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Chen, Qian; Deng, Xuhui; Elzenga, J. Theo M.; van Elsas, Jan Dirk

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Effect of soil bacteriomes on mycorrhizal colonization by *Rhizophagus irregularis*—interactive effects on maize (*Zea mays* L.) growth under salt stress

Qian Chen^{1,2} · Xuhui Deng^{3,4} · J. Theo M. Elzenga² · Jan Dirk van Elsas¹

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Abstract

In this study, we investigated the interactive effects of the arbuscular mycorrhizal fungus (AMF) *Rhizophagus irregularis* and soil bacteriomes on maize growth under salt stress (100 mM NaCl) and also the effect of salt and bacteriomes on the mycorrhizal infection levels. We found that soil bacteriomes directly promoted the growth of maize and indirectly enhanced maize biomass by increasing mycorrhizal colonization levels, irrespective of salt stress. Although *R. irregularis* by itself had no maize growth-promoting effect even at a high mycorrhizal colonization level in roots, its benefits to maize were reflected in other aspects, evidenced by the significantly increased rate of arbuscule formation (a proxy for a functional plant-AMF nutritional exchange) under salinity. A negative correlation between arbuscule colonization and root biomass suggested *R. irregularis* expands the role of maize roots. Besides, the positive correlation between the overall AMF colonization level and shoot biomass supported the tenet of a positive contribution of *R. irregularis* to maize growth. Our findings suggest that soil bacteriomes interactively work with *R. irregularis*, modulating the growth of maize by affecting the colonization of AMF in roots.

Keywords Arbuscular mycorrhizal fungi · Bacteria · Interaction · Root colonization · Arbuscule

✉ Jan Dirk van Elsas
j.d.van.elsas@rug.nl

Qian Chen
1260123360@qq.com

Xuhui Deng
x.h.deng@qq.com

J. Theo M. Elzenga
j.t.m.elzenga@rug.nl

- ¹ Microbial Community Ecology, Groningen Institute for Evolutionary Life Sciences, University of Groningen, 9747 AG Groningen, The Netherlands
- ² Plant Ecophysiology, Groningen Institute for Evolutionary Life Sciences, University of Groningen, 9747 AG Groningen, The Netherlands
- ³ Jiangsu Provincial Key Lab of Solid Organic Waste Utilization, Jiangsu Collaborative Innovation Center of Solid Organic Wastes, Educational Ministry Engineering Center of Resource-Saving Fertilizers, Nanjing Agricultural University, Nanjing, 210095 Jiangsu, People's Republic of China
- ⁴ The Key Laboratory of Plant Immunity, Nanjing Agricultural University, Nanjing, 210095 Jiangsu, People's Republic of China

Introduction

Over 6% of soil in the world and around 20% of the area used for agriculture is subjected to salinity problems ($EC_e \geq 4$ ds m^{-1} , ~40 mM NaCl), and this percentage is expected to increase due to improper cultivation practices, such as irrigation with salty water, continuous utilization of fertilizer and more frequent inundation of coastal lands (Munns 2005; Shrivastava and Kumar 2015). Unfortunately, many crop species are relatively intolerant to salt stress, especially at high salt levels. The damage caused by salt to plants is generally divided into (1) osmotic stress that can directly reduce plant growth and (2) ionic toxicity, with the accumulation of ions in plant shoots (Munns and Tester 2008). In general, plants can cope with abiotic stress by adjusting physiologically (Munns 2005; Munns and Tester 2008), but their adjustability is limited under high salt conditions.

Soil harbors large amounts of microorganisms, including bacteria, fungi and protozoa. Some of these microorganisms, such as plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungus (AMF), have become used as biological fertilizers, which may relieve biotic and

abiotic stress on plants (Evelin et al. 2019; Ilangumaran and Smith 2017). Plants can release up to 20–30% of their photosynthates to the rhizosphere, thus attracting beneficial soil microbes through root exudates, influencing the interactions (that can be neutral, positive or negative) (el Zahar et al. 2014). Beneficial interactions, such as those exerted by rhizobia or PGPR, promote plant growth and/or enhance stress tolerance and pathogen resistance. PGPR can exert a direct effect on plant growth by supplying nutrients (i.e. N, P, K, Fe and other essential minerals) and promoting the production of phytohormones such as auxins, ethylene, cytokinins and gibberellins (Gupta et al. 2015; Nadeem et al. 2014). Compared with AMF, soil bacteria need less C from plants and take less time to solubilize mineral substrate (Saia et al. 2020). The cooperation of bacteria with plants mainly occurs in the rhizosphere, whereas for the soil outside the rhizosphere, their assistance is very limited.

AMF could make up for this ‘shortcoming’ of soil bacteria as it develops a hyphal network (2.7 to 20.5 m/g of soil) in soil (Giovannetti and Avio 2002; Mikkelsen et al. 2008; Pepe et al. 2018), exploring the soil for nutrients beyond what can be easily reached by the plant root system itself. The fungal hyphae, which colonize the root cortical cells, differentiate into different structures, i.e. the arbuscules (specific sites for nutrient exchange) and vesicles (functioning as storage sites) (Engelmoer et al. 2014). The mycorrhizal plants take up nutrients in two ways: directly through epidermal cells and root hairs and indirectly, through the fungal hyphal cells that transfer nutrients to the arbuscules (Wipf et al. 2019). AMF colonize nearly 80% of all terrestrial plant species, exerting beneficial effects on plant growth, in particular under stress conditions (Smith and Read 2008). They do so by increasing water uptake (Sheng et al. 2008), accumulating phytohormones and reprogramming metabolism (Rivero et al. 2018), facilitating nutrient uptake (Willmann et al. 2013) and improving ion homeostasis (Estrada et al. 2013).

AMF are obligate biotrophs that are unable to complete their life cycle without a host plant, receiving carbohydrates from their host in exchange for nutrients, such as N, and P that is poorly mobile for plants and thus perceived as scarce by plants (Hodge and Storer 2015; Smith et al. 2009). Although AMF colonize plant litter in various ecosystems, there is no evidence to suggest that they can degrade organic compounds by themselves, as they have been found to be largely unable to produce the lytic enzymes required to break down plant-derived organic molecules (Bunn et al. 2019; Smith and Read 2008). Therefore, they depend on other microbes such as soil bacteria to release nutrients.

Regarding the combined effects of PGPR and AMF on plant growth, it has often been proposed that co-inoculation may be a good strategy, as it is presumably more efficient than single inoculation of bacteria or fungi

(Hashem et al. 2016; Magallon-Servin et al. 2020; Nacoon et al. 2020; Wang et al. 2011). Specific AMF-associated bacteria, such as *Pseudomonas* spp. and *Bacillus* spp., have been found to promote mycorrhizal development (Pivato et al. 2009). Most previous studies have focused on the effect of specific bacterial strains on mycorrhizal developmental stages (Artursson et al. 2006). Bacteria may improve AM fungal spore germination by degrading cell envelopes or through releasing volatile substances (Agnolucci et al. 2015; Turrini et al. 2018). It can facilitate hyphae growth and subsequent mycorrhizal colonization by producing organic acid chelators or secreting phosphatase to mobilize P (Ezawa and Saito 2018; Ordoñez et al. 2016). However, how the overall soil bacterial community influences mycorrhizal development is still poorly understood. Moreover, in natural soil systems, plant roots and AMF encounter abiotic stresses such as salinity, and soil bacterial communities potentially play a role in the mycorrhization process under such conditions. Based on our knowledge, there is no study that addressed the interactive effect of salinity and microbial factors (i.e. soil bacteria) on mycorrhizal colonization. In recent studies, soil microbiomes have been found to suppress the activity of AM mycelium, instead of influencing root colonization (Cruz-Paredes et al. 2019; Svenningsen et al. 2018). However, these studies did not exclude other fungi in the soil, notwithstanding the fact that AMF interact not only with soil bacteria but also with such fungi. Considering that soil bacteria and AMF are important microbial participants in soil and plant growth, it is necessary to study them separately, next to their interactions, in order to understand their respective functions, excluding the influence of other microorganisms.

Maize (*Zea mays* L.), the third most important crop after wheat and rice, is considered to be moderately sensitive to salt stress. *Rhizophagus irregularis* is commonly found in various soil ecosystems and types (Xie et al. 2018), and so we selected this AMF to study its interactions with soil bacteriomes as these affect maize plant growth in the presence of salt stress. To avoid the influence of other soil fungi in the present study with soil-derived bacteriomes, we used a filtering method to exclude these. We hypothesized that both AMF and soil bacteria promote maize growth under salt stress and that co-inoculation has a positive synergistic effect. On the basis of these hypotheses, we carried out research to answer the following questions: (i) Does the combination of soil bacteriomes and *R. irregularis* perform better than the single inoculation in improving maize growth in the presence of salt stress? (ii) What is the influence of soil bacteriomes on mycorrhizal colonization in maize roots under salt and non-salt conditions? (iii) If salt or bacteriomes affect mycorrhizal colonization, is there any correlation between mycorrhizal colonization and maize biomass?

Materials and methods

Soil bacterial inocula

Whole bacterial communities were isolated from soil (53°28'N, 6°12'E; EC = 0.75 ms/cm; pH = 7.54; loamy soil) sampled in Schiermonnikoog, the Netherlands. Following removal of the fine top layer of soil, the 5–15-cm layers were collected and taken to the lab. Of these, 30 g was added to 200 ml 0.1% sodium pyrophosphate solution (pH 7.0), after which the mixture was blended four times within 8 s to release soil bacteria, cooling on ice for 2 min each time (Scheublin et al. 2010). The suspension in a sterile centrifuge tube (50 ml) was centrifuged at 150×g (5 min, 4 °C), after which supernatants were passed sequentially through different mesh size filters (25 µm, 20 µm, 16 µm, 11 µm, 8 µm, 5 µm, 3 µm) to remove (most of) the soil fungi (Rudnick et al. 2015). Bacterial cell density was determined by using a Bürker-Türk counting chamber. About 10⁶ cells were added per gram of 'soil/sand growth substrate,' consisting of γ-sterilized (50 kGy, 4 mm) soil and autoclaved sand (2 mm; 121 °C, 20 min on two consecutive days), at a ratio of 2:3 (v:v). The soil used here was taken from Buinen (52°55'N–6°49'E; pH = 5.5; loamy sand soil; 5% organic C), the Netherlands (Inceoğlu et al. 2011). After 1 month (allowing bacterial establishment in the new environment, 10⁷–10⁸ CFU/g of soil), the bacterially colonized soil/sand growth substrate was used as the bacterial inoculum (referred to as 'starter soil').

Experimental setup

Maize (*Zea mays* L, SY Milkytop) seeds were surface-sterilized with 5% NaClO (v/v) for 10 min and rinsed eight times with sterile demineralized water. A volume of 250 µl of water was sampled from the third and eighth rinse and spread onto a TSA plate, incubating at 30 °C to check for possible contamination. Following sterilization, all seeds received a cold treatment at 4 °C for 48 h in the dark to synchronize germination; they were then germinated on moist sterile filter paper at 28 °C in darkness for 72 h.

The experiment examined three factors: (1) the levels of salt (0 and 100 mM NaCl); (2) the presence or not of AMF (with or without added *R. irregularis*); (3) the presence or absence of an added soil bacteriomes. Each treatment had five biological replicates. In total, four microbial treatments were used: control (without added microbes), B (single inoculation of soil bacteriomes), RI (single inoculation of *R. irregularis*) and RI + B (co-inoculation of *R. irregularis* and soil bacteriomes).

The experiment was conducted in the greenhouse as from July 2019. The growth substrate used for growing maize was a mixture of soil isolated from Buinen and sand (1:10, v:v; referred to as the 'soil'). Both soil (4 mm) and sand (2 mm) were sterilized by γ-irradiation and autoclave method, respectively, as we described above. All pots (2.5 l) containing 'soil' were adjusted to 65% soil water holding capacity (WHC) before bacterial inoculation. The 'starter soil' was added to the bacterial treatments, whereas the treatments without added bacteriomes were treated with similar amounts of non-bacterial colonized 'soil/sand growth substrate' (v:v = 2:3, γ-irradiation). Then, a 4-week rest period was used to allow bacterial community establishment (van Elsas et al. 2012). Commercial inoculum of *R. irregularis* (Symbiom Ltd, www.symbiom.com) was then introduced into the systems, together with germinated maize seeds (one plant/pot). The AMF inoculum includes *R. irregularis* spores, hyphae and root fragments embedded in calcined diatomaceous earth (diatomite), no other additives included. All *R. irregularis* (AM) treatments used 1800 spores per pot, while non-AM treatments only received 10 ml aqueous filtrate (< 10 µm) of non-sterilized AMF inocula to homogenize microbial community. Eighteen days following the onset of maize growth, we applied salt treatment, by daily increases of 25 mM NaCl, until reaching the desired 100 mM NaCl to avoid osmotic shock. All pots were placed in the same climate chamber with a 14/10-h light/dark cycle and temperatures of 22/18 °C (day/night). Pots were supplied with sterile water daily and 50% modified Hoagland solution (Hoagland and Arnon 1950) with 10% phosphate (nutrient composition: 3.25 mM KCl, 2.5 mM CaCl₂, 0.1 mM KH₂PO₄, 1 mM MgSO₄, 3.75 mM NH₄NO₃, 23.4 µM H₃BO₃, 4.8 µM MnCl₂, 0.48 µM ZnSO₄, 0.16 µM CuSO₄, 0.26 µM Na₂MoO₄ and 45 µM Fe³⁺EDTA).

Leaf relative water content (RWC) measurement

As a proxy for the plant water status, leaf relative water content values (RWC%) were determined the day before harvest. Two leaflets from four different plants per treatment were taken. The fresh weight (FW) was measured directly. Leaves were then transferred into petri dishes containing de-ionized water, incubating for 24 h at 4 °C in the dark. Hereafter, the turgid weight of the leaflet (TW) was weighted, and then the leaflet was dried (DW) for 48 h at 80 °C. RWC (%) was calculated as

$$\frac{FW - DW}{TW - DW} \times 100$$

Plant harvest and mycorrhizal colonization measurement

Maize shoots and roots were harvested together after growth for 50 days (V15 stage, Fig S1). A weighed subsample of the fresh roots was stored in 50% ethanol for quantification of mycorrhizal colonization. Fresh weight of shoot and remaining fresh root were recorded and then dried at 70 °C for 4 days. Mycorrhizal colonization in roots was assessed in both AM and non-AM treatments (checking if there is any contamination). Approximately 2-g root subsamples were cut into 1-cm pieces, cleared with 10% KOH in autoclave liquids cycle at 121 °C for 15 min, acidified with 2% HCl for 15 min and stained with 0.05% (m/v) trypan blue in lactic acid: glycerol:water (1:1:1, v/v/v) (modified from Phillips and Hayman 1970). The extent of root length colonized by hyphae, arbuscule and vesicle was determined with the magnified intersection method (McGonigle et al. 1990). Total mycorrhizal colonization level (M%), arbuscule colonization level (A%), and vesicle colonization levels (V%) were calculated separately.

$$M\% = \frac{T - N}{T} \times 100$$

$$A\% = \frac{A + AV}{T} \times 100$$

$$V\% = \frac{V + AV}{T} \times 100,$$

where T = total number of intersections sampled; N = number of intersections without any fungal structure; A = number of intersections where arbuscules were present; V = number of intersections where vesicles were present; AV = number of intersections where both arbuscule and vesicle were present. Note that the percentage values relate to the number of intersections, not the number of

structures (when an intersection intersected more than one fungal structure, it was still only scored as one in that specific structure).

Statistical analyses

All data were checked for normality and homogeneity of variance by using Shapiro–Wilk’s test and Levene’s test, respectively, before analysis. Three-way analysis of variance (ANOVA) was performed to analyze the effects of salt, AMF (*R. irregularis*), soil bacteriomes and their interactive effects on the shoot dry weight, root dry weight and leaf relative water content RWC%. To assess the interactions between AMF (*R. irregularis*) and soil bacteriomes on shoot and root dry weight under non-salt or salt conditions respectively, we performed two-way ANOVA analysis. A following Tukey’s honest significant difference (HSD) test was used to check the differences in shoot dry weight, root dry weight and leaf relative water content RWC% between different treatments under non-salt and salt conditions. Two-way ANOVA was conducted to check the effects of salt, soil bacteriomes and their interactions on the total mycorrhizal colonization (M%) and arbuscule colonization (A%). Student’s t tests were then conducted to analyze the differences of mycorrhizal colonization parameters between treatments with single inoculation of soil bacteriomes (B) and the co-inoculation of *R. irregularis* and bacteriomes (RI + B) under different salt levels. Pearson correlation analysis was used to assess the relationship between shoot dry weight and total mycorrhizal colonization (M%), and also the relationship between root dry weight and arbuscule colonization (A%). Variance partitioning analysis (VPA) was conducted to determine the effects of salt and soil bacteriomes on the total mycorrhizal and arbuscule colonization levels, using the ‘varpart’ function in the vegan package. All analyses were performed with R version 3.5.1 (R Core Team 2017).

Table 1 Three-way ANOVA results of the effect of salt, AMF, soil bacteriomes and their interactions on shoot dry weight and root dry weight

Factors	Shoot dry weight (g)			Root dry weight (g)		
	df	F	P value	df	F	P value
Salt	1	368.589	<0.001	1	115.180	<0.001
RI	1	21.702	<0.001	1	0.001	0.9794
B	1	58.203	<0.001	1	16.251	<0.001
Salt × RI	1	1.631	0.2107	1	3.336	0.0771
Salt × B	1	1.006	0.3235	1	0.432	0.5158
RI × B	1	6.118	<0.05	1	0.038	0.8464
Salt × RI × B	1	2.224	0.1457	1	0.080	0.7796

RI *R. irregularis*, B soil bacteriomes

Results

Plant biomass and leaf relative water content

The first objective of this study was to determine the effects of *R. irregularis* and soil bacteriomes, alone or in combination, on maize growth under different salt levels. Salinity (applied as 100 mM NaCl) had a significant inhibitory effect on the growth of the maize plants in all treatments (*three-way ANOVA*, salt: $P_{shoot} < 0.001$, $P_{root} < 0.001$; Table 1, Fig. 1). The presence of soil bacteriomes, either alone or in combination with *R. irregularis* (RI+B), enhanced the growth of both shoots and roots (*three-way ANOVA*, Bacteriomes: $P_{shoot} < 0.001$, $P_{root} < 0.001$; Table 1, Fig. 1). The increase of shoot biomass (RI+B vs. bacterial alone) was higher under non-salt (13.8%) than under salt conditions (8%). Two-way ANOVA further showed a positive interactive effect on shoot dry weight in the absence of salt ($F_{non-salt} = 6.118$, $P_{non-salt} < 0.05$; Fig. 1). Upon inoculation with bacteriomes but without *R. irregularis*, the maize shoot dry weights increased by 6.9% and 11.5% in comparison with the controls under non-salt and salt conditions (Table S1), respectively. Thus, the effect of added soil bacteriomes on maize shoot growth was higher in the salt than in the corresponding non-salt condition. Adding *R. irregularis* alone (without an added bacteriomes) did not affect maize growth, while co-inoculation with bacteriomes (RI+B) increased shoot dry weight by 18.2% under non-salt and 14.8% under salt stress, indicating the helper effect of the bacteriomes. The shoot biomass in the co-inoculation treatment (RI+B) showed the highest shoot biomass, with an approximately 20% increase over the control, under both non-saline and saline conditions (Table S1). This indicated that the synergistic shoot-growth-promoting effect of *R.*

irregularis and soil bacteriomes was more efficient in the presence of salt.

Exposure to salt decreased the RWC% values (*three-way ANOVA*, salt: $F = 31.835$, $P < 0.001$; Fig. S2). However, although these values were higher in the plants that had received bacterial inoculum than those in the controls, this effect was not statistically significant (Fig. S2).

Effects of salt and bacteriomes on maize root colonization by *R. irregularis*

In the treatments that had received *R. irregularis*, maize plants were abundantly colonized by this fungus, with values exceeding 80%. We did not find AM fungal contamination in the non-AM treatments. Total mycorrhizal colonization was not affected by salinity, whereas co-inoculation with bacteriomes increased this value in the root (*two-way ANOVA*, bacteriomes: $F = 13.085$, $P < 0.01$, Table S2; *t*-test, $P_{non-salt} = 0.057$, $P_{salt} < 0.05$, Fig. 2a), explaining 42% of the variance in total mycorrhizal colonization (Fig. 2c). The density of vesicles in the root was not affected by either salinity or bacteriomes (Table S2, Fig. S3).

Exposing maize roots to salt affected the density of arbuscules, being that salinity led to an increased arbuscule density (*two-way ANOVA*, salt: $F = 25.356$, $P < 0.001$, Table S2; Fig. 2b). In the co-inoculation treatment, this effect was diluted, to a certain extent, in the presence of bacteriomes, given that plants treated with *R. irregularis* (RI) alone had a greater increase than plants treated with both *R. irregularis* and bacteriomes (RI+B), after addition of salt (*t* test, $P_{RI} < 0.01$, $P_{RI+B} = 0.049$; Fig. 2b). Thus, salt and bacteriomes are both key factors that drive arbuscule colonization, explaining 42% and 25% of the variance in this parameter, respectively (Fig. 2d).

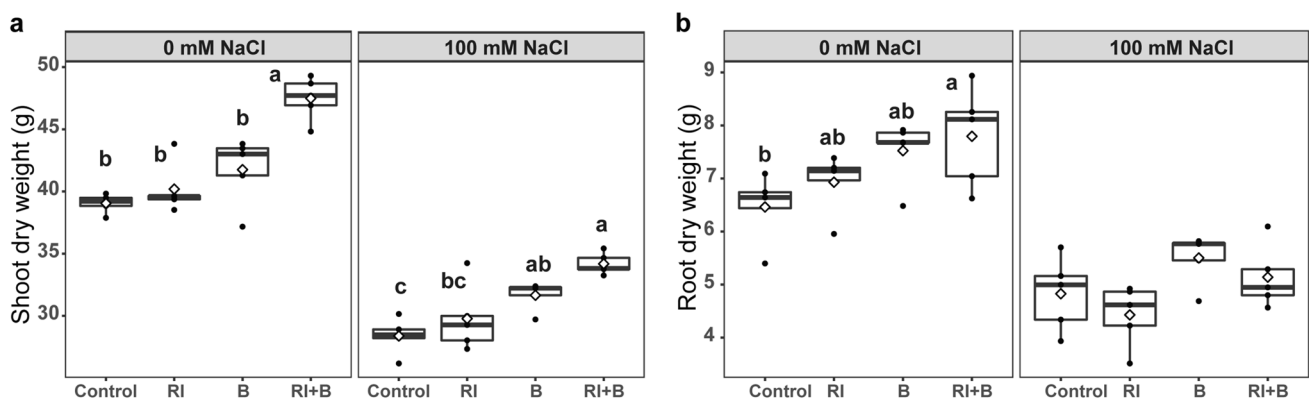


Fig. 1 Shoot (a) and root (b) dry weight influenced by different microbial treatments under non-salt (0 mM NaCl) and salt stress (100 mM NaCl). Control: without any microbial inoculum; B: single inoculation of soil bacteriomes; RI: single inoculation of *R. irregularis*; RI+B: co-inoculation of *R. irregularis* and bacteriomes. Different lower letters indicate statistically significant differences among microbial treatments within each salt concentration for each measured parameter (Tukey’s HSD tests, $P < 0.05$)

laris; RI+B: co-inoculation of *R. irregularis* and bacteriomes. Different lower letters indicate statistically significant differences among microbial treatments within each salt concentration for each measured parameter (Tukey’s HSD tests, $P < 0.05$)

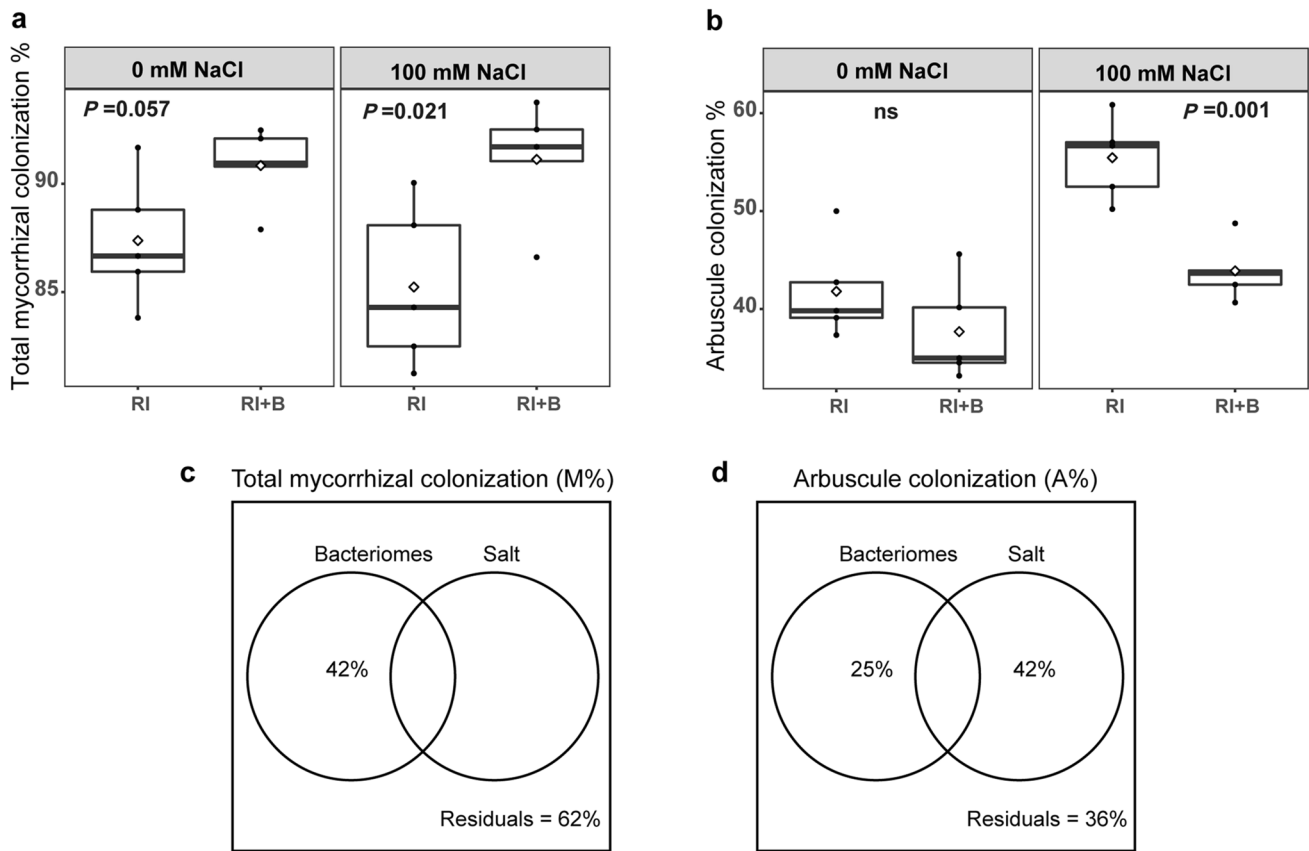


Fig. 2 Influence of salt and soil bacteriomes on mycorrhizal colonization by *R. irregularis* under non-salt (0 mM NaCl) and salt stress (100 mM NaCl). Boxplots showing the total mycorrhizal hyphal colonization (**a**) and arbuscule colonization (**b**) in different treatments. RI: single inoculation of *R. irregularis*; RI+B: co-inoculation of *R. irregularis* and bacteriomes. Significant difference was examined by

Student's *t* test ($P < 0.05$) and non-significant difference was indicated by 'ns'. Variation partitioning modeling showing the percentage of the variance of the total mycorrhizal colonization (**c**) or arbuscule colonization (**d**) explained by salt and bacteriomes (values less than 0 were not displayed)

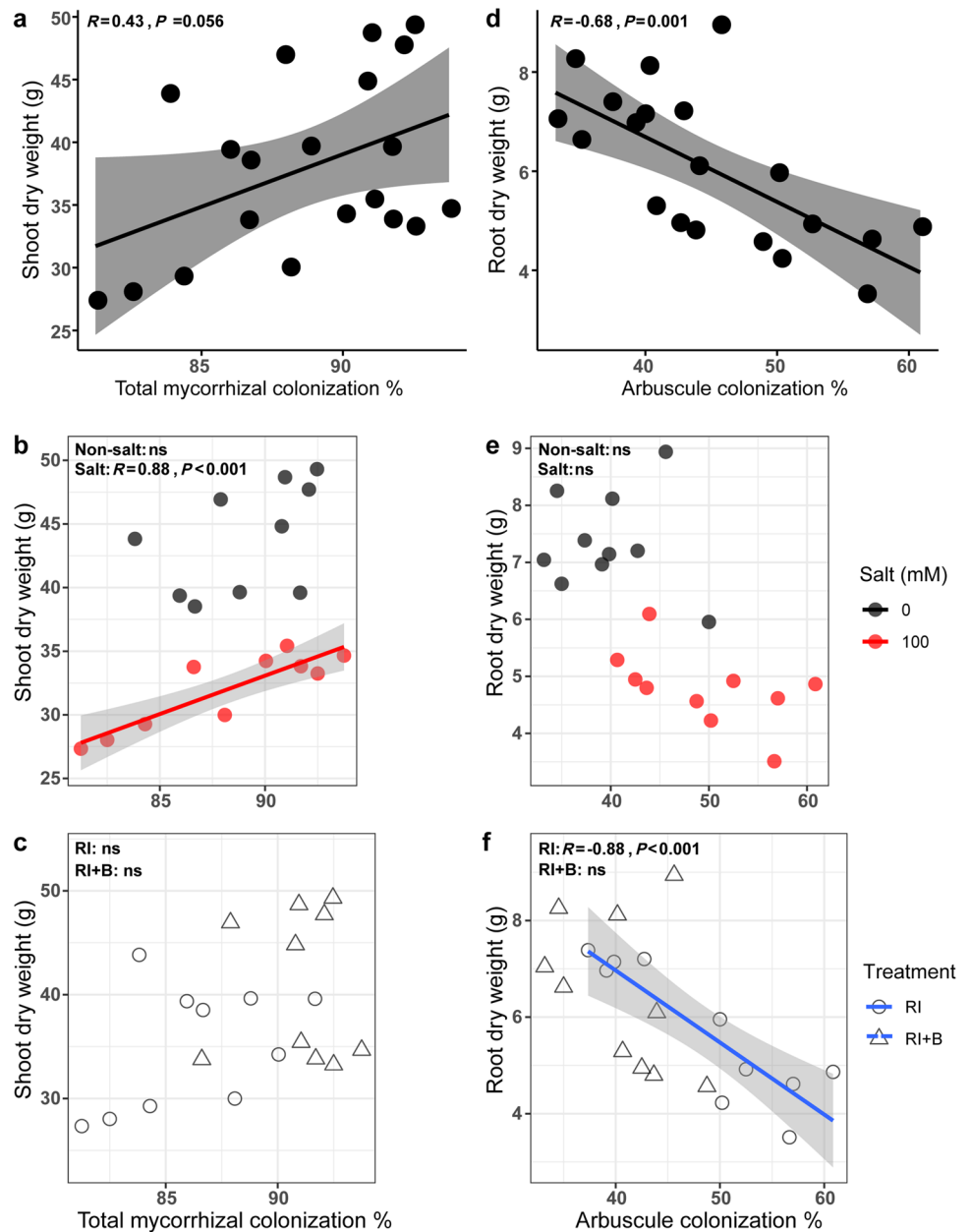
Relationship between mycorrhizal colonization and plant biomass

As the effects of salinity and soil bacteriomes on plant growth and AMF colonization might be correlated, the correlation between total mycorrhizal colonization level (M%) and shoot dry weight was assessed in all *R. irregularis*-treated plants. Using the whole dataset of mycorrhizal plants, the correlation between the total mycorrhizal colonization (M%) and shoot biomass was at the border of significance with the *P* value near 0.05 ($R = 0.43$, $P = 0.056$; Fig. 3a). When we examined the maize plants exposed to non-salinity and salinity separately, the total mycorrhizal colonization values (M%) were positively correlated with shoot biomass levels ($R = 0.88$, $P < 0.001$; Fig. 3b); however, this correlation was not found in maize plants from the non-salinity treatments (Fig. 3b). In the *R. irregularis* alone or co-inoculation treatments, we did not find significant associations between total mycorrhizal colonization

level (M%) and shoot dry weight levels (Fig. 3c), which may be because salinity did not influence total mycorrhizal colonization in both *R. irregularis* treatments with or without bacteriomes (Fig. 2a).

For the arbuscules levels (A%), we found a negative correlation with root dry weight ($R = -0.68$, $P = 0.001$; Fig. 3d). Considering the finding of a marginal interactive effect of salt and bacteriomes on A% (*two-way ANOVA*, salt \times bacteriomes: $F = 3.565$, $P = 0.077$; Table S2), we conducted a Pearson correlation analysis between A% and root dry weight levels in separate, according to different salt treatments or different *R. irregularis* treatments (with or without bacteriomes inoculation). We found no correlation between these two parameters under both non-salt and salt stress, respectively (Fig. 3e). A significant negative correlation was found in the RI treatment (Fig. 3f, $R = -0.88$, $P < 0.001$), but not in RI+B treatment, indicating that the presence of bacteriomes affected the density of the arbuscules along the root.

Fig. 3 Relationship between maize dry weight (shoot, root) and mycorrhizal colonization levels (total mycorrhizal colonization, arbuscule colonization). Pearson correlation analyses were conducted on all the mycorrhizal plants (a, d). Pearson correlation analyses were performed separately according to different salt treatments (b, e), or different treatments with or without bacteriomes (c, f). Different colors represent different salt levels (0 mM NaCl, 100 mM NaCl), and different shapes indicate different mycorrhizal treatments. RI: single inoculation of *R. irregularis* (indicated by blue solid line); RI+B: co-inoculation of *R. irregularis* and bacteriomes (indicated by blue dotted line). The shaded area shows 95% confidence intervals for the fitted line. The non-significant correlations were indicated by ‘ns’



Discussion

Soil bacteriomes and AMF are important microorganisms that can affect plant growth under stressful conditions, such as salinity. To make better use of microbes, it is necessary to understand the mechanisms of the interactions between these microorganisms, and between these and the plants (Zhang et al. 2021). To explore their effects on maize growth under salinity and the influence of salinity and soil bacteriomes on maize root mycorrhizal colonization, we introduced soil-derived bacteriomes to sterilized soil/sand systems with or without added *R. irregularis*. The introduced soil bacteriomes enhanced the effect of *R. irregularis* on the growth of

maize plants. In addition, both salt and bacteriomes affected maize roots colonization by *R. irregularis*.

The role of bacteriomes in *R. irregularis* colonized maize growth

For maize, it has been well established that association of its roots with mycorrhizal fungi can be beneficial for growth under low nutrient and abiotic stress conditions (Estrada et al. 2013; Liu et al. 2016, 2018; Sawers et al. 2017; Willmann et al. 2013). However, in the experiments performed in this study, colonization by the single inoculation of AM fungus *R. irregularis*, did not stimulate the growth of the

maize cultivar SY Milkytop, neither in the non-saline control, nor under saline conditions.

A stimulatory effect of AMF on crop plants is not always observed, and inhibitory effects on plant growth can even occur (Jacott et al. 2017). Thus, plant–mycorrhiza symbioses can have, next to positive, neutral or even negative effects on plant growth, depending on factors like plant genotype and fungal specificity, plant developmental stage and environmental conditions (Chen et al. 2018; Johnson et al. 1997). Different maize lines or cultivars respond variably to the presence of AMF (*R. irregularis*, *Funneliformis mosseae*) in terms of plant growth (Chu et al. 2013; Sawers et al. 2017). Under particular soil conditions, mycorrhizal symbioses with soybean and sunflower were found to be marginally beneficial or even negative during the early stages of plant growth (Bethlenfalvay 1982; Koide 1985). This was, potentially, due to low mycorrhizal infection rates during the early growth stage, with the AMF investing few resources into their host in comparison with non-AM plants, in order to reach the reproductive stage of AMF. In our study with a low P nutrient input, maize was harvested 50 days after seed germination and maize plants were no longer in a juvenile stage, with the mycorrhizal colonization level being high. Thus, we assume that our maize cultivar (SY Milkytop) has a low growth response to *R. irregularis*. Besides, we grew one maize plant per pot (2.5 l); thus, the benefits of introducing *R. irregularis* alone may be limited.

Even though the high colonization levels of *R. irregularis* did not greatly stimulate maize growth, the root colonization levels indicated the existence of a trade-off between the two partner organisms. The benefits of *R. irregularis* colonization to maize biomass may have been counteracted by the increasing C consumption by the increasing fungal biomass (Sawers et al. 2008). Maize root biomass inoculated with *R. irregularis* alone was lower than that inoculated with bacteriomes alone. It is possible that with the single inoculation of bacteriomes, the growth of roots is stimulated to recruit microbes thus obtaining a wider range of substances from soil. Alternatively, the extraradical hyphae in the soil may have functioned as the classical AMF-driven root extensions, enabling to acquire nutrients (instead of investing much energy in developing root systems), or the direct nutrient uptake way via roots may have been depressed by the AM fungal pathway (Grace et al. 2009).

Even though we did not see a clear stimulatory effect of *R. irregularis* alone on the growth of maize, the introduction of bacteriomes alone or the dual inoculation did boost maize growth. This finding reflects the helper role of soil bacteriomes in promoting maize growth, either alone or in combination with *R. irregularis*. Such effects have been attributed to mycorrhization helper bacteria (MHB), i.e. bacteria that aid in the establishment of an effective plant–fungal interaction, specifically under stress conditions

(Frey-Klett et al. 2007). It has been reported that the benefit of AMF (*Rhizoglyphus irregularis*) on maize growth and nutritional status can be intensified by the presence of the PGPB *Pseudomonas reactans* EDP28 and *Pantoea alli* ZS 3–6 under salinity (0–5 g NaCl kg⁻¹ soil). Phosphate solubilizing bacteria (PSB), which can grow on the hyphal surface of *R. irregularis*, could collaborate with their fungal host to increase P mobilization (Taktek et al. 2015). A recent study highlights the cooperation between AMF and bacteria, showing that a water film (2–10 µm) developing around the hyphae of *R. irregularis*, could bring PSB close to the organic P source with the energy provided by fungal exudates (Jiang et al. 2021). Our study did not prove the presence of specific mycorrhization helper bacteria contributing to root functioning, but our observations clearly show an increasingly important interactive effect on stimulating maize growth in the joint inoculations of *R. irregularis* and bacteriomes. This study is a good model to study the interaction between AMF and soil bacterial community, but we explore only one bacterial community that was inoculated to a particular soil type. It is possible that bacterial communities from other soils behave differently. In this study, we did not check the effect of the original soil (with the whole microbiome) from which we obtained the soil bacteriomes on the colonization of *R. irregularis* and the growth of maize colonized by this AM fungus. However, addressing how the whole soil biome affects mycorrhizal colonization and plant growth is besides the issue of studying effects of isolated soil bacteriomes. Clearly, work based on other isolated soil bacteriomes would shed light on questions as to the generality or specificity of the effects seen.

Salt and bacteriomes affect mycorrhizal colonization of maize

Salinity had no effect on the overall *R. irregularis* infection rate in this study. Although salinity, to some extent, has adverse effects on fungal spore germination, hyphal growth and colonization ability (Juniper and Abbott 2006), AMF are generally not very sensitive to salt stress. Diverse AMF have been found in strongly saline environments, such as salt marshes with sodium contents of 3500 to 6400 mg dm⁻³ (Dini-andreote et al. 2016) and colonized halophytes (Hildebrandt et al. 2001). Colonization of roots by AMF is related to plant genotype, AMF behavior and factors such as the origin of AMF, soil nutrient status and stress factors (Nadeem et al. 2014; Säle et al. 2021). The influence of salt (66–100 mM NaCl) on mycorrhizal colonization of maize roots is different between different types of AMF (Estrada et al. 2013). Moreover, the influence of salt on AMF colonization of roots may depend on the salt levels applied (Sheng et al. 2008; Wang et al. 2019). However, salt increased the *R. irregularis* arbuscule levels, especially in the *R. irregularis*

treatment without added bacteriomes, suggesting that *R. irregularis* assists maize to obtain nutrients under salinity. Secondly, there was a negative association between maize root biomass and arbuscule colonization levels. This can be interpreted as root growth, but not arbuscule development, being affected by salinity, resulting in arbuscules becoming ‘denser’ in the resulting root biomass. One thing that was consistent in this study was that, with or without salt stress, soil bacteriomes consistently reduce arbuscule colonization and increase overall mycorrhization levels. Despite the bacterial effect on arbuscule abundance, we did not find a relationship between this parameter and maize growth. There is no consensus at this moment to highlight the positive relationship between total mycorrhizal colonization and plant growth, which is probably related to plant genotype, AM fungal species and environmental factors. However, our correlation analysis results suggest that the promotion of overall mycorrhizal colonization contributes to the increase in maize shoot biomass under saline stress but not under non-saline environment.

Regarding the reduced arbuscule colonization rates with the introduced bacteriomes under salt stress, there are several possible explanations. There might be an increase in nutrient availability when bacteria are present. In the presence of bacteriomes, *R. irregularis* seems to shift the allocation of resource from the formation of arbuscules to the development other fungal structures (e.g. hyphae) as indicated by the enhanced total fungal colonization levels of maize roots. In this study, arbuscule colonization (%) was used as a relative quantification, and so one may imagine a situation in which the absolute quantity does not change, only due to the contribution of bacteria to the increased root biomass (Fig. 1b). We observed a positive correlation between total mycorrhizal colonization and maize shoot biomass levels under salt stress, indicating that *R. irregularis* mycorrhization is beneficial to the growth of maize plants exposed to salinity stress, at least to a limited extent. However, the specific mechanisms underlying the bacterial promotion of mycorrhiza-stimulated plant growth are still enigmatic. For instance, do the added bacteriomes directly promote maize growth? Or do they enhance the effectiveness of mycorrhiza-plant interactions and thus influence maize growth indirectly, or is it a combination of the two mechanisms?

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Author contribution QC, JTME and JDVE designed this study. QC performed the experiment, collected and analyzed the data, and wrote the manuscript. XD helped with the VPA analysis. JTME and JDVE

reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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Declarations

Ethics approval Not applicable.

Conflict of interest The authors declare no competing interests.

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