

3.3. Results

Dry biomass results revealed increases of 2.1 and 2.6-fold in root dry weight (RDW) under moderate drought when compared to severe drought and no drought conditions,

respectively. Shoot dry weight (SDW) did not differ between moderate drought and no drought, differing them only from severe drought. Therefore, under moderate drought, a 2-fold increase in the total plant dry weight was observed when compared to no drought treatment (Figure 1).

There was no difference ($p > 0.05$) in nutrient uptake between treatments and, overall, N e P was allocated more in RDW than in SDW. There was no clear allocation pattern for the other nutrients (Table 2).

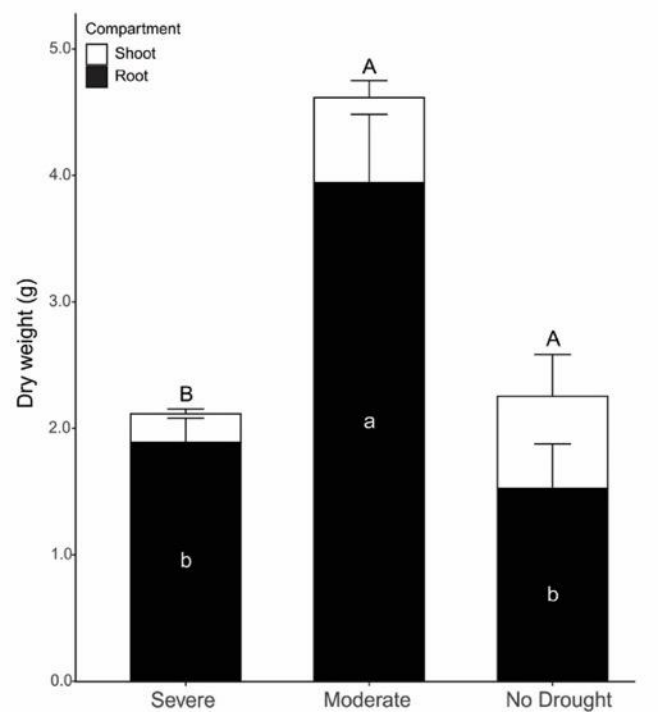

Figure 1. Plant dry weight under severe, moderate and no drought conditions, considering root (black bar) and shoot (white bar) compartments. Uppercase letters compare differences in shoot compartment, while lowercase letters compare differences in root compartment by Tukey's test at 5 % ($p < 0.05$). Standard deviation are shown ($n = 5$).

Table 2. Nutrient uptake by maize, considering shoot, root and total dry weight under severe, moderate and no drought conditions.

Compartment/ Drought levels	N	P	K	Ca	Mg	S
Shoot			*mg SDW^{-1}*			
Severe	3.80 ± 1.90 a*	0.22 ± 0.07 a	7.16 ± 1.97 a	1.08 ± 0.35 a	1.13 ± 0.42 a	0.43 ± 0.13 a
Moderate	8.58 ± 4.00 a	0.61 ± 0.40 a	23.13 ± 11.65 a	3.24 ± 1.63 a	3.21 ± 1.75 a	1.09 ± 0.54 a
No Drought	7.45 ± 5.31 a	0.74 ± 0.75 a	21.43 ± 17.00 a	3.12 ± 2.86 a	3.33 ± 3.61 a	1.23 ± 1.26 a
Root			*mg RDW^{-1}*			
Severe	12.10 ± 6.02 a	1.43 ± 0.74 a	6.70 ± 3.26 a	1.68 ± 0.90 a	0.89 ± 0.48 a	5.22 ± 2.66 a
Moderate	15.47 ± 9.78 a	2.61 ± 1.61 a	7.54 ± 6.28 a	1.96 ± 1.30 a	0.93 ± 0.71 a	8.25 ± 5.48 a
No Drought	10.01 ± 10.22 a	2.97 ± 2.69 a	21.03 ± 17.04 a	12.34 ± 11.51 a	3.03 ± 2.74 a	3.79 ± 3.05 a
Total dry weigh			*mg plant^{-1}*			
Severe	15.90 ± 6.66 a	1.65 ± 0.75 a	13.86 ± 3.22 a	2.76 ± 0.99 a	2.02 ± 0.71 a	5.65 ± 2.65 a
Moderate	24.05 ± 9.45 a	3.22 ± 1.57 a	30.67 ± 9.45 a	5.20 ± 1.67 a	4.14 ± 1.54 a	9.34 ± 5.36 a
No Drought	17.46 ± 13.86 a	3.71 ± 3.33 a	42.46 ± 30.63 a	15.46 ± 13.78 a	6.36 ± 6.13 a	5.02 ± 4.08 a

*Lowercase letters compare differences between drought levels within the same compartment, (shoot, root or total), by Tukey's test at 5 % ($p < 0.05$). Means are followed by standard deviation ($n = 5$)

In soil, acid phosphatase and inorganic pyrophosphatase activities differed ($p < 0.05$) according to drought levels, with severe drought showing the highest activities (991 μg PNF g^{-1} soil h^{-1} and 555 μg P g^{-1} soil $5h^{-1}$, respectively). Still, the lowest activities were observed under moderate drought (785 μg PNF g^{-1} soil h^{-1} and 313 μg P g^{-1} soil $5h^{-1}$) and no drought (749 μg PNF g^{-1} soil h^{-1} and 153 μg P g^{-1} soil $5h^{-1}$) and did not differ ($p > 0.05$) from each other. Alkaline phosphatase activity remained constant between drought levels (Figure 2 A-C).

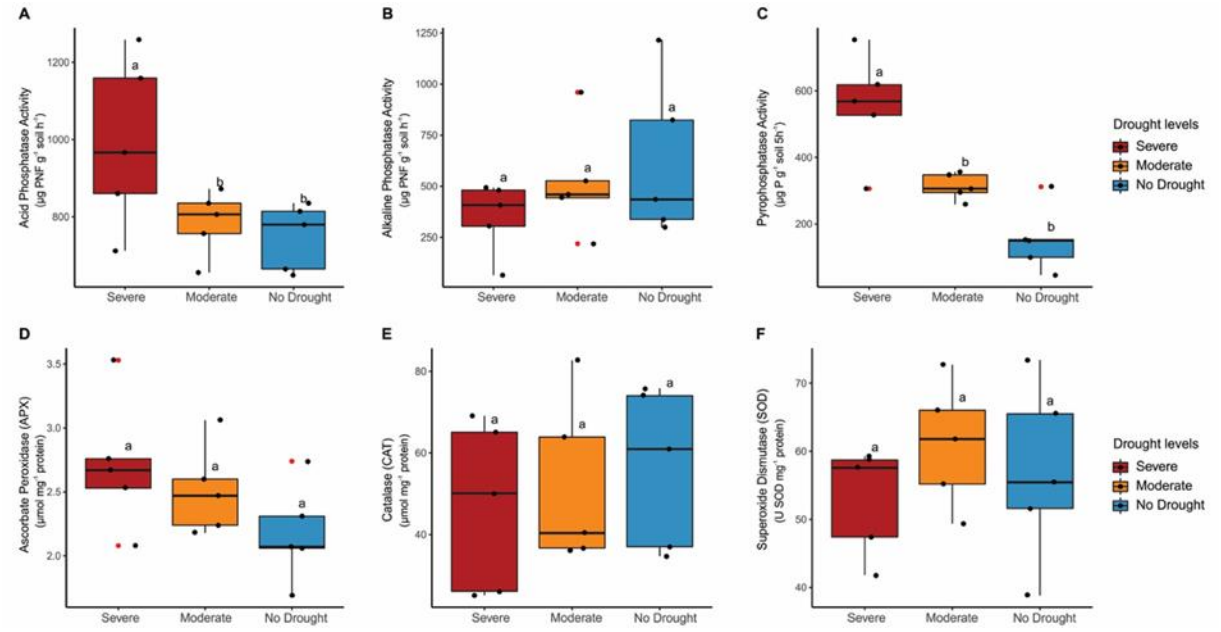

Figure 2. Response of soil (A-C) and plant (D-E) enzyme activities under severe, moderate and no drought conditions. (A) acid phosphatase activity, (B) alkaline phosphatase activity, (C) inorganic pyrophosphatase activity, (D) ascorbate peroxidase (APX) activity, (E) catalase (CAT) activity, and (F) superoxide dismutase (SOD) activity. Boxplot displays the minimum, first quartile, median (vertical line), third quartile, and maximum, respectively. Black dots indicate replicates (n = 5), while red dots indicate the presence of outlier. Lowercase letters compare differences between drought levels by Tukey's test at 5 % ($p < 0.05$).

In plant, no difference ($p > 0.05$) was observed in the enzyme activities related to the antioxidant defence system (APX, CAT, and SOD). On average, APX tended to decrease with increasing soil water content, ranging from 2.7 µmol mg^{-1} protein (severe drought) to 2.1 µmol mg^{-1} protein (no drought).

On the other hand, CAT tended to increase with increasing soil water content, ranging from 47 µmol mg^{-1} protein (severe drought) to 56 µmol mg^{-1} protein (no drought). For SOD was observed activity of 53 U SOD mg^{-1} protein for plants under severe drought, 61 U SOD mg^{-1} protein for plants under moderate drought, and 57 U SOD mg^{-1} protein for plants under no drought (Figure 2 D-F).

Although microbial biomass carbon (MBC) and glomalin content did not differ ($p > 0.05$) between treatments, they appeared to be higher in the moderate and no drought treatments. Whilst microbial biomass phosphorus (MBP) and AMF root colonisation showed differences ($p < 0.05$) between treatments but opposite behaviour. The highest MBP was found under severe drought, while moderate drought and no drought had the lowest values and did not differ from each other.

The highest AMF root colonisation was found in plants under no drought, not differing from plants under moderate drought. Whilst the lowest AMF root colonisation was found in plants under severe drought (Figure 3 A-D).

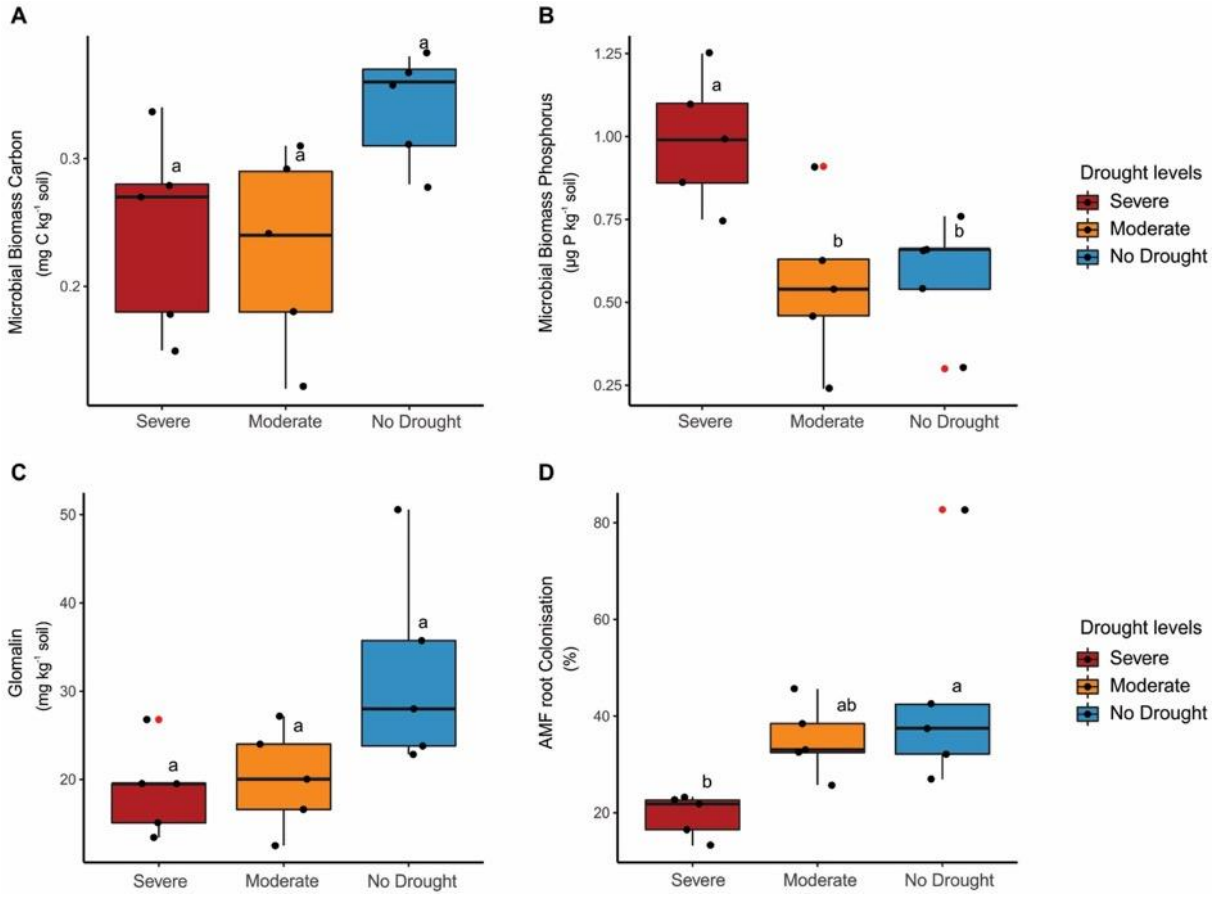

Figure 3. Response of (A) microbial biomass carbon, (B) microbial biomass phosphorus, (C) glomalin content, and (D) arbuscular mycorrhizal fungi (AMF) root colonisation under severe, moderate and no drought conditions. Boxplot displays the minimum, first quartile, median (vertical line), third quartile, and maximum, respectively. Black dots indicate replicates (n = 5), while red dots indicate the presence of outlier. Lowercase letters compare differences between drought levels by Tukey's test at 5 % ($p < 0.05$).

According to the principal component analysis (PCA), the first two components explained 62 % (PCA1 = 40 %, and PCA2 = 22 %) of the data variation, ensuring better interpretation of the results (Figure 4). Data dispersion between replicates was lower under severe and moderate drought compared to no drought samples. MBP, APX, acid phosphatase and pyrophosphatase activities were highly correlated with each other and more associated with the severe drought. Whilst MBC, glomalin content, AMF root colonisation, Mg, K, and Ca uptake were more associated with the no drought condition.

The total plant dry weight and nutrient uptake (N, P and S) correlated with each other and were associated with plants under moderate drought. The PCA results corroborate and complement the results seen in the aforementioned one-way ANOVA.

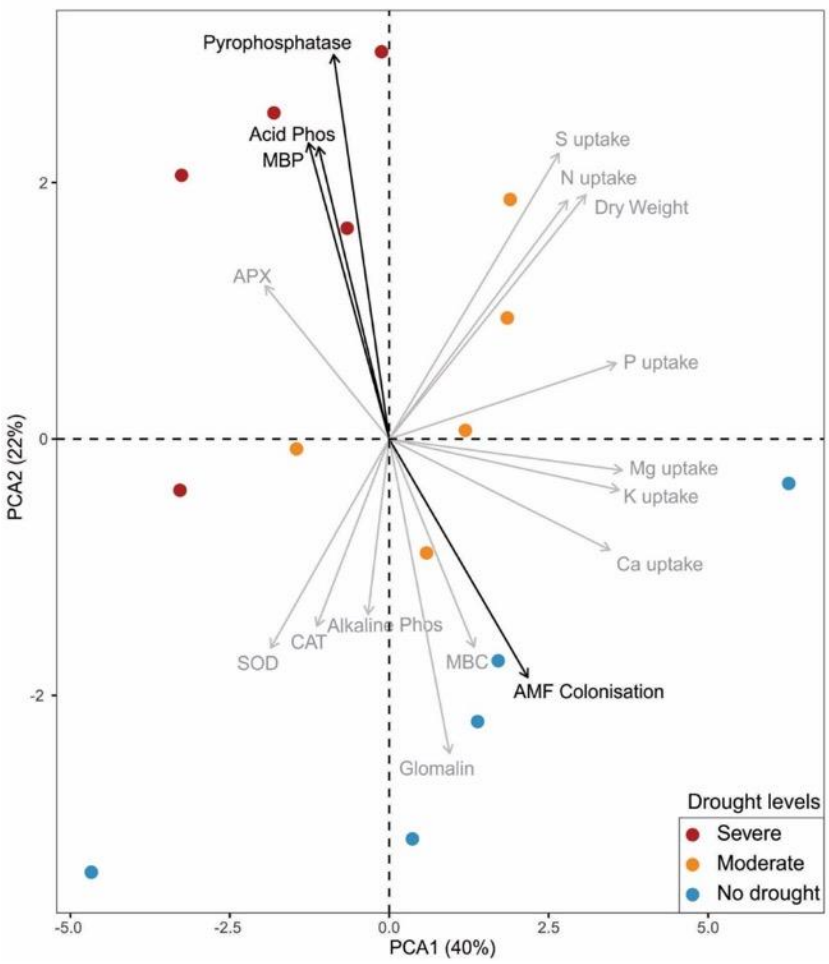

Figure 4. Principal component analysis (PCA) displaying relationships between the variables evaluated and treatments under severe, moderate and no drought conditions. Black arrows indicate significance in on-way ANOVA analysis, while grey arrows indicate non-significance. Pyrophosphatase: inorganic pyrophosphatase activity; Acid Phos: acid phosphatase activity; MBP: microbial biomass phosphorus; APX: ascorbate peroxidase activity; SOD: superoxide dismutase activity; CAT: catalase activity; Alkaline Phos: alkaline phosphatase activity; MBC: microbial biomass carbon; AMF colonisation: arbuscular mycorrhizal fungi root colonisation; Dry weight: total plant dry weight.

3.4. Discussion

Our findings demonstrated that the use of the AMF inoculum from a harsh environment was able to increase maize biomass and change the response of soil attributes, especially under moderate drought. We partially accepted our initial hypotheses, since the effectiveness of the native AMF inoculum varied according to drought levels, but did not promote significant changes in the plant's antioxidant system. This general understanding is vital

for planning the better use of AMF inoculum not only to increase crop resilience to drought, but also to maximize crop yields, especially in the context of rising drought events.

According to Xiao et al. [26] moderate drought-stress exerts a positive effect on soil quality in the grass's species due to the increased fine root turnover and rhizodeposition. Likewise, previous studies reported that moderate drought stress facilitated the growth of fine roots, leading an increase in the root-to-shoot biomass ratio [54,55]. In our investigation, we observed a significant increase in root biomass under moderate drought, indicating a higher efficiency of the AMF inoculum when compared to the optimal water condition (no drought). This result may be associated with the intrinsic characteristic of the AMF inoculum, since it was obtained in a hostile environment and, therefore, the moderate drought here guarantees the best performance of the AMF species that compose it.

According to Torres-Arias et al. [14], investigating different abiotic factors for AMF inoculum production, the native AMF species were well adapted to the edaphoclimatic conditions of the area that were screened and, therefore, ensuring the success of the symbiosis and better development of the plants. This is leading different research groups to postulate the choice of native AMF inoculants instead of commercial or AMF isolates [56, 57]. Therefore, local adaptation can represent a powerful factor in establishing new combinations of fungi and plants [58]. Indeed, according to the recent meta-analysis of Liu et al. [59], the use of microbial consortium increased plant growth by 48%, whilst single inoculation increased only 29% compared with uninoculated treatments. Furthermore, the interaction between AMF and other microbes, such as bacteria, has been shown to increase nutrient uptake by plant and alleviating drought [9.60-62].

Overall, under drought, the soil microbial activity used to decline, including decreasing in enzyme production (e.g., phosphatases, pyrophosphatase) and nutrient cycling, leading to a decrease in soil fertility followed by lower plant productivity and loss in economy [63]. This is also supported by Hosseini et al. [64], which found that severe drought considerably decreased the phosphatase activity by 67% in comparison with optimum moisture.

On the other hand, our findings showed an increase in soil microbial activity based on increases of acid phosphatase (up to 32 %) and pyrophosphatase activities (up to 263 %) in the soil under severe drought compared to no drought. These results suggest a greater demand for P under severe drought, which was not converted into biomass, since no increase in plant weight was observed. Meanwhile, a greater amount of microbial biomass phosphorus (MBP) was observed under severe drought, indicating that under severe drought events in the soil, the increase in the enzyme activities related to P availability was associated with P allocation in the

soil microbial biomass, i.e., a greater P microbial immobilization. This result corroborates Sardans and Peñuelas [65] who found a decrease in phosphorus availability with increasing drought.

Although P immobilization is sensitive to drought, due to decreased diffusion and, subsequently, microbial uptake [66,67], our findings suggest that P immobilization was facilitated under severe drought. However, it is worth noting that microbial P immobilization may be temporary and P may be released during microbial biomass turnover in response to moisture conditions or when carbon becomes limiting, for example [68].

In our investigation, increases in AMF colonisation, soil microbial carbon and glomalin content were observed in plants under no drought. According to de Vries et al. [69], plants under no water stress are actively growing and allocating photosynthate carbon to AMF in the rhizosphere, allowing different AMF taxa to colonize plants and, therefore, a higher AMF root colonisation is expected. On the other hand, plants under moderate drought have their photosynthesis and root exudation down-regulated, beneficing only direct interactions with AMF and bacteria, as well as reducing the heterotrophic microbial activity [69]. Here, these direct interactions would be linked to the increase in plant biomass observed under moderate drought (up to 104 %) compared to no drought. According to Chareesri et al. [70], AMFs under drought stress are able to increase the plant hormone-driven pathways, conferring them drought tolerance and high yields.

Here, no difference in plant enzyme activity related to the antioxidant defence system was observed and can be understood as an alleviation of oxidative stress in plants due to AMF inoculum [71]. On the other hand, if considering the tendency observed, the better effect of the AMF inoculum could be more related to the decrease in ascorbate peroxidase (APX) activity or increase of superoxide dismutase (SOD) activities as observed in plants under the moderate drought that stood out in the growth of plant biomass.

Likewise, Amer et al. [72] showed that especially APX and catalase activities significantly decreased after AMF inoculation when water deficit was applied. These authors explained that this probably occurred due to the increased absorption of water provided by AMF hyphae and its transfer to the host plant, decreasing the generation of reactive oxygen species. However, Chandrasekaran [73] argues that the benefits of AMF inoculation to alleviate drought stress are more related to the reduction of non-enzyme antioxidants such as hydrogen peroxide, malondialdehyde, and electrolyte leakage.

Altogether, our investigation showed that the native AMF inoculum effectiveness varied according to soil water content based on biomass growth, but did not provide evidence that AMF inoculation could increase nutrient uptake in maize plants. On the other hand, changes in

soil parameters related to phosphorus cycling and no change in the plant response of antioxidant system led us to argue that an AMF legacy effect can be considered. These findings reinforce the importance of our efforts in this investigation, especially given the strong scientific consensus that water scarcity will have severe long-term effects on crop yields.

3.5. Conclusions

This study underscores the potential of AMF inoculum from the harsh environment to increase plant biomass under moderate stress. Furthermore, our findings reveal an important association between microbial biomass phosphorus, acid phosphatase and pyrophosphatase activity shifts in soil and lower plant performance in terms of biomass under severe drought. We argue that a better understanding of mycorrhizal responsiveness to enhance plant performance would be achieved in a further investigation that addresses AMF inoculum effectiveness considering its origin and soil water content, crops with different rhizodeposition patterns and genotypes, and wide edaphoclimatic conditions.

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Supplementary Materials

Appendix A

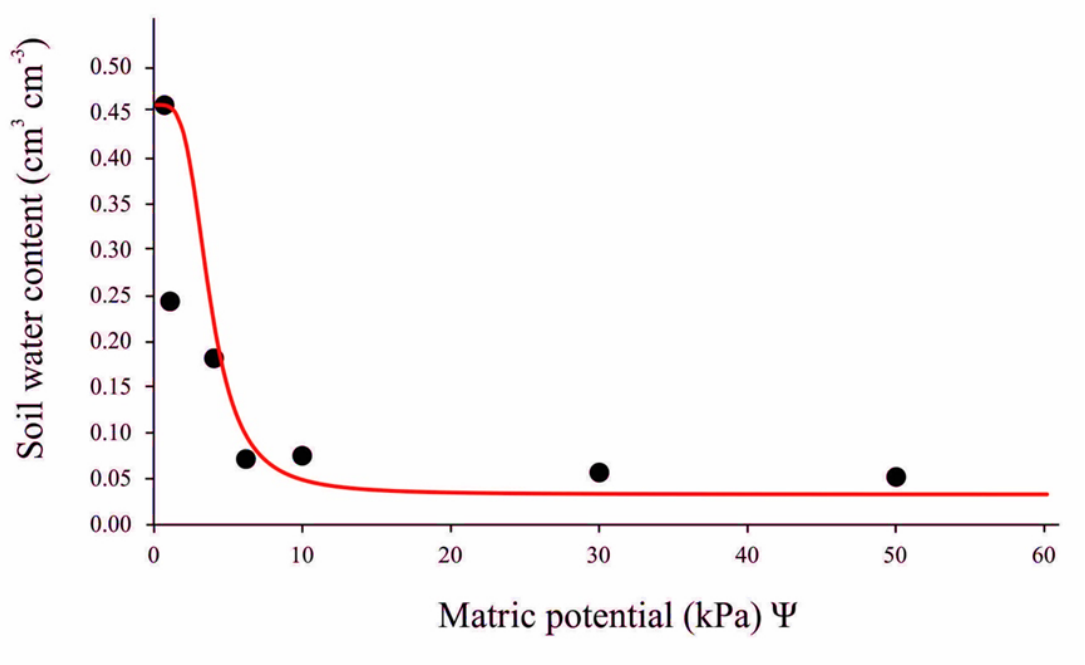

Figure A1. Water retention curve of the soil used for the experiment.

Appendix B

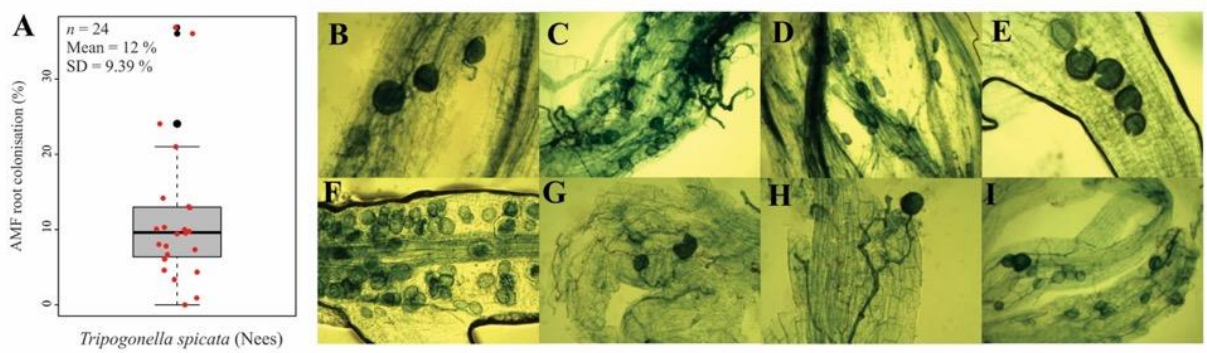

Figure A2. Root arbuscular mycorrhizal colonisation (AMF) traits. (A) boxplot showing all samples (n = 24) that composed the AMF inoculum (red dots) and outliers' presence (black dots). SD: standard deviation. (B) AMF spores inside the root. (C) fungal mycelium. (D) AMF vesicles (oval shaped). (E and F) spores and fungal hyphae. (G to I): spores germinated inside the root.

Appendix C

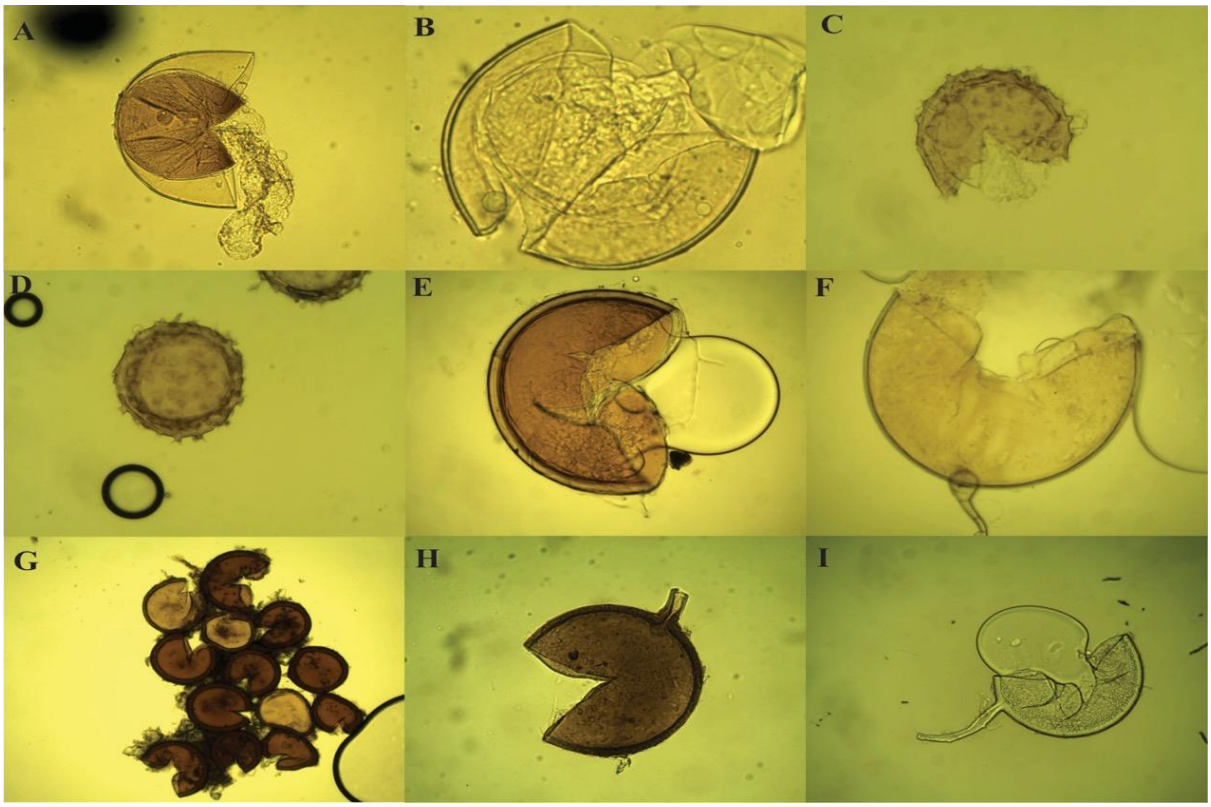

Figure A3. Arbuscular mycorrhizal fungi that composed the soil inoculum. (A) *Acaulospora morrowiae* (PVLG+Melzer) 20x. (B) *Acaulospora morrowiae* (PVLG) 40x. (C and D) *Ambispora* sp. (PVLG) 20x. (E) *Gigaspora decipiens* (PVLG+Melzer) 20x. (F) *Gigaspora gigantea* (PVLG) 10x. (G) *Glomus glomerulatum* (PVLG+Melzer) 10x. (H) *Glomus* sp. (PVLG+Melzer) 20x. (I) *Rhizophagus clarus* (PVLG) 10x.

4. CAN ARBUSCULAR MYCORRHIZAL FUNGI AND RHIZOBACTERIA FACILITATE ^{33}P UPTAKE IN MAIZE PLANTS UNDER WATER STRESS? [3]

Abstract

Arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) are able to provide key ecosystem services, protecting plants against biotic and abiotic stresses. Here, we hypothesized that a combination of AMF (*Rhizophagus clarus*) and PGPR (*Bacillus* sp.) could enhance ^{33}P uptake in maize plants under soil water stress. A microcosm experiment using mesh exclusion and a radiolabeled phosphorus tracer (^{33}P) was installed using three types of inoculation: i) only AMF, ii) only PGPR, and iii) a consortium of AMF and PGPR, alongside a control treatment without inoculation. For all treatments, a gradient of three water-holding capacities (WHC) was considered i) 30 % (severe drought), ii) 50 % (moderate drought), and iii) 80 % (optimal condition, no water stress). In severe drought conditions, AMF root colonization of dual-inoculated plants was significantly lower compared to individual inoculation of the AMF, whilst ^{33}P uptake by dual-inoculated plants or plants inoculated with bacteria was 2.4-fold greater than the uninoculated treatment. Under moderate drought conditions the use of AMF promoted the highest ^{33}P uptake by plants, increasing it by 2.1-fold, when compared to the uninoculated treatment. Without drought stress, AMF showed the lowest ^{33}P uptake and, overall, plant P acquisition was lower for all inoculation types when compared to the severe and moderate drought treatments. The total shoot P content was modulated by the water-holding capacity and inoculation type, with the lowest values observed under severe drought and the highest values under moderate drought. The highest soil electrical conductivity (EC) values were found under severe drought in AMF-inoculated plants and the lowest EC for no drought in single or dual-inoculated plants. Furthermore, water-holding capacity influenced the total soil bacterial and mycorrhizal abundance over time, with the highest abundances being found under severe and moderate drought. This study demonstrates that the positive influence of microbial inoculation on ^{33}P uptake by plants varied with soil water gradient. Furthermore, under severe stress conditions, AMF invested more in the production of hyphae, vesicles and spore production, indicating a significant carbon drain from the host plant as evidenced by the lack of translation of increased ^{33}P uptake into biomass. Therefore, under severe drought the use of bacteria or dual-inoculation seems to be more effective than individual AMF inoculation in terms of ^{33}P uptake by plants, while under moderate drought, the use of AMF stood out.

Keywords: Phosphate nutrition, isotope tracer, water shortage, plant symbiosis, soil-dwelling microbes.

4.1. Introduction

According to the Food and Agriculture Organization of the United Nations (FAO), drought is now recognised as the primary reason for agricultural production losses globally, costing the sector USD 37 billion overall from 2008-2018. However, other extreme events caused by climate change, such as floods and heatwaves, are also contributing to ongoing issues with food security (FAO, 2021). Therefore, improved management approaches are urgently required to improve agricultural sustainability. This is particularly relevant for the supply and exploitation

[3] Article published in Microbiological Research (https://doi.org/10.1016/j.micres.2023.127350)

of soil nutrients which have a finite supply, such as phosphorus (P). This is even more important when we consider the dramatic rise in fertilizer prices over the past year (Smith, 2022). To overcome the combined impact of drought stress and low nutrient use efficiency in cropping systems and the design of new management systems requires a greater fundamental understanding of plant-soil-microbial interactions. Mawarda et al. (2020) highlighted that arbuscular mycorrhizal fungi (AMF) and rhizobacteria may provide an environmentally friendly solution to this combined problem.

In highly weathered soils, where the exchange surfaces are dominated by aluminium and iron oxides/hydroxides, a large proportion of the applied phosphate fertiliser (ranging from 15-30 %) becomes rapidly immobilized on the solid soil phase by adsorption and precipitation processes (Dhillon et al., 2017; Zavaschi et al., 2020). AMF may provide a tool to exploit native soil P reservoirs or residual fertiliser-derived P ("legacy P") that has accumulated over the past 50 years in these soils (Scrase et al., 2019; Pavinato et al., 2020a; Pavinato et al., 2020b). AMF is a key group of soil microorganisms that form symbiotic associations with more than 80 % of all land plants and play an important role in the acquisition of nutrients (Smith and Read, 2008). For example, in maize (*Zea mays* L.), AMF is more important than root hairs for seedling growth under low P availability (Ma et al., 2021). Concurrently, it is widely acknowledged that AMF and plant growth-promoting rhizobacteria (PGPR) can play an important role in the amelioration of a wide range of plant biotic and abiotic stresses such as drought, salinity, heavy metal exposure, and soil-borne pathogens (Pérez-de-Luque et al., 2017; Santoyo et al., 2021; Chen et al., 2022). There is also evidence that co-inoculation with AMF and PGPR can increase plant growth and health through additive and/or synergistic effects between them (Saia et al., 2015; Battini et al., 2017; Dutta and Neog, 2017; Nanjundappa et al., 2019). Understanding the plant-mycorrhizae-rhizobacteria interactions is crucial, as plants dedicate 5-30 % of their photo-assimilate to supporting bacterial growth in soil (Carvalhais et al., 2011; Almeida et al., 2020). A further 20 % of photo-assimilate is allocated to the maintenance of symbiotic AMF networks (Smith and Read, 2008).

Some studies have demonstrated the ability of AMF or PGPR alone to promote plant growth under water shortage events. It was postulated that AMF mechanisms, such as improvements in soil aggregation, photosynthetic efficiency, and nutrient uptake are primarily responsible for this response (Ji et al., 2019; Quiroga et al., 2019; Al-Arjani et al., 2020). In the case of PGPR, the main reported mechanisms include direct (e.g., changes in hormonal signalling, P solubilisation, biological nitrogen fixation) and indirect mechanisms (e.g., antibiotic production, cell wall degrading enzymes, induced systemic resistance, osmotic adjustment,

quorum quenching, and siderophore production) (Glick, 2012; Olanrewaju et al., 2017; Naylor and Coleman-Derr, 2018; Araújo et al., 2020). Overall, the majority of crop plant species are responsive to mycorrhizal symbiosis and rhizobacteria inoculation. This discovery has subsequently led to the search for novel microbes with the potential to increase crop yields, especially maize, as this represents one of the most important global crops (Zhao et al., 2017; Li et al., 2021). Recently, the use of maize has become more inviting due to the current scenario of bio-economy, which has incentives for production of biofuels to reduce CO_2 emissions. Thus, there is the possibility to intensify the biofuels market, making it more prosperous and stable (Eckert et al., 2018). A myriad of earlier studies (Rhodes and Gerdemann, 1975; Jakobsen et al., 1992; Pearson and Jakobsen, 1993; Battini et al., 2017; Jongen et al., 2022) have demonstrated the ability of AMF hyphae to recover and translocate $^{32/33}$P located beyond the immediate root zone.

Nevertheless, these studies do not consider how the efficiency of AMF-mediated P absorption process is affected under a gradient of soil water availability, especially when considering the presence of PGPR capable of tolerating low water activity. Here, we combine experiments that simultaneously addressed i) the P dynamics (sorption and diffusion) using radiolabeled phosphorus tracer (^{33}P), ii) the soil water gradient and iii) the presence or absence of specific AMF and PGPR strains. Our investigation was set up to test the hypothesis that the combined use of AMF and PGPR would enhance P uptake in maize plants under drought stress. For this, we evaluated ^{33}P uptake in maize plants, the response of soil phosphatase activity, and key soil chemical attributes, as well as monitoring the abundance and dynamics of soil mycorrhizal and bacterial communities.

4.2. Materials and Methods

4.2.1. Experimental design

The microcosm experiment was set up in a completely randomized design, comprising a double factorial scheme (4 × 3) with three replicates. The first factor was the inoculation of microorganisms (either only AMF, only PGPR, or the consortium of AMF and PGPR, besides a control without any inoculation). The second factor was water stress (80, 50, and 30 % of the water-holding capacity, simulating no drought, moderate drought, and severe drought, respectively). We established the water-holding capacity of 30 %, 50 % and 80 % as severe, moderate and no drought, respectively, according to previous investigations (Kavamura et al., 2013; Araujo et al., 2020; Santos et al., 2020). Each experimental unit comprised a plastic pot (8 cm internal diameter × 7 cm high), containing 200 g (dry weight) of sterilized soil (at 121 ºC for 2

h) (Figs. 1a and 1b), ensuring that bacteria and fungi that were traced were only those from the inoculum.

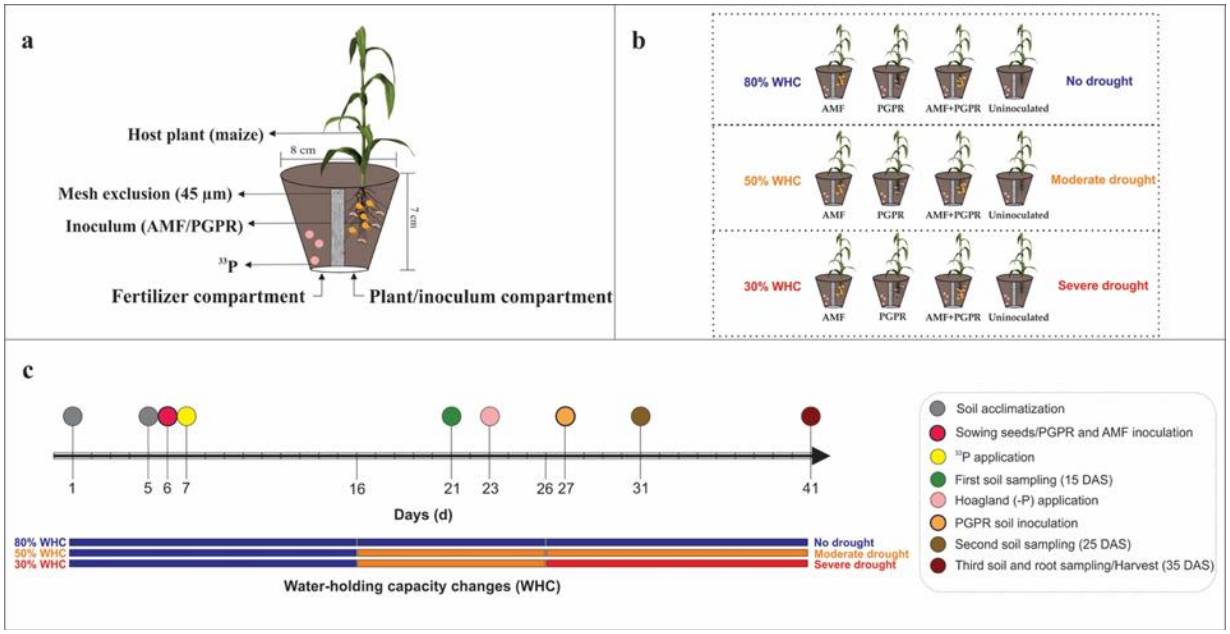

Fig. 1 Experimental representation, (a) pot using a root exclusion mesh to create two compartments, one of which was fertilized and the other, planted and inoculated. (b) Summary of the treatments evaluated in the experiment. (c) Timeline of the experiment. WHC: water-holding capacity; DAS: days after sowing; PGPR: plant growth-promoting rhizobacteria; AMF: arbuscular mycorrhizal fungi

The soil was obtained from the Ah horizon (0-10 cm deep) of a field site located in Brazil (22°42' S, 47°38' W) and classified as an Arenosol (WRB-FAO, 2015), with low P content (Table 1). The A horizon has been used in the diagnosis of the main epipedons in soil taxonomy and here we use the Ah horizon as the most representative of the sampled area. Soil chemical characterization was evaluated according to van Raij et al. (2001).

Table 1. Physico-chemical characterization of the initial studied soil

Material	pH*	O.M	P	S	K	Ca	Mg	Al	H+Al	SB	CEC	V*	Sand	Silt	Clay
		g dm^{-3}	-mg dm^{-3}-		----------	----------	$mmol_c$ dm^{-3}	----------	----------	----------	----------	%	----------	g dm^{-3}	----------
Soil	4.1	15.0	<6.0	18.0	0.8	4.0	3.0	7.0	28.0	7.8	35.8	22.0	751	23	226

pH: measured in $CaCl_2$. O.M: organic matter - colorimetric method. P: phosphorus with anion exchange resin. S: sulfur - 0.01 mol L^{-1} calcium phosphate. K, Ca and Mg: potassium, calcium and magnesium measured in anion exchange resin. Al: aluminum in 1 mol KCl L^{-1}. H + Al: potential acidity in SMP buffer. SB: sum of bases (K + Ca + Mg). CEC: cation exchange capacity. V: base saturation. $mmol_c$ kg^{-1}: millimoles of charge per kilogram of soil according to SI unit (International Standard of Units). *Soil pH was increased to 6.5 and base saturation to 70 % after liming performed according to van Raij et al. (1997) using dolomitic lime (1.71 Mg ha^{-1}) with 100% relative power of total neutralization (80% Ca^{2+} and 20% Mg^{2+}).

Mesh exclusion (45 µm) was utilized to divide the pot into two compartments: a fertilized compartment and a planted/inoculated compartment, each of them receiving 100 g of sterilized soil. This mesh allowed fungal hyphae to pass through and absorb nutrients but prevented the ingrowth of roots from the plant compartment, inoculated or not. This approach has been used

to investigate the role of mycorrhizae on plant growth and water supply (Cardoso et al., 2004; Neumann and Matzner, 2013; Scrase et al., 2019; Kakouridis et al., 2020). Nevertheless, we set up a preliminary experiment (Experiment 1) to confirm that roots were not able to pass through the mesh, while simultaneously also determining seed germination rate in the soil and the correction factor needed when calculating water-holding capacity over the course of subsequent experiments (Supplementary Note 1).

The mode of fertiliser amendment in the compartment was based on the application of 2.8 mL of 6 mM KH_2PO_4 (equivalent to 30 mg P kg^{-1} soil, as recommended for this type of weathered soil; van Raij et al., 1997) with a ^{33}P activity of 185 kBq. The fertiliser amendment was carried out after the soil acclimatization, sowing of seeds and inoculation of microbes in the planted/inoculated compartment (Fig. 1c). Furthermore, we set up an additional microcosm experiment to determine the distance that P can diffuse in the soil to confirm that the presence of ^{33}P in the planted/inoculated compartment could only occur via microbial transfer. In addition, we also measured P sorption to the soil to characterize the P-dynamics in this soil (Supplementary Note 2).

4.2.2. Fungal and bacterial inoculum

Fungal and bacterial strains were previously isolated from Serra do Ouricuri, Petrolina, Pernambuco, Brazil (39°3' S, 8°28 W) in the Caatinga Biome, where a bio-prospecting program was developed to find microbes with the potential of helping crop plants to tolerate drought stress (Kavamura et al., 2013; Fernandes-Júnior et al., 2015). Initially, a pool of fungi and bacteria was isolated from the rhizosphere of *Tripogonella spicata* (Nees) plants, the so-called resurrection grass, due to its surprising rehydration capacity after a drought period (Fernandes-Júnior et al., 2015; Aidar et al., 2017). Then, those microrganisms were selected for their plant growth-promoting properties, such as the ability to grow under reduced water availability (Hallsworth et al., 1998), indole-3-acetic acid (IAA) production (Bric et al., 1991; Kuss et al., 2007) and calcium phosphate solubilization (Verma et al., 2001).

Bacillus sp. was cultivated in 10 % (w/v) TSB (trypticase soy broth) culture medium at 30 °C and 150 rpm for 48 h. The inoculum was homogenized at OD_{550} = 0.2 and washed twice in a 0.85 % (w/v) saline solution to obtain the bacterial suspensions (at 10^8 CFU mL^{-1}). A second bacterial inoculation (3 mL pot^{-1} at 10^8 CFU mL^{-1}) was done when the water-holding capacity was changed to 30 % (i.e., twenty-one days after sowing). The second bacterial inoculation was performed due to the drastic reduction in soil water content, thus maintaining bacterial activity in

the soils (Jeong et al., 2013; Armada et al., 2018; Mawarda et al., 2020). The same amount of sterile saline solution was provided to the AMF and control treatments (Fig. 1c).

Rhizophagus clarus spores were obtained from the pure trap culture, using maize as host plant, and transferred to the soil after surface-disinfection, directly under the seeds, in the form of 5 mL of sterile water containing 50 spores at the time of sowing. Spores of AMF were surface-sterilized by exposing them to 0.5 % sodium hypochlorite in a filter unit allowing contact for 15 minutes and then repeatedly rinsing in sterile water (Johnson and Pfleger, 1992; Habte and Osorio, 2001; Maia and Yano-Melo, 2001). The germination rate of *R. clarus* spores in the soil was around 85 % according to our third additional microcosm experiment, as reported in Supplementary Note 3.

4.2.3. Plant material, cultivation, and water-holding capacity management

Uniformly sized seeds of *Zea mays* L. (cv. BRS Gorotuba) obtained from the Brazilian Agricultural Research Corporation (EMBRAPA) were surface sterilized twice in 2 % (v/v) sodium hypochlorite solution for 7 min, 70 % (v/v) ethanol for 1 min, and rinsed thoroughly with sterile MilliQ water. The germination rate of seeds in soil and Petri dishes was around 90 %. Seeds inoculated with PGPR or for doubled-inoculated plants with PGPR and AMF were soaked in the bacterial suspension for 2 h, whilst seeds for only AMF or with uninoculated plants were soaked in 0.85 % (w/v) sterile saline solution for the same time (Kavamura et al., 2013). Two seeds were sown in the right compartment in each pot and thinning was done when one of the seedlings presented two true leaves. Soil bacterial inoculation was done 21 days after sowing, in PGPR or consortium treatments, when pots were reduced to 30 % of the water-holding capacity, according to the water content management described below. At the same time, 0.85 % (w/v) sterile saline solution was applied to the AMF-inoculated or the uninoculated plants.

Plants were cultivated in a Conviron Adaptis® CMP 6010 growth chamber (Controlled Environments Ltd, Winnipeg, Manitoba, Canada) at the Environment Centre Wales, Bangor University, United Kingdom (53º13' N, 4º7' W). Plants were maintained under a day/night cycle of 16/8 h, 25/20 °C, 70 % relative humidity, receiving artificial lighting at a photosynthetic photon flux density of 500 µmol m^{-2} sec^{-1}. Hoagland solution (without phosphorus) was applied 17 days after sowing, to keep nutritional balance of the plants. The final solution of pH 5.5 was composed of 4 mM Ca $(NO_3)_2$, 6 mM KNO_3, 2 mM $MgSO_4$, 1 mM Fe-EDTA, and 1 mM trace elements (Hoagland and Arnon, 1950).

The microcosms were randomized daily to ensure equal growth conditions and were weighed for 35 days, and the desired moisture was maintained with the addition of deionized and sterilized water when needed. At the beginning of the experiment, all microcosms (n = 36) were kept at 80 % water-holding capacity to ensure seed germination. Ten days after sowing, $^{2}/_{3}$ of the microcosms (n = 24) were reduced to 50 % water-holding capacity. Finally, twenty days after sowing, $^{1}/_{3}$ of the microcosms (n = 12) were reduced to 30 % water-holding capacity (Fig. 1c). This approach was used to facilitate understanding the potential of inoculated microbes in the context of a decreasing gradient of soil water content (Ahmad et al., 2018; Czarnes et al., 2020; Lopes et al., 2021).

After 35 days of growth, the plants were harvested, and separated into shoot and root material, whilst the soil was separated into fertilized and planted/inoculated compartments. In addition, soil was sampled in the planted/inoculated compartment five days after changing the water-holding capacity (i.e., on the 15th and 25th day after sowing) to monitor soil bacteria and mycorrhiza total abundance via quantitative polymerase chain reaction (qPCR). Sampling for assessing bacterial and fungal abundances were executed with a sterile polypropylene cylinder (8 mm inner diameter) to avoid disturbances during plant growth and not to affect the fungal hyphae.

4.2.4. Analytical procedures

At the end of the experiment (i.e., 35 days after sowing) plant height was measured using a metal ruler (Westcott Ltd., Chichester, England, United Kingdom) placed on the ground next to the tallest stem, while basal diameter was measured using a digital micrometer (Moore and Wright Ltd., Sheffield, England, United Kingdom). Shoot was cut off at the soil surface. Later, it was dried at 80 ºC in paper bags, for 16 h before, quantifying the dry weight. The roots were separated from soil by gently shaking them and rinsing them with water. Then, 5 g of roots were placed in a 50 cm^{3} polypropylene tube with 70 % ethanol for analysis of mycorrhizal colonization percentage, and the remainder was dried to obtain the dry weight. For phosphorimager analysis to visualize the ^{33}P, dried plant tissue was placed in a 20 × 25 cm cassette for 1 h, and then analyzed in a Bio-Rad Molecular Imager® FX (Bio-Rad Laboratories Inc., Hercules, California, United States of America).

Soil available phosphorus was extracted using 0.5 M acetic acid (1:5 w/v) according to Fisher et al. (1998). Samples were extracted by shaking (200 rpm for 30 min at room temperature), centrifuging for 15 min (18,000 *g*), filtering and the supernatant was recovered for

analysis. ^{33}P activity of the samples was determined in counts per minute (CPM) of ^{33}P using 1 mL of soil extract and 4 mL of HiSafe 3 Scintillation cocktail (PerkinElmer, Waltham, Massachusetts, United States of America) in a Wallac 1404 scintillation counter with automated quench correction (Wallac EG&G, Milton Keynes, United Kingdom). For plant tissue, the extract was obtained by placing 0.2 g in a muffle furnace and ashing at 500°C overnight. Later, this was dissolved in 1 mL of 20 % HCl and 9 mL of deionized water was added, according to Adrian (1973). The colorimetric P determination in soil and plant tissue was determined according to Murphy and Riley (1962).

The soil pH and electrical conductivity were determined according to Thomas (1996), whereby 10 g of 2 mm-mesh sieved soil was mixed with 25 ml of water and shaken (10 min at 200 rpm) and then allowing the sample to settle for 10 min before taking measurements with standard electrodes.

The acid and alkaline phosphatase activities (EC 3.1.3.2 and EC 3.1.3.21, respectively) were measured using the methodology described by Marx et al. (2001). For evaluation of AMF root colonization, the roots were prepared according to Vierheilig et al. (1998), with the roots dispersed in a Petri dish with a grid background and scored using a stereomicroscope according to Giovannetti and Mosse (1980).

4.2.5. Molecular analysis

Soil (0.25 g) was utilized for DNA extraction using DNeasy® PowerSoil® Pro Kit (QIAGEN Inc., Germany) according to the manufacturer's protocol. Extracted DNA was stored at -80 °C before quantitative PCR analysis. DNA concentrations were determined using the Qubit quantification platform with Qubit 1X dsDNA HS Assay Kit (Invitrogen, Carlsbad, California, United States of America).

The quantitative PCR (qPCR) was used to determine gene copy number per gram of soil for bacteria (16S rRNA) and AMF (LSU rDNA region), using the StepOnePlus™ Real-Time PCR System (Applied Biosystems Inc., Carlsbad, California, United States of America) with the fluorescent marker GoTaq® qPCR Master Mix (Promega, Madison, Wisconsin, United States of America). All samples were analysed in triplicate.

16S rRNA reactions were run in 10 µL comprising 5 µL of GoTaq® qPCR Master Mix, received 1 µL (5 µM) of each primer (Eub338 5'-CCTACGGGAGGCAGCAG-3' and Eub518 5'-ATTACCGCGGCTGCTGG-3'), 0.1 µL of CXR Reference Dye, 2 µL of DNA template, and 0.9 µL nuclease free sterile water in the same conditions as described by Muyzer et al. (1993).

Standard curves were obtained using 7-fold serial dilutions of purified PCR (10^2 to 10^8 copies) containing the targeted gene. The reliability of the standard curves was controlled by verifying reproducibility of the Ct values, the quality of the dilution series, and the efficiency (101.42 %, R^2 = 0.993). The specificity of the primers was confirmed by melting curves analysis.

AMF reactions were run in 10 µL comprising 5 µL of GoTaq® qPCR Master Mix, received 1 µL (5 µM) of each primer (FLR3 5'-TTGAAAGGGAAACGATTGAAG T-3' and FLR4 5'-TAC GTCAACATCCTTAACGAA-3'), 0.1 µL of CXR Reference Dye, 2 µL of DNA template, and 0.9 µL free sterile water. FLR3 is localized between the D1 and D2 domains of LSU rRNA, whilst FLR4 is in the D2 domain (Gollotte et al., 2004). Standard curves were obtained using 7-fold serial dilutions of purified PCR (10^2 to 10^8 copies) containing the targeted gene. The reliability of the standard curves was controlled by verifying reproducibility of the Ct values, the quality of the dilution series and the efficiency (101.46 %, R^2 = 0.975). The specificity of the primers was confirmed by melting curves analysis.

4.2.6. Data analyses

Data were tested for normal distribution using the Shapiro-Wilk test, followed by the homogeneity of variances tests, using the Bartlett test. Having met the criteria (residuals normality and variance homoscedasticity), a two-way analysis of variance (ANOVA) was performed and, when appropriate, Tukey's posthoc pairwise comparison (cut-off significance at $p < 0.05$) was applied to determine individual differences between means.

Principal components analysis (PCA) was performed using the statistical packages FactoMineR and factoextra in the R® program (R Core Team, 2017). In PCA, to meet the premise of multivariate normality, the data were transformed into log (x + 1), and the attributes subject to collinearity were removed (Ramette, 2007). Additionally, using k-means clustering algorithm, an unsupervised machine learning method of identifying and grouping similar data points, we classified our variables into groups (Jansson et al., 2022).

4.3. Results

In both soil compartments i.e., planted/inoculated and the fertilized compartment, we determined their ^{31}P and ^{33}P content alongside soil pH, electrical conductivity, and soil acid and alkaline phosphatase activity. In addition to plant P content, AMF root colonization and number of spores for the planted/inoculated compartment were determined. Here, we primarily

concentrate on the results from the planted/inoculated compartment, while results from the fertilized compartment are present in the supplementary material. Data are reported on P uptake by arbuscular mycorrhizal hyphae, as revealed by the use of radioactive P (^{33}P), added to the fertilized compartment. Overall, inoculation of AMF or PGPR positively influenced plant growth, radiolabelled P uptake, mycorrhization and soil characteristics, when compared to the uninoculated control. Nevertheless, the positive influence of microbial inoculation varied with soil water gradient.

4.3.1. ^{33}P uptake by plants, biomass, and P pool under different treatments

Soil water content greatly affected ^{33}P uptake by the plants, with the highest ^{33}P uptake (0.4 kBq.plant^{-1}, on average) observed in moderate drought (50 % WHC) with the lowest uptake (0.2 kBq.plant^{-1}, on average) observed in the absence of drought (80 % WHC) (Figs. 2a and 2b).

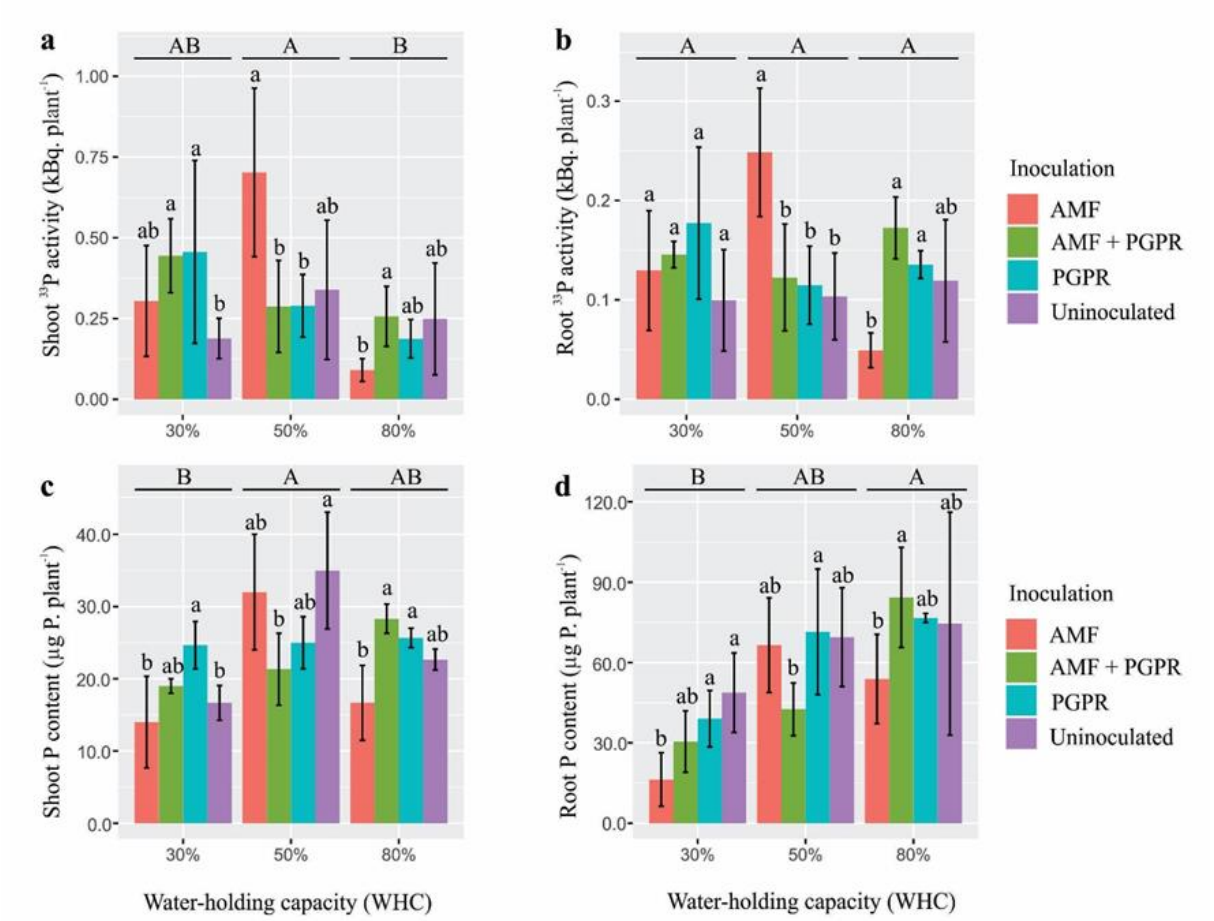

Fig. 2 The impact of arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) inoculation on P acquisition under three different soil water-holding capacities (WHC). (a) ^{33}P activity in shoot and (b) root. (c) Total P content (^{31}P + ^{33}P) in the shoot and (d) root. Uppercase letters compare differences in WHC, while lowercase letters compare differences according to inoculant types by Tukey's test at 5 % ($p \leq 0.05$). Standard errors are shown ($n = 3$).

Overall, for the planted/inoculated compartment, the highest levels of ^{33}P activity in soil were found in the presence of mycorrhizal inoculum under severe or moderate water stress, but this was not detected in soil from the fertilized compartment (Figs. S1a and S1b). In addition, for shoot biomass, the main difference among the inoculum types occurred in severe drought, with the highest biomass found in the presence of bacterial inoculum and the lowest biomass in the presence of mycorrhizal inoculum (Fig. S2a). This same pattern was detected for morphological traits, such as height and diameter (Fig. S2b and S2c).

Under severe drought (30 % WHC), ^{33}P uptake in shoot was 2.4-fold greater ($p \leq 0.05$) in the PGPR and AMF+PGPR treatments than in uninoculated control. Whilst under moderate drought (50 % WHC), ^{33}P uptake in shoot of the AMF treatment was 2.1-fold greater ($p \leq 0.05$) than in the uninoculated control, outperforming the other inoculation types. On the other hand, under optimal conditions (80 % WHC), the highest ($p \leq 0.05$) ^{33}P uptake in shoot was found in

AMF+PGPR and the lowest ($p \leq 0.05$) in AMF treatment (Fig. 2a, Fig. S3). The same pattern of ^{33}P uptake was seen in the root (Fig. 2b, Fig. S4).

The total shoot P content was modulated by the water-holding capacity and inoculation type, with the lowest values ($p \leq 0.05$) observed under severe drought (18.6 μg P plant^{-1}, on average) and the highest values ($p \leq 0.05$) under moderate drought (28.3 μg P plant^{-1}, on average), almost reflecting the results of those for ^{33}P uptake (Fig. 2c). However, this did not occur in the roots, in which there was an increase in P content with the increase of water content (Fig. 2d). Under severe drought, the highest shoot P content was observed in the PGPR treatment ($p \leq 0.05$) whilst under moderate drought the uninoculated control was, in general, superior to all other inoculation types. Under no water stress, shoot P content in AMF+PGPR and PGPR was 1.7 and 1.5-fold greater than in the AMF treatment, respectively (Fig. 2c). Regarding the P pool, it was observed that, under severe drought, in the AMF, PGPR, AMF+PGPR and uninoculated treatments the contents were 2.40, 2.74, 2.49, and 3.33 mg P.100g soil^{-1}, respectively. Whist under moderate drought, following the same sequence of treatments, P contents were 2.07, 2.98, 3.45, and 3.06 mg P.100g soil^{-1}. Finally, for no drought, the following P contents were observed: 2.98, 3.22, 2.77, and 2.26 mg P.100g soil^{-1} (Fig. S1c). Overall, soil P contents were higher in the planted/inoculated compartment than in the fertilized compartment (Figs. S1c and S1d).

4.3.2. AMF root colonization and number of spores in soil

AMF root colonization was higher under severe (20.9 %, on average) and moderate water stress (21.8 %, on average) than in no drought (6.4 %, on average). Under severe drought, the AMF treatment showed a higher ($p \leq 0.05$) AMF root colonization percentage (56.7 ± 7.4) than the AMF+PGPR treatment (26.7 ± 16.1). Whilst under moderate and no drought, there was no difference ($p > 0.05$) between them (Fig. 3a). The number of spores differed only between the AMF and AMF+PGPR treatments under severe drought ($p \leq 0.05$), where the highest value was found in the AMF treatment (18 ± 6). Overall, the water-holding capacity did not influence ($p > 0.05$) the number of spores (Fig. 3b).

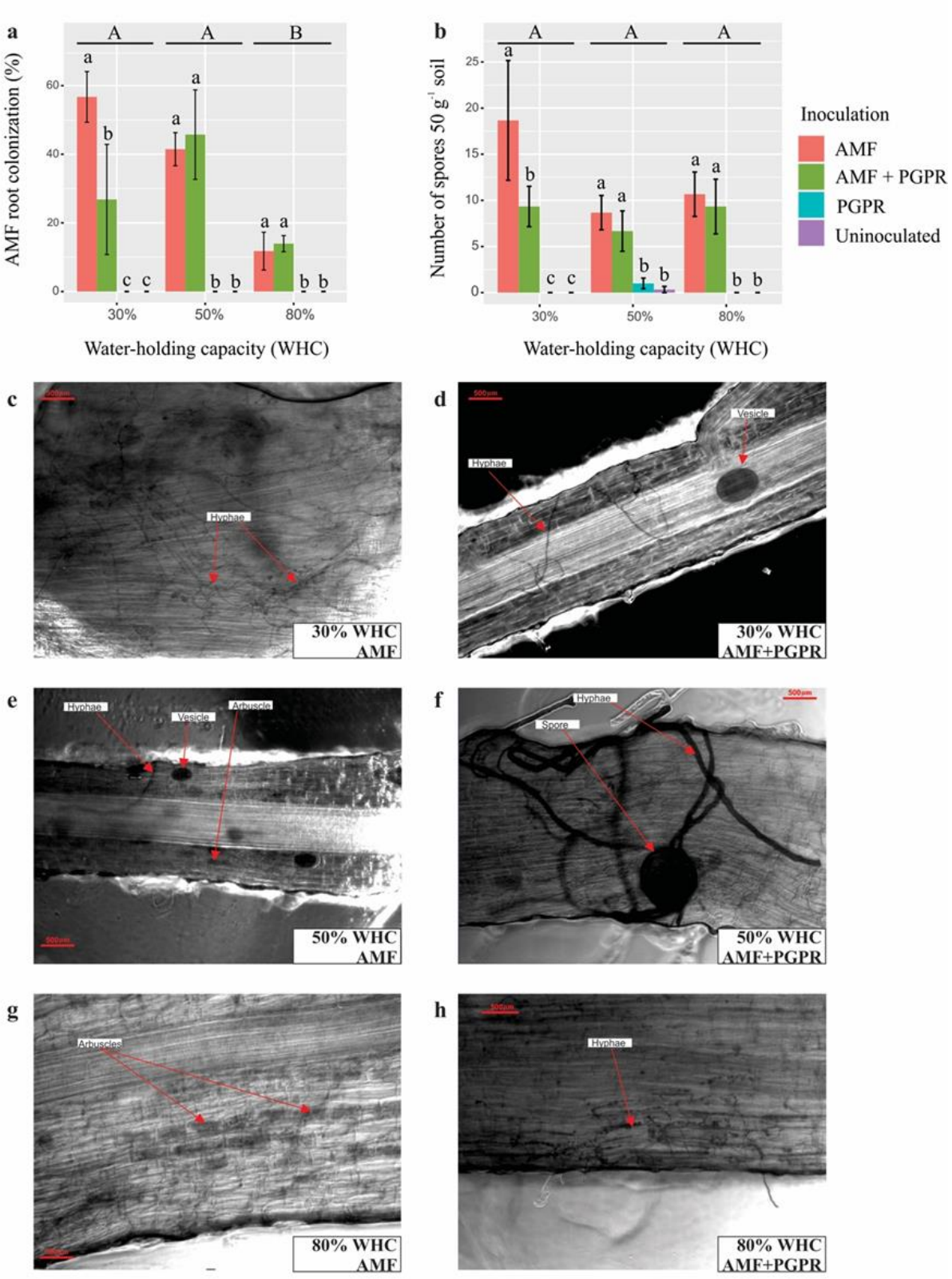

Fig. 3 Mycorrhization results, (a) Arbuscular mycorrhizal root colonization. (b) Number of spores in soil. (c to h) microscopy results showing the arbuscular mycorrhizal structures (extraradical hyphae, vesicle, arbuscules, and spore) in the treatments inoculated with arbuscular mycorrhizal fungi (AMF) or consortium of AMF and plant growth-promoting rhizobacteria (AMF+PGPR) under three different water-holding capacities (WHC). Uppercase letters compare differences in WHC, while lowercase letters compare differences according to inoculant types by Tukey's test at 5 % ($p \leq 0.05$). Standard errors are shown ($n = 3$).

According to the microscopy results, a different pattern in WHC response occurred in the presence of AMF structures inside the roots. Considering a severe drought in the AMF treatment, there was a large presence of hyphae (60 %), whilst in the AMF+PGPR treatment, we observed only about 40 % of hyphae and 20 % of vesicles (Figs. 3c and 3d, respectively). On the other hand, under a moderate drought, for AMF treatment, there was a higher presence of vesicles (50 %), hyphae (35 %), and arbuscules (10 %) (Fig. 3e). Considering the AMF+PGPR treatment, there was the presence of spores inside the root (20 %), as well as hyphae (20 %) and vesicles (30 %) (Fig. 3f). Without water stress, in the AMF treatment, there was a higher presence of arbuscules (25 %), while in the AMF+PGPR we only noticed the presence of hyphae (20 %) (Figs. 3g and 3h, respectively).

4.3.3. Soil pH and electrical conductivity, soil phosphatases activity, and microbial monitoring sampling time

The water-holding capacity did not influence the soil pH ($p > 0.05$), but it did influence ($p \leq 0.05$) the soil electrical conductivity (EC). The highest soil EC values were found under severe drought (86.1 $\mu S.cm^{-1}$, on average), and the lowest for moderate (64.7 $\mu S.cm^{-1}$, on average) and no drought (61.4 $\mu S.cm^{-1}$, on average). A subtle difference was found in soil pH between inoculum types, within the same soil water-holding capacity. Compared with the other treatments, the AMF treatment showed the lowest pH value under severe and moderate stress ($p \leq 0.05$), whilst non-water restriction showed the highest pH value ($p \leq 0.05$). The main difference in electrical conductivity (EC) was found under severe drought, where the AMF+PGPR treatment presented the lowest EC (Table 2).

The highest soil acid phosphatase activity was found in the uninoculated treatment without any water restriction, which was on average 3-fold higher than in the other treatments. There was no difference between treatments under severe drought, while under moderate drought, the uninoculated and PGPR treatments showed higher values compared to all other inoculation types. For soil alkaline phosphatase activity, the highest values were found, both, under moderate and non-stress conditions. An opposite behaviour was observed in the AMF+PGPR treatment, in which, under moderate stress, there was higher phosphatase activity, whereas in non-stress conditions, there was the lowest activity, when compared to the other treatments with the same water-holding capacity (Table 2). The results for pH, EC, acid, and alkaline phosphatase in the fertilized compartment are presented in Supplementary Table S1.

Table 2. Soil pH, electrical conductivity (EC), acid phosphatase activity (Ac. Phosphatase) and alkaline phosphatase activity (Alk. Phosphatase) in the planted/inoculated compartment at three contrasting soil water-holding capacities (WHC) and with different types of inoculum (arbuscular mycorrhizal fungi (AMF), plant growth-promoting rhizobacteria (PGPR), and consortium of AMF and PGPR)

WHC	Inoculum	pH	EC ($\mu S\ cm^{-1}$)	Ac. Phosphatase ($nmol\ g^{-1}\ soil\ h^{-1}$)	Alk. Phosphatase ($nmol\ g^{-1}\ soil\ h^{-1}$)
30% SD	AMF	6.5 ± 0.1 bA*	102.0 ± 10.0 aA	28.9 ± 10.7 aB	6.4 ± 0.7 bB
	AMF+PGPR	6.7 ± 0.1 abA	62.0 ± 6.0 bA	16.3 ± 7.9 aB	6.7 ± 0.7 bB
	PGPR	6.8 ± 0.04 aA	90.0 ± 4.0 abA	17.3 ± 4.6 aB	6.2 ± 0.2 bB
	Uninoculated	6.6 ± 0.1 abA	91.0 ± 22.0 abA	24.7 ± 8.1 aB	9.1 ± 1.0 aB
50% MD	AMF	6.8 ± 0.1 bA	65.0 ± 6.0 aB	18.5 ± 4.6 bB	7.9 ± 0.1 bA
	AMF+PGPR	7.1 ± 0.1 aA	69.0 ± 4.0 aB	26.3 ± 3.5 bB	12.0 ± 2.4 aA
	PGPR	6.5 ± 0.1 bcA	69.0 ± 17.0 aB	42.7 ± 5.5 aB	10.3 ± 0.3 abA
	Uninoculated	6.5 ± 0.02 cA	55.0 ± 2.0 bB	41.7 ± 4.7 aB	10.9 ± 0.5 aA
80% ND	AMF	7.0 ± 0.02 aA	63.0 ± 6.0 abB	38.0 ± 3.6 cA	11.1 ± 0.7 bA
	AMF+PGPR	6.7 ± 0.1 bA	60.0 ± 3.0 abB	64.1 ± 5.4 bA	9.3 ± 0.2 cA
	PGPR	6.7 ± 0.1 bA	64.0 ± 2.0 aB	39.4 ± 8.5 cA	12.3 ± 1.0 aA
	Uninoculated	6.8 ± 0.02 bA	58.0 ± 2.0 bB	114.2 ± 46.5 aA	10.7 ± 0.7 bA

*Uppercase letters compare differences in water-holding capacity, while lowercase letters compare differences according to inoculant types by Tukey's test at 5 % ($p \leq 0.05$). Mean values are followed by standard errors ($n = 3$). SD: severe drought, MD: moderate drought, ND: no drought.

Overall, water-holding capacity influenced the total soil bacterial and mycorrhizal abundance over time (Table 3 and Table 4). Soil bacterial abundance increased from 15 days after sowing (DAS) to 25 DAS and decreased from 25 DAS to 35DAS (Table 3).

Table 3. Total soil bacterial abundance (gene copy number $g\ soil^{-1}$) over sampling time based on the qPCR of 16S rRNA gene, considering the water-holding capacities (WHC), type of inoculum (arbuscular mycorrhizal fungi (AMF), plant growth-promoting rhizobacteria (PGPR), and consortium of AMF and PGPR), and soil sampling taken 15, 25, and 35 days after sowing (DAS)

WHC	Inoculum	Sampling		
		15 DAS	25 DAS	35 DAS
30% SD	AMF	$3.2 \times 10^9 \pm 7.3 \times 10^8$ abA	$2.9 \times 10^9 \pm 1.4 \times 10^9$ aA	$2.0 \times 10^9 \pm 3.1 \times 10^8$ aA
	AMF+PGPR	$3.3 \times 10^9 \pm 7.8 \times 10^8$ abAB	$3.1 \times 10^9 \pm 1.6 \times 10^9$ aA	$1.4 \times 10^9 \pm 2.0 \times 10^8$ aB
	PGPR	$4.1 \times 10^9 \pm 1.2 \times 10^9$ aA	$4.5 \times 10^9 \pm 4.6 \times 10^7$ aB	$1.6 \times 10^9 \pm 5.1 \times 10^8$ aA
	Uninoculated	$1.9 \times 10^9 \pm 4.5 \times 10^7$ bA	$3.2 \times 10^9 \pm 4.4 \times 10^8$ aA	$2.0 \times 10^9 \pm 6.3 \times 10^8$ aAB
50% MD	AMF	$3.1 \times 10^9 \pm 9.6 \times 10^8$ abA	$4.1 \times 10^9 \pm 8.6 \times 10^7$ bA	$1.3 \times 10^9 \pm 2.3 \times 10^8$ aAB
	AMF+PGPR	$3.8 \times 10^9 \pm 1.7 \times 10^9$ aA	$2.9 \times 10^9 \pm 8.6 \times 10^8$ bA	$1.5 \times 10^9 \pm 3.1 \times 10^8$ aB
	PGPR	$2.0 \times 10^9 \pm 2.6 \times 10^7$ abB	$8.0 \times 10^9 \pm 5.0 \times 10^9$ aA	$1.5 \times 10^9 \pm 1.4 \times 10^8$ aA
	Uninoculated	$1.9 \times 10^9 \pm 2.2 \times 10^8$ bA	$2.1 \times 10^9 \pm 1.8 \times 10^8$ bA	$1.3 \times 10^9 \pm 1.8 \times 10^8$ aB
80% ND	AMF	$1.4 \times 10^5 \pm 6.3 \times 10^4$ bB	$2.9 \times 10^9 \pm 1.4 \times 10^8$ aA	$1.2 \times 10^9 \pm 2.1 \times 10^8$ cB
	AMF+PGPR	$2.2 \times 10^9 \pm 4.3 \times 10^8$ aB	$2.7 \times 10^9 \pm 3.3 \times 10^8$ aA	$2.9 \times 10^9 \pm 2.3 \times 10^8$ aA
	PGPR	$1.5 \times 10^9 \pm 7.4 \times 10^8$ abB	$2.2 \times 10^9 \pm 4.3 \times 10^8$ aB	$1.9 \times 10^9 \pm 6.7 \times 10^8$ bcA
	Uninoculated	$1.5 \times 10^9 \pm 6.0 \times 10^8$ abA	$4.8 \times 10^9 \pm 2.8 \times 10^9$ aA	$2.5 \times 10^9 \pm 7.5 \times 10^8$ abA

*Uppercase letters compare differences in water-holding capacity, while lowercase letters compare differences according to inoculant types by Tukey's test at 5 % ($p \leq 0.05$). Mean values are followed by standard errors ($n = 3$). SD: severe drought, MD: moderate drought, ND: no drought.

Whilst mycorrhizal fungal abundance decreased from 15 DAS to 25 DAS (only in severe and moderate drought) and increased substantially from 25 DAS to 35DAS (Table 4) under absence of stress. For both, bacterial and mycorrhizal inoculum, the highest abundances were found under severe and moderate drought.

Table 4. Total soil mycorrhizal abundance (gene copy number g soil^{-1}) over sampling time based on the qPCR using FLR3 and FLR4 primers, considering the water-holding capacities (WHC), type of inoculum (arbuscular mycorrhizal fungi (AMF), plant growth-promoting rhizobacteria (PGPR), and consortium of AMF and PGPR), and soil sampling taken 15, 25, and 35 days after sowing (DAS)

WHC	Inoculum	Sampling		
		15 DAS	25 DAS	35 DAS
30% SD	AMF	$3.78 \times 10^3 \pm 2.20 \times 10^3$ aA	$1.75 \times 10^3 \pm 1.04 \times 10^2$ aB	$1.04 \times 10^5 \pm 9.15 \times 10^4$ aB
	AMF+PGPR	$2.89 \times 10^3 \pm 9.07 \times 10^2$ abA	$2.52 \times 10^3 \pm 6.82 \times 10^2$ aB	$1.20 \times 10^5 \pm 5.30 \times 10^4$ aA
	PGPR	$1.84 \times 10^3 \pm 4.01 \times 10^2$ abA	$1.55 \times 10^3 \pm 5.57 \times 10^2$ aB	$2.15 \times 10^4 \pm 1.20 \times 10^4$ aA
	Uninoculated	$1.12 \times 10^3 \pm 1.65 \times 10^2$ bA	$1.49 \times 10^3 \pm 1.70 \times 10^2$ aA	$1.02 \times 10^4 \pm 2.98 \times 10^3$ aA
50% MD	AMF	$5.63 \times 10^3 \pm 4.14 \times 10^3$ aA	$2.59 \times 10^3 \pm 7.75 \times 10^2$ aB	$2.36 \times 10^5 \pm 1.82 \times 10^5$ aA
	AMF+PGPR	$2.22 \times 10^3 \pm 1.90 \times 10^2$ bAB	$1.86 \times 10^3 \pm 3.64 \times 10^2$ aB	$3.65 \times 10^4 \pm 1.52 \times 10^4$ bAB
	PGPR	$1.10 \times 10^3 \pm 1.35 \times 10^2$ bA	$1.42 \times 10^3 \pm 3.32 \times 10^2$ aB	$1.50 \times 10^4 \pm 2.65 \times 10^3$ bA
	Uninoculated	$1.35 \times 10^3 \pm 7.21 \times 10^1$ bA	$1.78 \times 10^3 \pm 2.51 \times 10^2$ aA	$6.83 \times 10^3 \pm 5.49 \times 10^2$ bA
80% ND	AMF	$8.49 \times 10^2 \pm 2.86 \times 10^2$ aB	$9.71 \times 10^3 \pm 1.83 \times 10^3$ bA	$4.77 \times 10^4 \pm 3.97 \times 10^4$ aB
	AMF+PGPR	$7.28 \times 10^2 \pm 4.33 \times 10^1$ aB	$1.87 \times 10^4 \pm 4.42 \times 10^3$ aA	$1.05 \times 10^4 \pm 6.01 \times 10^2$ aB
	PGPR	$1.48 \times 10^3 \pm 1.94 \times 10^2$ aA	$7.26 \times 10^3 \pm 1.75 \times 10^3$ bA	$6.83 \times 10^3 \pm 5.52 \times 10^2$ aA
	Uninoculated	$2.00 \times 10^3 \pm 5.64 \times 10^2$ aA	$3.46 \times 10^3 \pm 1.52 \times 10^3$ cA	$5.66 \times 10^3 \pm 2.48 \times 10^2$ aA

*Uppercase letters compare differences in water-holding capacity, while lowercase letters compare differences according to inoculant types by Tukey's test at 5% ($p \leq 0.05$). Mean values are followed by standard errors ($n = 3$). SD: severe drought, MD: moderate drought, ND: no drought

4.3.4. Principal components analysis (PCA)

Principal components analysis (PCA) was conducted to address relationships between water-holding capacity and inoculation type and to determine the major trait components that explain the variation in the original data. The water-holding capacity and inoculum type influenced the attribute dynamics according to the visualization of residuals in the PCA, which explained about 48 % in the 2 first components and 60 %, when considering the three main components (Fig. 4a).

Overall, fungal parameters (AMF root colonization and number of spores), and 33P uptake, besides soil EC and pH, were more correlated to the inoculum type under severe drought (square dots). Whilst plant parameters (biomass, diameter, and height), phosphatase dynamics, and phosphorus contents were more correlated with moderate stress and no-drought (triangular and circular dots, respectively). Despite the dispersion within the replicates, a subtle difference

was evident in relation to the inoculum type, where the AMF+PGPR, only PGPR, and uninoculated treatments showed better clustering than the AMF treatment.

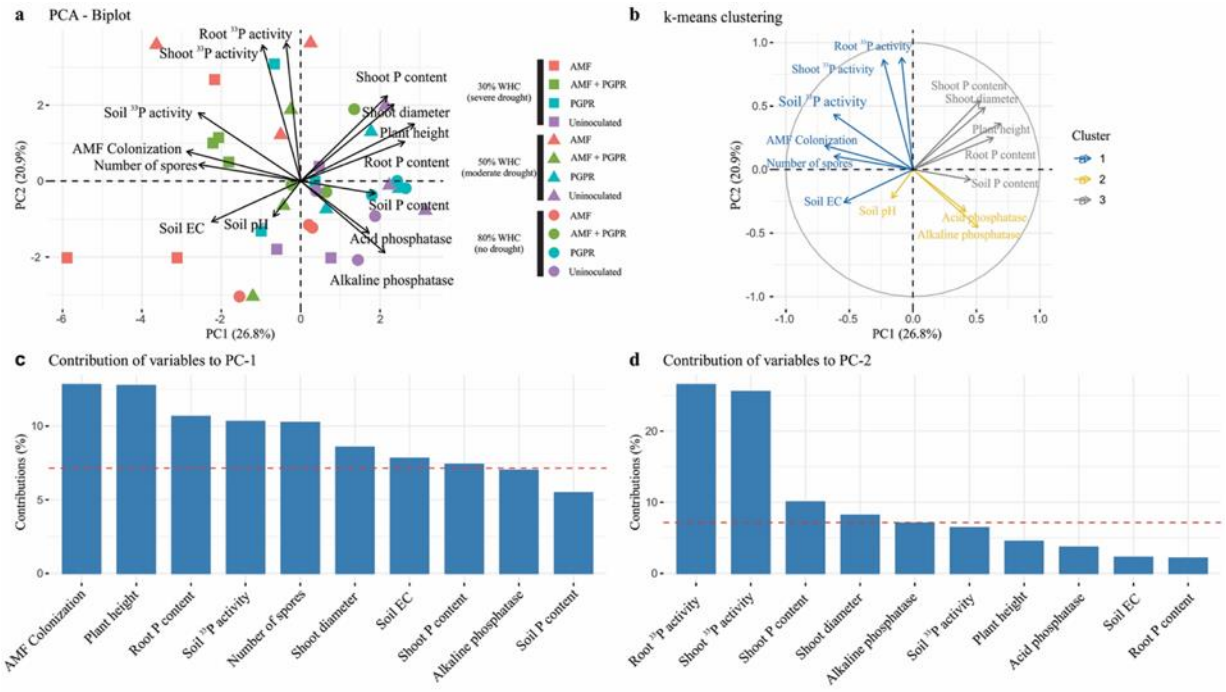

Fig. 4 Principal component analysis (PCA), (a) displaying relationships between the variables evaluated and treatments in the planted/inoculated compartment at three contrasting soil water-holding capacities (WHC). Equally, other factors, as different types of inoculum (arbuscular mycorrhizal fungi (AMF), plant growth-promoting rhizobacteria (PGPR), and consortium of AMF and PGPR) were evaluated. (b) k-means clustering algorithm ordination, clustering similar variables based on an unsupervised machine learning method. (c and d) specific contribution of the variables to the main principal components (i.e., PC-1 and PC-2) of PCA. The red dashed line on the graph above indicates the expected average contribution. The third principal component explained 15 % of the data variation.

According to the results of the k-means clustering algorithm, three clusters of variables were identified, the first being composed of AMF root colonization, soil electrical conductivity, number of spores and 33P activity (in soil and plant), which was more correlated to the AMF+PGPR treatment under severe drought. The second cluster was composed of plant parameters (diameter and height), and plant P content, while the third was composed of phosphatase activities and soil pH (Fig. 4b). We assessed the most important variables in explaining the variability in our data set according to the contribution level. The variables that contributed the most to the definition of the principal component 1 were AMF root colonization (12.81 %), plant height (12.75 %), root P content (10.65 %), soil ^{33}P activity (10.31 %), and number of spores (10.24 %) (Fig. 4c). Whilst for the principal component 2, the most important variables were root and shoot 33P activity (26.53 % and 25.57 %, respectively) (Fig. 4d).

4.4. Discussion

Inoculated plants outperformed the uninoculated plants in terms of ^{33}P uptake, especially under drought conditions. Still, dual-inoculation or PGPR inoculation showed higher efficiency under severe drought compared to individual inoculation of AMF, which was more efficient under moderate drought. This was observed equally in instances where the measured plant growth variables of plants inoculated with either AMF or PGPR were higher when compared to co-inoculated plants. Therefore, considering our one-to-one model, the results indicate that the PGPR (*Bacillus* sp.) used here did not act as a mycorrhizal helper bacteria under moderate drought, at least not considering the time of evaluation. Thus, we partially rejected our initial hypothesis that the co-inoculation of AMF and PGPR enhances plant nutrient acquisition in treatments under any drought.

Nevertheless, we found that the co-inoculation (*Rhizophagus clarus* and *Bacillus* sp.), under severe drought, enhanced ^{33}P uptake 2.4-fold more intensely, than individual inoculation of *R. clarus*. In a similar approach, Battini et al. (2017) found that AMF inoculated plants showed higher ^{33}P uptake when also co-inoculated with bacteria. Karimi et al. (2017) and Karimi et al. (2018) also demonstrated the benefits of dual inoculation (AMF and PGPR) for phytoremediation of Pb-contaminated soils, showing that inoculated plants outperform non-inoculated ones in terms of growth and photosynthetic parameters. Furthermore, Hestrin et al. (2022) demonstrated that AMF inoculation has a protective effect on bacterial communities exposed to water limitation, thus ensuring plant growth and nutrition in droughted soils. Other studies have also demonstrated this strong synergistic effect between mycorrhizae and bacteria on plant biomass production (Larimer et al., 2014; Zhou et al., 2022). According to Jiang et al. (2021), mycorrhizae can control the interaction with the bacteria and actively recruit, transport and stimulate them to mineralize organic nutrients with benefit to the fungi, mainly in a region called mycorrhizosphere (a microhabitat in soil where plant roots are surrounded by fungal hyphae; Johansson et al., 2004). Conversely, the bacteria benefit from the release of carbon in hyphal exudates, representing cooperation between them, becoming allied with the symbiosis between the AMF and the plant root.

It is a well-known and proven response that the efficiency of this microbial synergism varies substantially with species identity of both fungi and plants, host phenology, soil nutrients or root exudation, which significantly impacts the rhizosphere and mycorrhizosphere microbial community (Pauwels et al., 2020; Jongen et al., 2022; Pérez-Castro et al,. 2019; Ulrich et al., 2019). In our study, due to the lack of indigenous microorganisms in the soil system, only straightforward interactions between *Rhizophagus clarus* and *Bacillus* sp. were addressed, and

therefore, most beneficial effects involving complex interactions harboured in the rhizosphere could not be exhibited, and different results can be obtained when these inoculants are challenged under realistic field conditions.

Although we have evidence of the ability of co-inoculation to increase ^{33}P uptake, this seems not to be related with the potential of drought mitigation effects on maize growth, as we did not observe increases in biomass production. Therefore, higher ^{33}P plant uptakes were not translated necessarily into higher growth. However, increase in biomass was present with individual inoculation of bacteria under severe drought. We argue that the absence of the beneficial effect of AMF on plant growth could be related to their rapid root colonization process, i.e., colonization of the plant root. There is a close association of the fungal life cycle with those of the plants, in which AMF sporulation happens at the end of the plant growth cycle, or when the plant growth slows down. Despite this, sporulation of the experimental AMF was observed at 35 days of plant growth when the plant was still in its vegetative phase, implying a significant carbon sink in the host plants (Smith and Read, 2008). This was also demonstrated by the results showing that AMF invested more in the production of hyphae and vesicles, in addition to the spore production, under severe and moderate stress conditions.

On the other hand, it is postulated, that the determination of C allocation to different fungal structures is driven by the severity of drought stress. Furthermore, the effectiveness of the plant–AMF interaction can lead to a plant physiological improvement and, consequently, to a higher C supply to the fungi even under drought stress, with severe drought increasing the C allocation to hyphae, and moderate drought to vesicles, and no stress investing in arbuscules (Kiers et al., 2011; Jongen et al., 2022). Likewise, in our study we observed the prevalence of arbuscules under no drought. Since, overall, arbuscules have a rapid turnover and are the exchange structures in mycorrhizal symbiosis (Smith and Read, 2008). Interestingly, although the highest presence of arbuscules found was under no drought stress, the highest ^{33}P uptake occurred under drought stress conditions (both severe and moderate). However, the arbuscules in the treatments under drought may have been formed and were active during earlier stages of the plant–AMF interaction, mainly due to the moment of water shortage experienced, explaining the observed AMF effects on the ^{33}P uptake.

In our study, we observed a higher ^{33}P uptake in shoots and roots of PGPR-inoculated maize plants than in AMF-inoculated plants under severe water-stress. Therefore, the use of bacteria as an agent mitigating the water stress seems to be more effective than using AMF, at least under the situation simulated in our investigation. Still, the second inoculation that took place 21 days after sowing (DAS) would potentiate these results, and, at this point, we are

gathering information on the several types of inoculation of bacteria screened from a harsh environment to mitigate water shortage in soil, and the results brought about, will shed light upon it (Mawarda et al., 2020).

The assessment of soil enzymes is crucial to understand the potential functioning response of the plant-microbe system since they are involved in the nutrient cycling. Thus, acid and alkaline phosphatase activities strongly control the biotic pathways of phosphorus (Margalef et al., 2017). Here, we observed an inverse relationship between enzyme activity and ^{33}P uptake by plants, which was somewhat expected, since the P source considered in our study (mono-potassium phosphate [KH_2PO_4]) is an inorganic compound, and the production of these enzymes in the soil is used to perform the acquisition of phosphate ions from organic molecules. Indeed, the high presence of inorganic P can repress the expression of *pho* genes, inhibiting soil phosphatase activities (Janes-Bassett et al., 2022).

In general, we observed that there was an increase in soil acid phosphatase activity with increasing soil water content, especially for the uninoculated treatment, which may be due to the high demand for P by plants since there was no microbial inoculation to facilitate the ^{33}P uptake. This result is interesting, considering that plants, although developing their adaptation to alleviate most biotic and abiotic stresses in nature, also rely on their microbial partners when they are present to absorb nutrients such as P (Hassani et al., 2018). In general, for soil alkaline phosphatase, the lowest activity was found in the presence of individual inoculation with AMF, which may be related to the facilitation of phosphorus nutrition promoted by AMF via hyphal network, which reflects the ^{33}P uptake results obtained in our experiments.

Regarding changes in soil pH and electrical conductivity (EC), our results showed that the EC was dependent on water content, whereas pH was not affected. Soil EC increased with decreases in the soil water content, which may be due to other factors, since soil EC is also modulated by a combination of soluble salts, and soil temperature, for example (Bai et al., 2013). Pankaj et al. (2020), using PGPR to improve plant growth and crop yield of *Bacopa monnieri* (L.) Nash observed that soil EC and pH decreased in inoculated soils. Likewise, Al-Enazy et al. (2018) demonstrated that the inoculation of maize plants with *Azotobacter chroococcoccum*, *Bacillus megaterium* or *Pseudomonas fluorescens* also decreased soil EC and pH.

Over time, we observed an increase in the bacterial abundance from 15 DAS to 25 DAS, which may be attributed to the fact that soil bacterial inoculation occurred at 21 DAS, to assist the plants when the water-holding capacity decreased during a severe drought. After that, the bacteria abundance decreased from 25 DAS to 35 DAS, indicating a transient rather than persistent effect. At the same time, i.e., 25 DAS and 35 DAS, we observed an increase in AMF

abundance under moderate drought, evidencing the complementary and synergistic effects provided by *R. clarus* and *Bacillus* sp. According to Mawarda et al. (2020), when inoculants are delivered into soil, they have low persistence due to a combination of high levels of competition from the native microbial community and a lack of available resources (e.g., C, water, nutrients). This transient effect, however, does not necessarily imply a lack of lasting legacy on plant growth. Therefore, the inoculant effects may not necessarily be due to the size of the inoculant populations, since various changes in soil community structure and functioning can be found, even though the number of inoculant cells declined following introduction into the soil. Florio et al. (2017) using rhizosphere soil from an experiment with maize seeds inoculated with *Azospirillum lipoferum* CRT1, under field conditions, could not detect the inoculated strain by qPCR at 37 DAS. Indeed, these authors maintain that, in general, bacterial inoculants stimulate root growth and modify plant metabolism at very early stages, and generate lasting effects on the root system, disappearing quickly, usually after a few weeks. Likewise, Silva et al. (2021), using bulk soil from an experiment with sugarcane, inoculated with a bacterial consortium, observed that bacterial abundance remains constant over time, while changes occur in its composition and functions.

In our study, a higher bacterial abundance was detected in the AMF treatments, even though the spores were previously disinfected, suggesting that the bacteria were located inside the spore walls. Indeed, bacteria belonging to the order *Bacillales*, which include the *Bacillus* strain used here, are intimately associated with AMF spores, increasing the AMF activity. Furthermore, they are often embedded in the outer or inner of the spore wall layers or the microniches formed by the peridial hyphae interwoven around the spores of various *Glomus* species, now assigned as *Rhizophagus* (Walley and Germida, 1995; Filippi et al., 1998; Rouphael et al., 2015; Selvakumar et al., 2016). In addition, it is important to consider that seeds have their microbiota, which comes from the flower microbiota (so-called anthosphere) and, therefore, bacteria can reside in and on seeds (Nelson, 2018; Johnston-Monje et al., 2021). In our investigation, as the seeds were sterilized, the bacteriome that lives inside the seeds may have contributed in some way to our results. However, there is still insufficient knowledge allowing us to determine which specific bacterial species would be helping AMF, as it will strongly depend on the associated soil and plant microbiome.

Our investigation is a breakthrough in the topic of dual microbe inoculation, shedding light on the beneficial use of *Rhizophagus clarus* and *Bacillus* sp. (potential new species) to increase the ^{33}P uptake by maize plants under severe drought stress. Furthermore, given that the obtained dataset was composed of three clusters of variables according to the k-means algorithm, we

concluded that AMF root colonization, soil EC, and the number of spores (first cluster) were the main drivers to explain the ^{33}P uptake, especially using AMF+PGPR under severe drought. Therefore, we reinforce that the largest synergism between mycorrhizae and bacteria were more prevalent under severe drought rather than moderate drought.

4.5. Conclusions

We conclude that *Rhizophagus clarus* and *Bacillus* sp. inoculation offers a potential strategy to promote nutrient acquisition by plants in the context of the increasing frequency of drought gradient. This is supported by the enhanced uptake of ^{33}P in all inoculated plants at all moisture regimes, in comparison to the uninoculated plants. The major achievements of our investigation were i) demonstrating that the synergistic response of *Rhizophagus clarus* and *Bacillus* sp. increased under severe drought conditions and ii) that the use of mycorrhizae alone in conditions of moderate drought stood out in plant ^{33}P uptake. Therefore, the microbes tested in our investigation are potential candidates for a microbial inoculant in the near future. However, we emphasize that the straightforward interactions examined in the present study under controlled conditions may not represent complex systems in the rhizosphere, which harbours many microbes. Thus, further research is suggested to better understand how the application of these microbes can affect plant responses (physiological and biochemical) under the field in various edaphoclimatic conditions.

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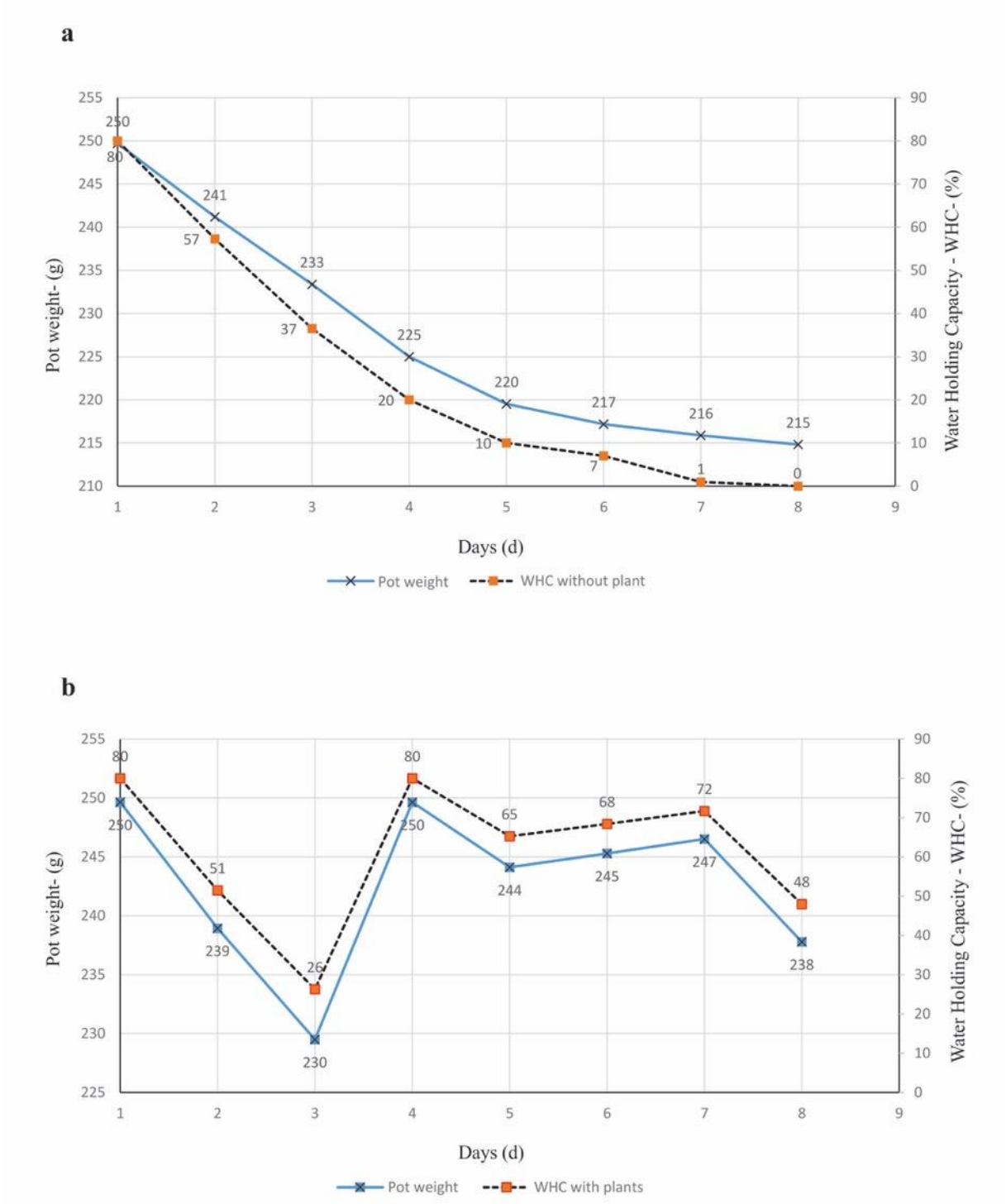
Supplementary Materials

Supplementary Note 1. Experiment 1: aims, design, and results.

The aim of Experiment 1 was: (i) to confirm whether the root was able to pass through a mesh with aperture of 45 μm. (ii) to ascertain how many days the maize seeds took to germinate in soil (also in Petri dishes). (iii) to verify how water-holding capacity decreases as a function of the soil volume. (iv) to verify the contribution of plant weight, since the control of the water content was based on the pot weight.

Two groups of treatments were considered, i.e., with and without a plant (n = 3), in order to learn about the contribution of the plant weight to the overall pot weight over time. Each experimental unit comprised a plastic pot (8 cm internal diameter × 7 cm high), containing 200 g of soil (dry weight). Mesh exclusion (45 μm) was used to divide the pot into two compartments, each one receiving 100 g of sterilized soil. Seeds were sown in the planted compartment at 80 % of water-holding capacity. There was no daily maintenance of the water content for the treatment without plants to understand how the water-holding capacity decreases as a function of the soil volume. In the treatment with plants, the soil was rehydrated to 80 % water-holding capacity (WHC) on the fourth day when the WHC was 26 %, followed by daily control to 80 % WHC using the addition of deionized and sterilized water (SN1 Figure 1a and 1b).

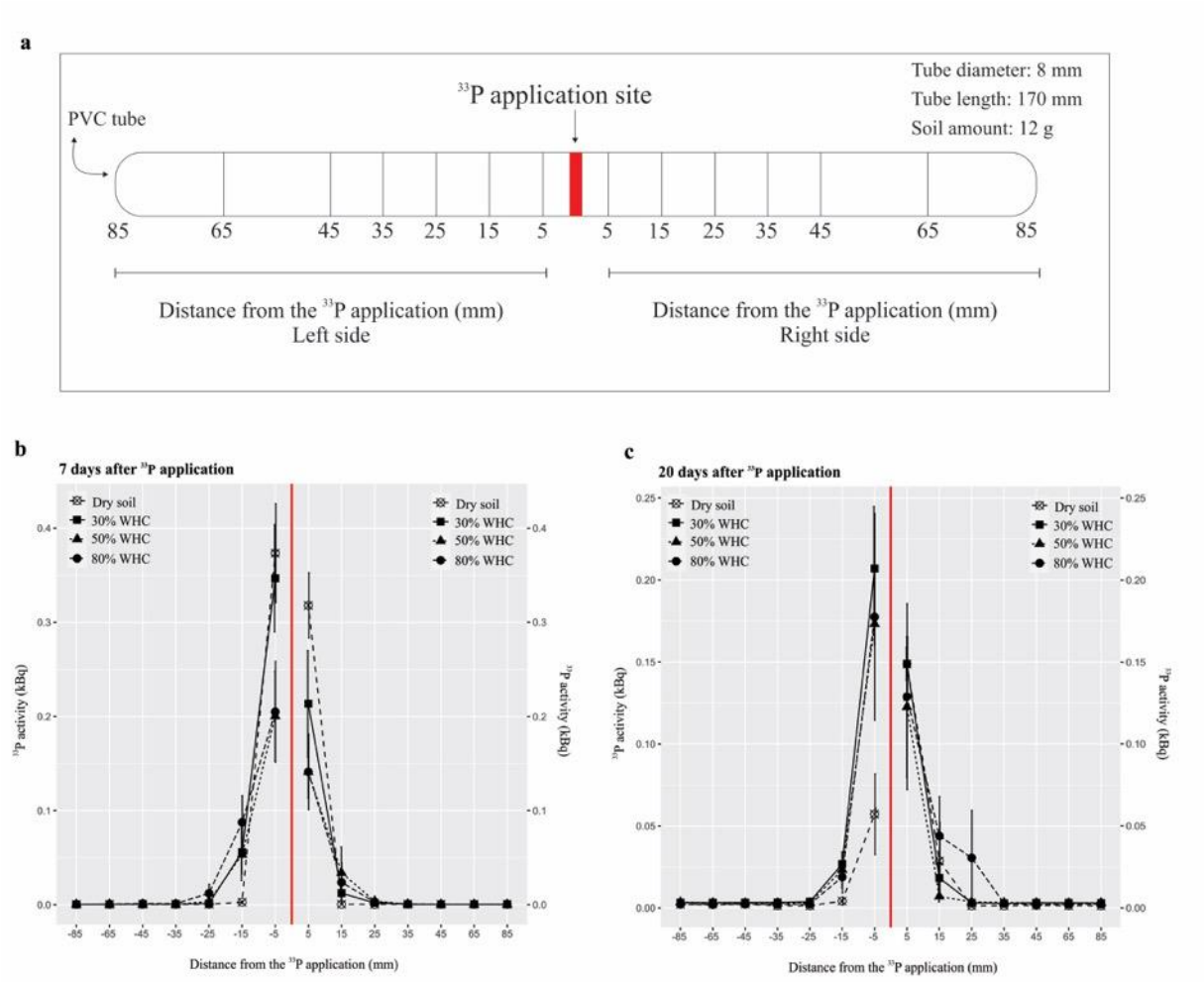
Overall, the seed germination rate was 98 %, six days after sowing. Furthermore, a large decrease in soil moisture was observed after one day. Based on this, it was decided that all pots required daily control of their water content for the main microcosm experiment. The plant weight contribution to the total pot weight followed the equation 1.47 ± 0.36 g (shoot, n = 3) and 2.49 ± 0.71 g (root, n = 3) on a wet basis.



SN1 Fig. 1 Representation of the pot weight (solid blue line) and the water-holding capacity (dashed black line) without plants (a) and with plants (b). Note: the increase in the water-holding capacity on the 4th day in panel (b) was due to the pot rehydration

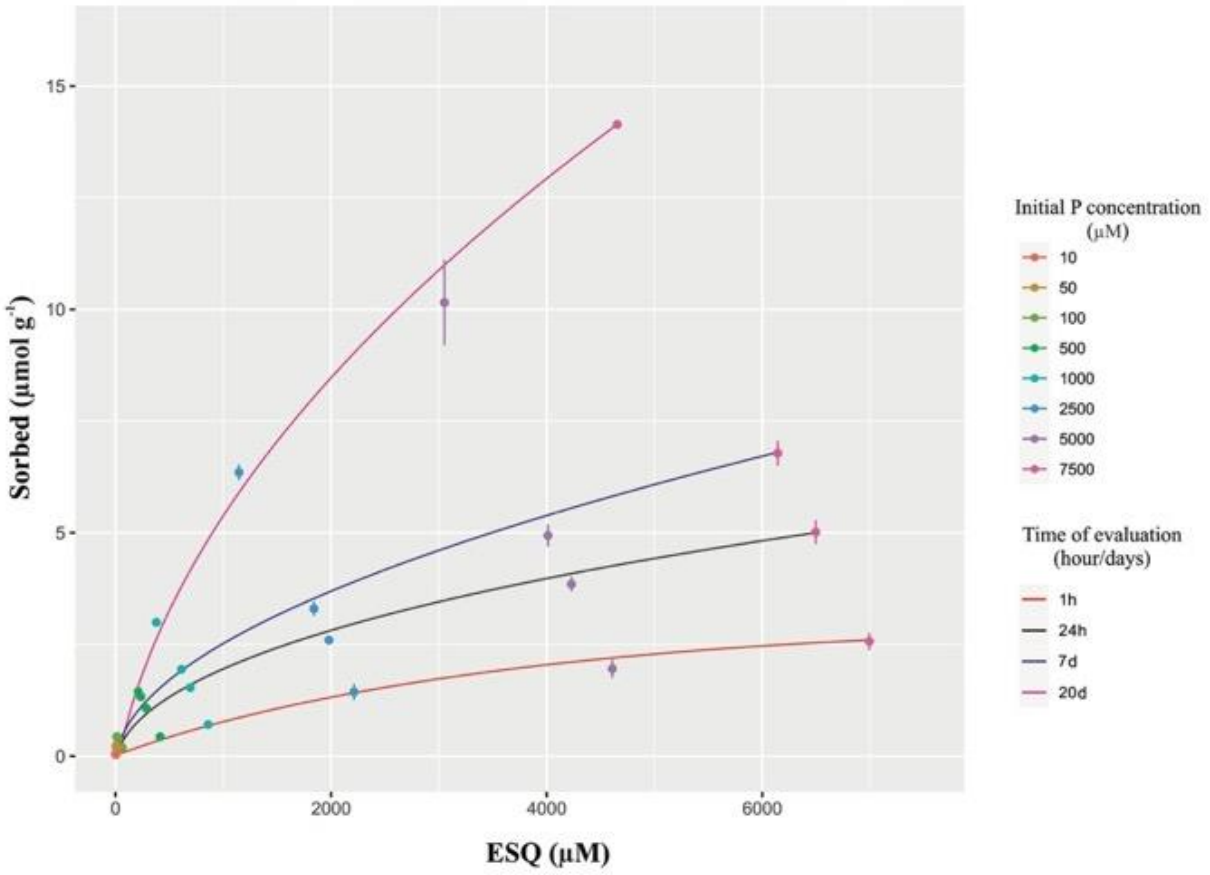
Supplementary Note 2. Experiment 2: aims, design, and results.

The soil diffusion microcosms were prepared by filling 8 mm inner diameter, 170 mm long polypropylene cylinders with air-dried soil (12 g of soil with a soil density of 1 g cm^{-3}). To retain the soil, one end of the cylinder was covered with gas-permeable polyvinyl chloride (PVC) film (Figure SN2 1a). Four types of treatment were considered, i) dry soil, ii) 30 % WHC, iii) 50 % WHC, and iv) 80 % WHC. Soil columns for each treatment were placed in a black box and then sealed (simulating the soil environment) and incubated in a growth chamber. This experiment was set up twice, as the first was evaluated at 7 days and the second at 20 days after ^{33}P application (100 µl). According to the results, regardless of soil water content and evaluation time, P diffusion in soil was limited to 15 mm from the point of addition (SN2 Figure 1b and 1c).



SN2 Fig. 1 Soil diffusion microcosms representation used in the experiment of diffusion of P to soil (a). Diffusion of P to soil at 7 days (b) and 20 days after 33P application (c). WHC: Water-holding capacity. Negative numbers represent the left side of the tube, while positive numbers represent the right side. The solid red line represents the 33P application site. Standard errors are shown. n = 3

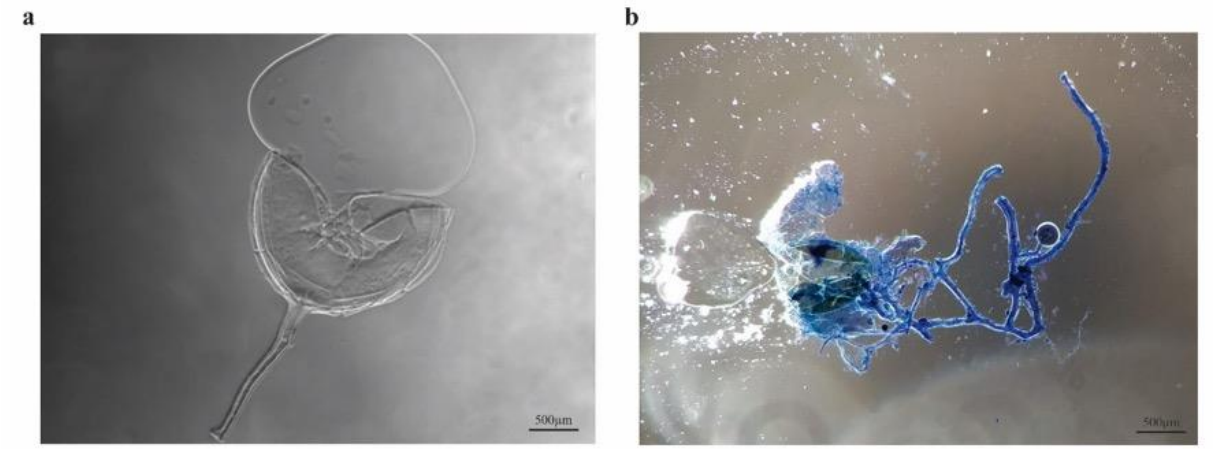
The sorption of P to the soil was also evaluated to ascertain the capacity of soil to remove phosphate from soil solution. Eight different target P concentrations were made from a 50 mM stock solution containing ^{33}P in a background of 0.01 M KCl ionic strength buffer. The solid phase sorption of each substrate was determined by shaking 2.5 g of soil with 12.5 ml of each ^{33}P-labeled solution. After known shaking times (i.e., 1 h and 24 h, 7 days, and 20 days), 1.5 ml were removed from the soil suspensions. The soil suspension removed was centrifuged (18,000 *g*, 5 min) and the supernatant (1 ml) recovered for ^{33}P determination. In the P sorption process, initially, P from the soil solution sorbs to the outermost surfaces of soil aggregate, and then gradually penetrates into the aggregate. However, it is important to take into account that after a long time (e.g., 7 and 20 days), P may also be taken up (immobilized) by microbes. In other words, it appears like absorption, but it is actually immobilization by the microbial community. In our results, we observed a high sorption of P, which would have been expected due to the high adsorption of P on iron and aluminium oxides/hydroxides, commonly found in Brazilian soils (SN2 Figure 2).



SN2 Fig. 2 Sorption of P to soil as a function of the equilibrium of P solution concentrations (ESQ), considering the different times of evaluation. Standard errors are shown. $n = 3$.

Supplementary Note 3. Experiment 3: aims, design, and results.

Arbuscular mycorrhizal fungi spore germination in soil was performed according to INVAM (International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi). For this, *Rhizophagus clarus* spores were extracted from the pure culture, surface-sterilized by exposing them to 0.5% sodium hypochlorite, washed repeatedly in sterile water, and agitated in a bead beater for 4-5 minutes, at 4800 rpm to remove surface debris. A membrane filter (0.45 μm pore size) was premoistened, and 25 spores were transferred to the filter under a stereomicroscope. Then, spores were redistributed, in a way that none were touching. Microcosms were filled with 200 g of sterile soil used in the previous experiment. Filters were folded in half and then in half again (with the spores inside). They were then buried in the soil mix of the microcosm, moistened with sterile distilled water, covered with foil, and then placed in a rack in growth chamber. Filters were removed after 3 weeks, opened, and placed in a glass Petri dish, containing hot 0.05 % direct blue stain. After immersion for 30 seconds, the filter was transferred to a clean Petri dish and spores transferred to glass slides for permanent mounts in PVLG (Polyvinyl Lacto Glycerol) and examined. Overall, a spore germination rate of 85 % was seen after three weeks, including new spore formation (SN3 Figure 1).



SN3 Fig. 1 *Rhizophagus clarus* spore extracted from pure culture (a) and collapsed and intact spores attached to the extramatrical hyphae after three weeks of incubation in soil (b).

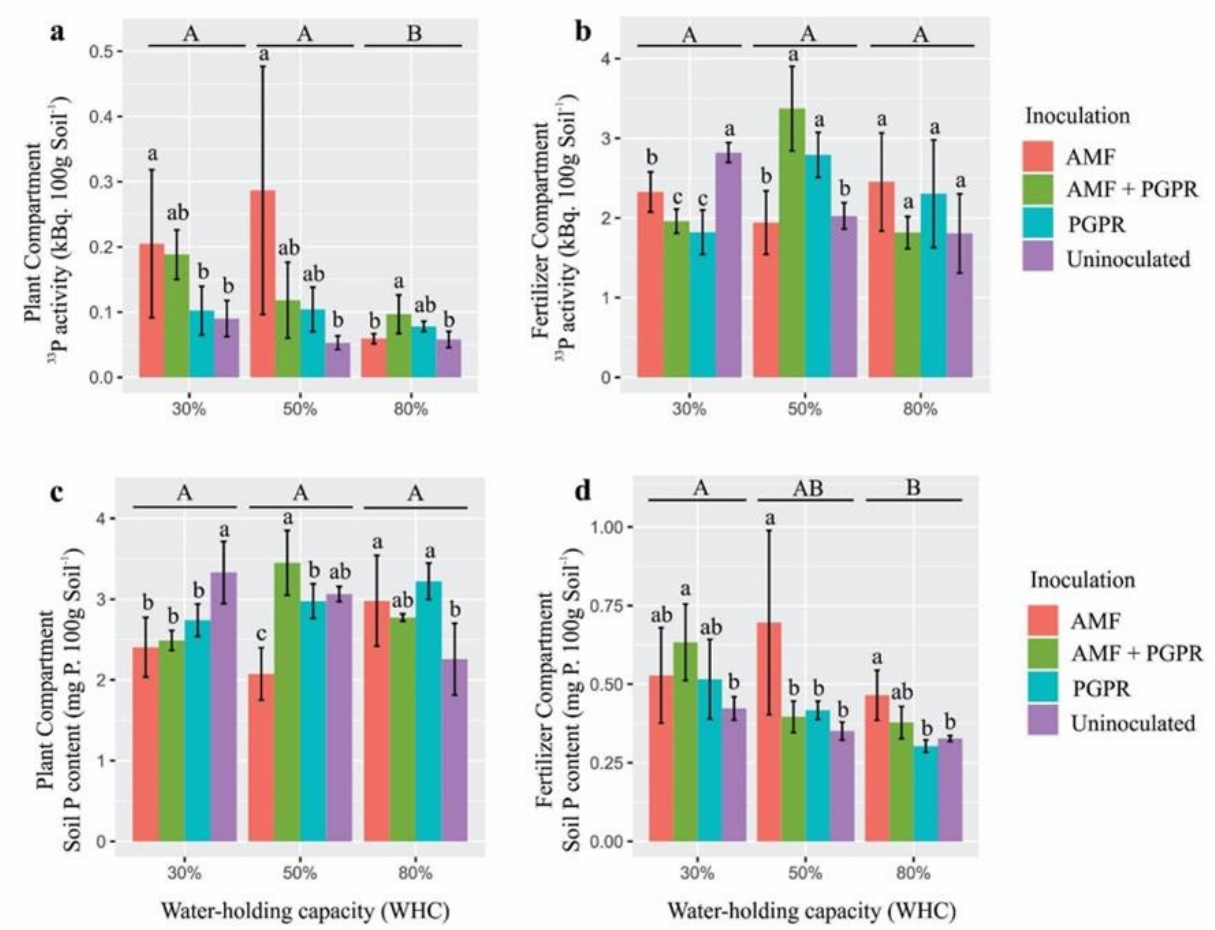


Fig. S1 Impact of arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) inoculation on P acquisition under three soil water-holding capacities (WHC). Soil ^{33}P activity in the plant compartment (a) and fertilizer compartment (b). Soil P content in plant (c) and fertilizer compartments (d). Uppercase letters compare differences in WHC, while lowercase letters compare differences according to inoculant types by Tukey's test at 5 % ($p \leq 0.05$). Standard errors are shown ($n = 3$).

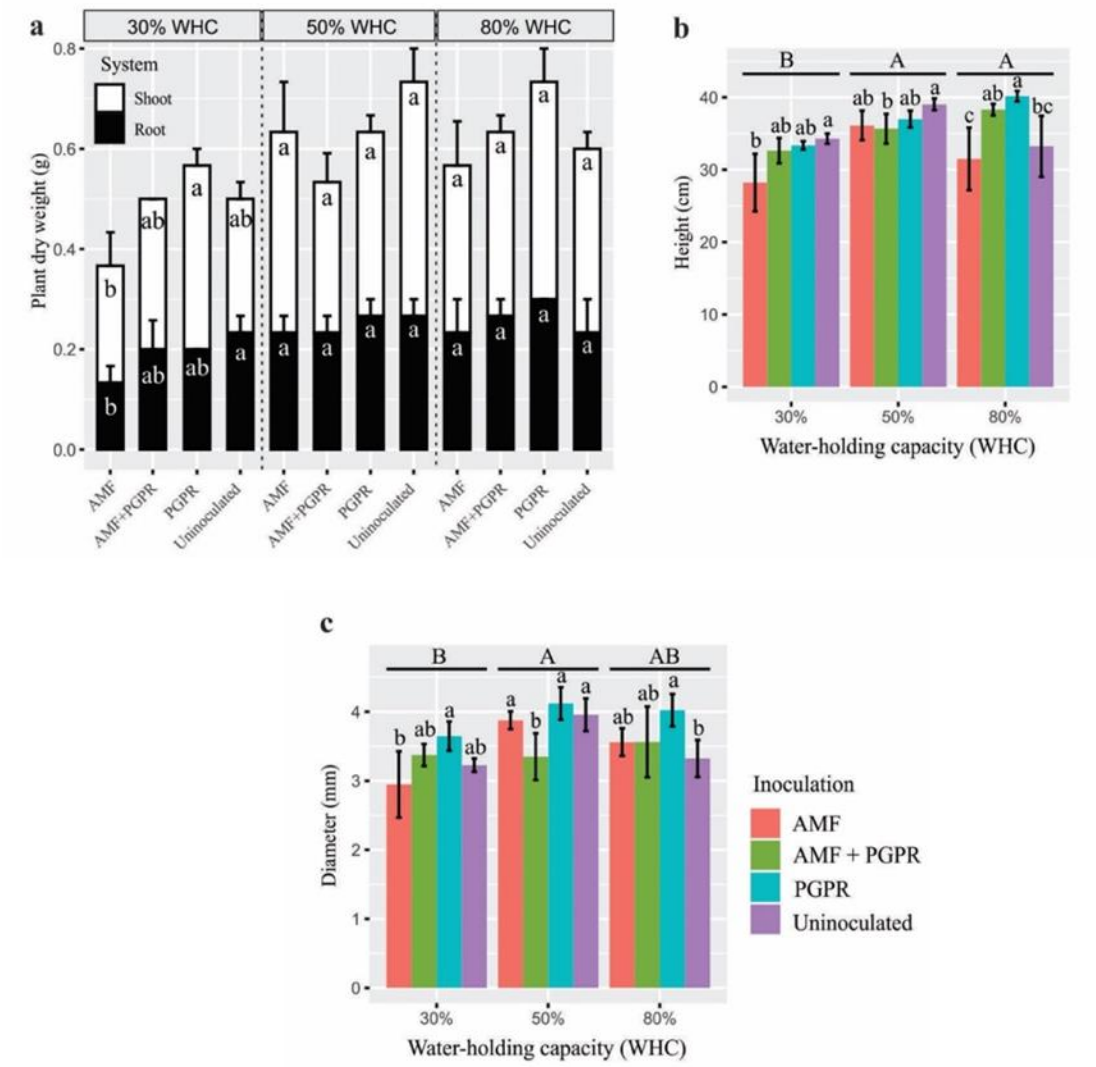


Fig. S2 Impact of arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) inoculation under three soil water-holding capacities (WHC) on plant dry weight, considering root (black bar) and shoot (white bar) systems (a). Plant height (b) and diameter (c). Uppercase letters compare differences in WHC, while lowercase letters compare differences in inoculant types by Tukey's test at 5 % ($p \leq 0.05$). Standard errors are shown ($n = 3$).

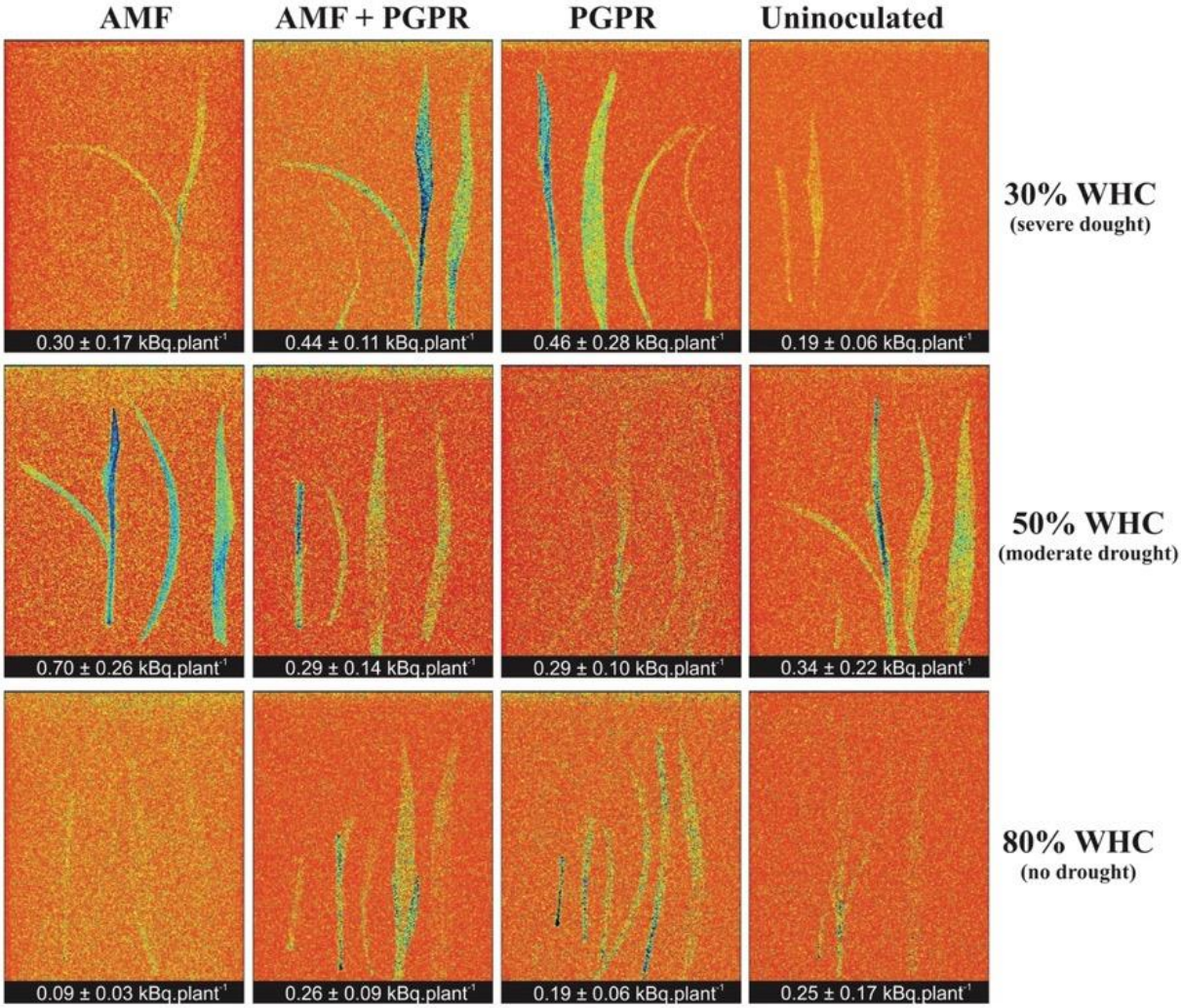


Fig. S3 Phosphorimager results of shoots, considering type of inoculum (in the column) and the water-holding capacity (in the row), where the intensity of the blue colour indicates the presence of ^{33}P activity. The mean values are followed by the standard error (n = 3). AMF: arbuscular mycorrhizal fungi; PGPR: and plant growth-promoting rhizobacteria; WHC: water-holding capacity.

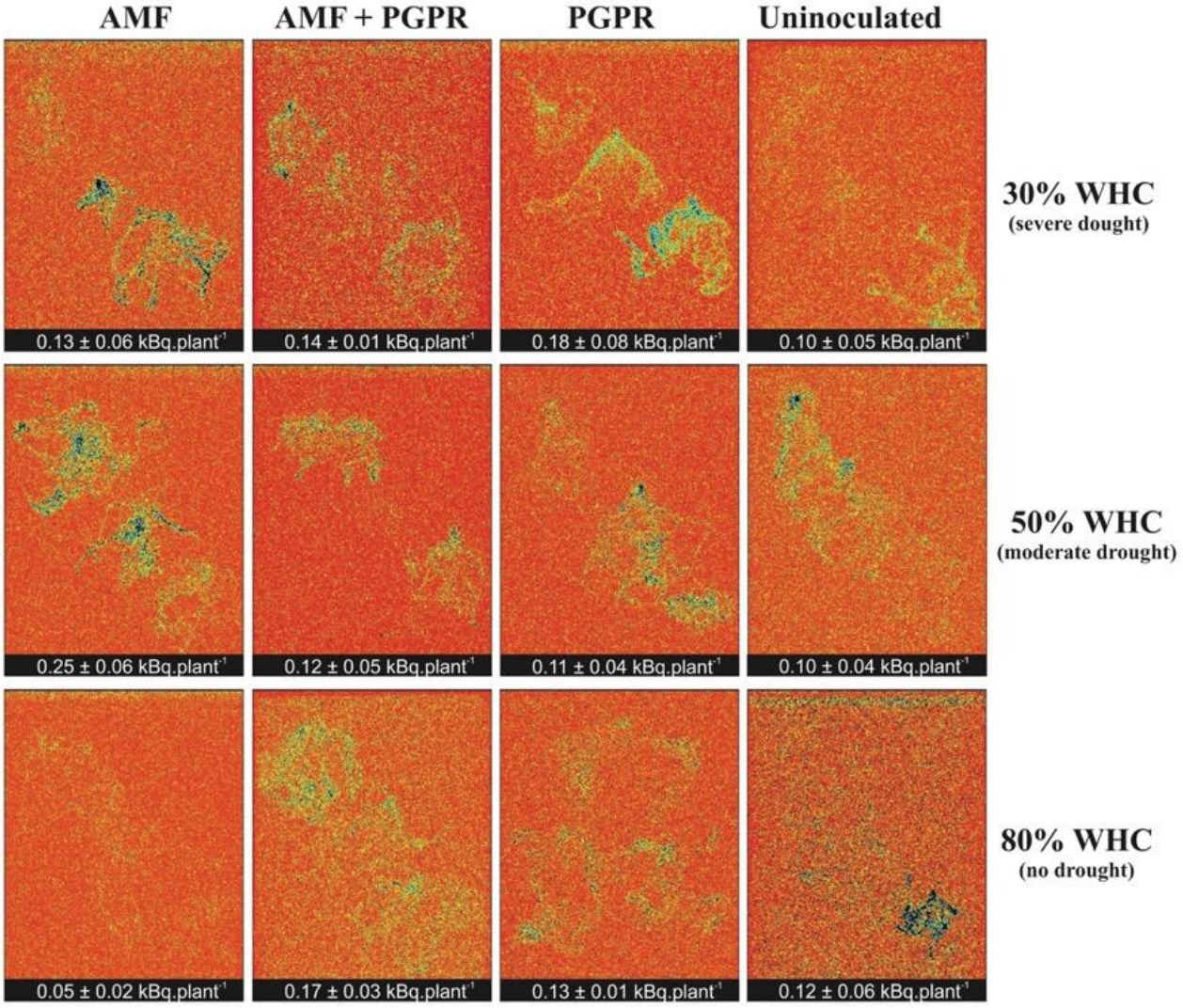


Fig. S4 Phosphorimager results of roots, considering type of inoculum (in the column) and the water-holding capacity (in the row), where the intensity of the blue colour indicates the presence of ^{33}P activity. The mean values are followed by the standard error (n = 3). AMF: arbuscular mycorrhizal fungi; PGPR: and plant growth-promoting rhizobacteria; WHC: water-holding capacity.

Supplementary Table S1. Soil pH, electrical conductivity (EC), acid phosphatase activity (Ac. Phosphatase) and alkaline phosphatase activity (Alk. Phosphatase) in fertilized compartment at three contrasting soil water-holding capacities (WHC) and with different types of inoculum (arbuscular mycorrhizal fungi (AMF), plant growth-promoting rhizobacteria (PGPR), and consortium of AMF and PGPR)

WHC	**Inoculum**	**pH**	**EC** ($\mu S\ cm^{-1}$)	**Ac. Phosphatase** (nmol g^{-1} soil h^{-1})	**Alk. Phosphatase** (nmol g^{-1} soil h^{-1})
30%	AMF	7.1 ± 0.1 bA	88.0 ± 4.0 aA	37.7 ± 6.8 aB	7.5 ± 1.0 aA
	AMF+PGPR	7.3 ± 0.04 abA	94.0 ± 9.0 aA	17.2 ± 2.6 bB	5.6 ± 0.7 cA
	PGPR	7.4 ± 0.1 aA	92.0 ± 6.0 aA	13.4 ± 1.6 bB	5.9 ± 0.6 bcA
	Uninoculated	7.4 ± 0.01 aA	71.0 ± 2.0 bA	14.9 ± 0.5 bB	7.4 ± 0.5 abA
50%	AMF	7.2 ± 0.04 cA	88.0 ± 8.0 aA	22.7 ± 4.3 bB	6.2 ± 0.4 bA
	AMF+PGPR	7.4 ± 0.1 aA	79.0 ± 14.0 abA	21.7 ± 3.7 bB	7.0 ± 0.5 bA
	PGPR	7.2 ± 0.03 bcA	75.0 ± 6.0 abA	36.1 ± 4.5 aB	8.4 ± 0.8 aA
	Uninoculated	7.3 ± 0.01 abA	65.0 ± 3.0 bA	35.0 ± 3.0 aB	6.8 ± 0.2 bA
80%	AMF	7.4 ± 0.04 aA	67.0 ± 8.0 abB	37.0 ± 0.8 bA	7.5 ± 0.8 bA
	AMF+PGPR	7.2 ± 0.04 bcA	55.0 ± 6.0 bB	47.4 ± 9.6 aA	7.4 ± 0.3 bA
	PGPR	7.4 ± 0.05 abA	56.0 ± 1.0 bB	49.5 ± 6.9 aA	8.8 ± 0.6 bA
	Uninoculated	7.2 ± 0.05 cA	74.0 ± 4.0 aB	51.6 ± 3.8 aA	12.0 ± 1.9 aA

*Uppercase letters compare differences in water-holding capacity, while lowercase letters compare differences according to inoculant types by Tukey's test at 5 % ($p \leq 0.05$). Means values are followed by standard errors ($n = 3$)

5. FINAL REMARKS

From the **first chapter**, the study revealed a lack of information about the mycorrhizal ecology of the sampled plants (*N. variegata* and *T. spicata*), particularly in relation to their potential to host microbes that aid in the tolerance of water shortages in soil. In addition, a difference in the arbuscular mycorrhizal fungal community was observed between the rhizosphere of the sampled plants, as indicated by the observed difference in network modularity. Overall, the study's results suggest that the diversity, richness, and modularity of mycorrhizal communities in the rhizosphere are mainly driven by the host species, as the sampled sites shared the same soil chemical and physical traits.

Based on the **second chapter**, the investigation conducted demonstrated that the efficacy of the native AMF inoculum varied upon drought levels, as inferred from biomass growth. However, no substantiation was procured to validate the hypothesis that the AMF inoculation was capable of enhancing nutrient uptake in maize plants. In contrast, modifications in soil parameters associated with phosphorus cycling, and the absence of any variation in the antioxidant system of the plants, support the notion of a lasting AMF impact. These findings emphasize the significance of the study, especially when considering the prevalent scientific consensus that water scarcity will have grave consequences on crop productivity over an extended period.

On the bases of the **third chapter**, it is asserted that the inoculation specific strains of AMF and PGPR presents a promising strategy to enhance nutrient uptake by plants in the context of the increasing incidence of drought. This assertion is supported by the increased absorption of ^{33}P in all inoculated plants across all moisture regimes, as compared to those that were not inoculated. However, it is emphasized that the simple interactions examined in the controlled study may not fully represent the complex rhizosphere system, which encompasses numerous microbes.

In conclusion, the growing threat of climate change and its effects on agriculture require the development of eco-friendly strategies to overcome or mitigate these issues. The use of microbial inoculants in agriculture has shown promising results in aiding plants during abiotic stresses such as drought. In particular, recent findings have highlighted the potential of using arbuscular mycorrhiza fungi and plant-promoting rhizobacteria as a combined strategy to promote plant growth and development under drought conditions. Further research is needed to explore the potential of these microbes, particularly those derived from arid habitats, to improve crop yields and soil biodiversity, which can contribute to reducing hunger and malnutrition worldwide.