

LETTER

Factors controlling the effects of mutualistic bacteria on plants associated with fungi

Daniel A. Bastías  | Emma R. Applegate  | Linda J. Johnson  | Stuart D. Card 

Grasslands Research Centre, AgResearch Limited, Palmerston North, New Zealand

CorrespondenceDaniel A. Bastías, Grasslands Research Centre, AgResearch Limited, Palmerston North, New Zealand.
Email: daniel.bastias@agresearch.co.nz**Funding information**

Ministry of Business, Innovation and Employment, Grant/Award Number: LVLX1702

Editor: Marc-André Selosse

Abstract

Plants interacting with mutualistic fungi (MF) or antagonistic fungi (AF) can form associations with bacteria. We assessed whether the performance gain conferred by mutualistic bacteria to fungal-associated plants is affected by the interaction between symbiont traits, type of bacterial-protective traits against AF and abiotic/biotic stresses. Results showed that (A) performance gain conferred by bacteria to MF-associated plants was greater when symbionts promoted distinct rather than similar plant functions, (B) bacterial-based alleviation of the AF's negative effect on plants was independent of the type of protective trait, (C) bacteria promoted a greater performance of symbiotic plants in presence of biotic, but not abiotic, stress compared to stress-free situations. The plant performance gain was not affected by any fungal-bacterial trait combination but optimised when bacteria conferred resistance traits in biotic stress situations. The effects of bacteria on fungal-associated plants were controlled by the interaction between the symbionts' functional traits and the relationship between bacterial traits and abiotic/biotic stresses.

KEYWORDS

antagonistic symbionts, mutualistic symbionts, plant-associated fungi, plant-microbe interactions, tripartite plant symbiosis

INTRODUCTION

Plants within natural and managed ecosystems commonly interact with mutualistic and antagonistic fungi (MF and AF respectively). Examples of MF include mycorrhizae and endophytes that belong to the families Glomeraceae (e.g. *Rhizophagus*) and Serendipitaceae (e.g. *Serendipita*) respectively. AF include biotrophic and necrotrophic pathogens within the orders Erysiphales (e.g. *Blumeria*), Pleosporaceae (e.g. *Alternaria*) and Sclerotiniaceae (e.g. *Botrytis*) (Balestrini, 2021; Porrás-Alfaro & Bayman, 2011). MF positively affect plant fitness by promoting plant growth, conferring protection against abiotic and biotic stresses and increasing

nutrient acquisition (Partida-Martínez & Heil, 2011). AF negatively affect plant fitness by disrupting the structure and function of plant organs and/or tissues (Doehlemann et al., 2017). As well as interacting with fungi, plants can simultaneously form associations with mutualistic bacteria. Examples include soil-borne and/or endophytic microbes that belong to phyla Actinobacteria (e.g. *Streptomyces*), Firmicutes (e.g. *Bacillus*) and Proteobacteria (e.g. *Rhizobium*) (Bastías et al., 2020; Bonfante et al., 2019). Bacteria can increase plant fitness by (1) directly enhancing the plant's ability to function and/or (2) indirectly, by affecting the growth/activities of plant-associated fungi (MF or AF) (Bangera & Thomashow, 1999; Frey-Klett et al., 2011; Glick, 2012).

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial](https://creativecommons.org/licenses/by-nc/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2022 The Authors. *Ecology Letters* published by John Wiley & Sons Ltd.

Bacteria can enhance the function of their plant hosts via growth promotion, stress protection, and/or by improving the host plant's nutrient acquisition (Glick, 2012). Bacteria can also affect plant-associated fungi by stimulating the growth of MF (e.g. providing essential vitamins) or suppressing the activities of AF (e.g. via antifungal compounds) (Bangera & Thomashow, 1999; Frey-Klett et al., 2011; Glick, 2012). The factors controlling the performance gain conferred by bacteria to plants associated with fungi have been scarcely studied (Larimer et al., 2010; Porter et al., 2020). We investigate whether bacteria affect the performance gain of plants that are simultaneously associated with fungi (MF or AF) and determine if this performance gain is affected by the trait interaction between symbionts, the type of protection traits conferred by bacteria against AF, and abiotic/biotic environmental stresses.

Plants typically form concurrent mutualistic tripartite symbioses with both fungi and bacteria (Larimer et al., 2014). Within these symbioses, plants can associate with fungi and bacteria that confer traits promoting distinct or identical/similar functions to that of their plant hosts. Associations with functionally distinct symbionts include plants simultaneously interacting with fungi and bacteria that enhance the plant's acquisition of nutrients and promote host plant growth via the production of hormones, respectively (e.g. Vivas et al., 2006). In contrast, associations with functionally equivalent symbionts include plants symbiotic with both fungi and bacteria that increase the plant's acquisition of nutrients from soil (e.g. Minaxi et al., 2013). The performance gain conferred by mutualistic bacteria to MF-associated plants depends on the interaction between the traits conferred by both symbionts (Afkhani et al., 2014). Fungal and bacterial symbionts that confer distinct functional traits to their plant hosts may enhance plant performance to a greater degree than those that confer equivalent functional traits to their plant hosts (Larimer et al., 2010). For example, tomato (*Solanum lycopersicum* syn. *Lycopersicon esculentum*) plants exhibited a higher biomass when they were simultaneously associated with a phosphorus-enhancing fungus and a growth-promoting bacterium (Gamalero et al., 2003), compared to tomato plants simultaneously associated with only phosphorus-enhancing microbes (Gamalero et al., 2004).

Plants can also form tripartite symbioses with AF and mutualistic bacteria (Kobayashi & Crouch, 2009). These bacteria are able to ameliorate the negative effects imparted by AF by conferring disease resistance and/or tolerance traits (Hol et al., 2013; Roy & Kirchner, 2000). Bacterial resistance traits can reduce AF-induced plant damage by directly affecting the phytopathogen, for example by the production of antifungal metabolites and/or promotion of host plant defences (Khan et al., 2018; Martínez-Hidalgo et al., 2015). Bacterial tolerance traits reduce the negative impact of AF by increasing plant fitness without directly affecting the phytopathogen, for

example by direct enhancement of plant growth via the production of growth-promoting hormones and/or the acquisition of nutrients from soil (Hashem et al., 2017). The performance gain conferred by mutualistic bacteria to AF-associated plants largely depends on the types of protection traits that bacteria confer to their host plants. Bacteria that confer a combination of resistance and tolerance traits may alleviate the negative effects caused by AF to a greater degree than those that provide only resistance or only tolerance traits. For example, the size of leaf lesions caused by the pathogen *Sclerotinia sclerotiorum* on rapeseed (*Brassica napus*) plants was significantly reduced when plants were associated with a bacterial strain that was able to produce a combination of resistance and tolerance traits (i.e. antifungal compounds and auxin growth-promoting hormones) compared to plants that were associated with another strain that only produced a tolerance trait (i.e. auxin production) (Sun et al., 2017).

The performance gain conferred by bacteria to plants associated with fungi (MF or AF) may also be influenced by abiotic and biotic stresses experienced by their hosts (Afkhani et al., 2014; Porter et al., 2020). Bacteria that confer stress protection traits may enhance the performance of plants associated with MF or AF to a greater degree in the presence of abiotic or biotic stresses compared to situations where the stress is absent. For example, in a tripartite plant-fungal-bacterial mutualism experiencing an abiotic stress, a halotolerant bacterium increased the growth of maize (*Zea mays*) plants associated with mycorrhizal fungi under salt stress, but this growth promotion was not observed in situations when this stress was absent (Selvakumar et al., 2018). Furthermore, within a plant-fungal-bacterial mutualism experiencing a biotic stress, tomato plants associated with mycorrhizal fungi and a nematocidal bacterium gained more foliar biomass in the presence of the root knot nematode (*Meloidogyne incognita*) than when these tripartite symbiotic plants were grown in the absence of this nematode (Siddiqui & Sayeed Akhtar, 2009). Similarly, in a tripartite symbiosis with AF and mutualistic bacteria, tomato plants associated with a bacterium that induced host plant defences showed a greater net gain of plant height when symbiotic plants were also infected by a phytopathogen (*Botrytis cinerea*) compared with symbiotic plants that were pathogen-free (Kim et al., 2017).

We evaluated the performance gain of plants conferred by mutualistic bacteria associated with MF or AF across three different symbiotic scenarios (Figure 1): (A) the interaction between traits conferred by MF and bacteria, that is symbionts conferring functionally distinct vs. functionally equivalent traits, (B) the types of protection traits conferred by bacteria against AF, that is resistance & tolerance versus only resistance versus only tolerance traits against AF and (C) the abiotic/biotic environmental stresses, for example drought, phytopathogen

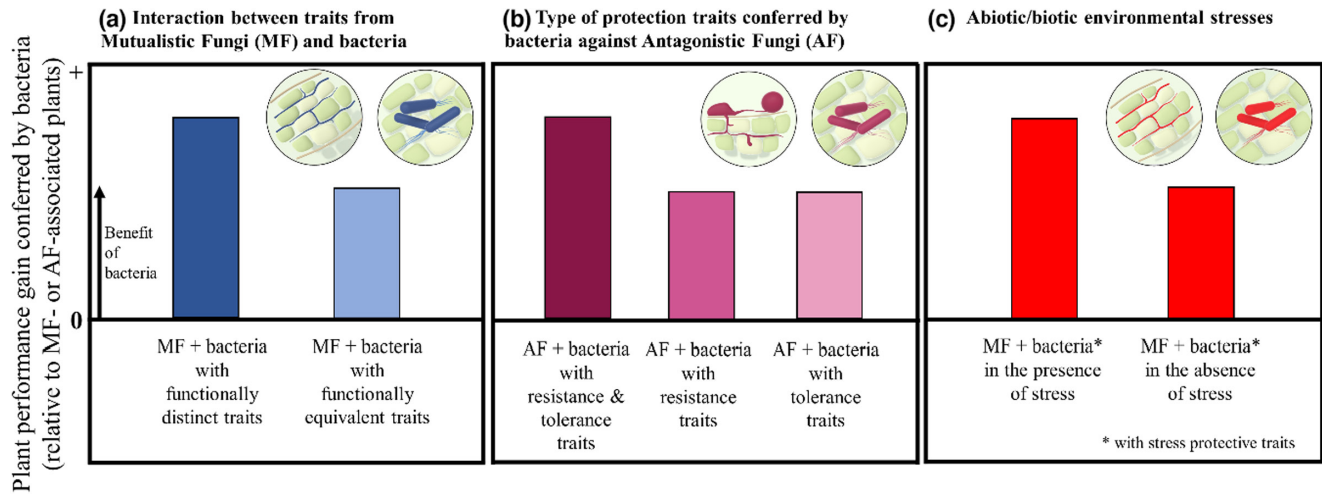


FIGURE 1 Predicted effects of three major factors on the performance gain conferred by bacteria to plants associated with Mutualistic Fungi (MF) or Antagonistic Fungi (AF). (a) Interaction between MF-bacterial traits: Fungi and bacteria that confer traits promoting distinct plant functions may lead to a greater gain in plant performance than symbionts that confer traits enhancing similar/identical plant functions (i.e. functionally distinct MF-bacteria vs. functionally equivalent MF-bacteria). (b) Type of protection traits conferred by bacterial symbionts: bacteria that confer both pathogen resistance and tolerance traits to plants interacting with AF may alleviate to a greater degree the AF's negative effect on plant performance than bacteria that confer these trait types separately. (c) Abiotic/biotic environmental stresses: bacteria that confer stress-protective traits to their MF-associated plant hosts may lead to a greater gain in plant performance in the presence of abiotic/biotic stresses compared to those plants in the absence of any stress.

infection. For this undertaking, we performed quantitative meta-analyses and general linear models (GLM) using data from published articles. We evaluated the following hypotheses (Figure 1). First, for plants interacting with MF, we evaluated whether bacteria conferring distinct functional traits than those provided by fungi to their host plants would increase plant performance to a greater degree than symbionts conferring equivalent functional traits (e.g. bacteria that stimulate plant nutrition via nitrogen fixation and fungi that produce plant growth-promoting hormones vs. bacteria and fungi that both stimulate plant nutrition) (Figure 1a). Additionally, we evaluated which combination or type of fungal-bacterial functional traits enhanced plant performance to a greater degree. Second, for plants interacting with AF, we evaluated whether bacteria conferring both resistance and tolerance traits simultaneously against AF would alleviate to a greater degree the negative effects of AF on plant performance than bacteria providing these trait types separately (e.g. bacteria that produce antifungal compounds and plant growth-promoting hormones vs. bacteria that produce only plant growth-promoting hormones) (Figure 1b). Finally, we evaluated whether bacteria that confer stress protection traits to their symbiotic plant hosts could promote greater performance in the presence of an abiotic or biotic stress as opposed to situations where this stress was absent (e.g. bacteria that produce nematocidal compounds that benefit their MF-associated host plants in the presence of nematodes vs. the same plant-fungal-bacterial association that lacks infection by nematodes) (Figure 1c). In studies where symbiotic plants experienced biotic stress, we also evaluated which type of stress protectional trait conferred by

bacteria promoted greater plant performance gain in the presence/absence of this stress.

MATERIALS AND METHODS

We reviewed and selected the literature for published journal articles describing investigations on tripartite plant-fungal-bacterial associations. The results of the selected articles were quantitatively analysed using meta-analyses and GLMs (see supplementary material and Table S1 for details about the study selection criteria, methods and the complete database).

We compiled a database composed of 139 published articles that included 357 case studies, of which 202 case studies evaluated the effects of bacteria on the performance of MF-associated plants and 155 case studies evaluated the effects of bacteria on the performance of AF-associated plants (see Table S1). The database included ~58 plant species, 60 fungal species (38 MF and 22 AF) and 84 bacterial species. The traits conferred by fungi and bacteria to their plant hosts included growth promotion (e.g. auxins, gibberellins), nutrient acquisition (e.g. nitrogen fixation) and protection against abiotic or biotic stresses (e.g. 1-aminocyclopropane-1-carboxylate [ACC] deaminase, antimicrobial compounds). In case studies involving tripartite plant symbioses with MF and bacteria ($n = 202$), the traits conferred by each microbial symbiont to their plant hosts were compared to determine whether plants were associated with functionally distinct or functionally equivalent symbionts. In certain case studies, the functional relationship between symbionts was unknown due to a lack of information relating

to fungal or bacterial traits. Functionally distinct symbionts conferred traits that promoted plant growth & nutrition, nutrition & stress protection, or growth & nutrition & stress protection. Within these categories, bacteria conferred traits that stimulated plant growth and/or stress protection while fungi added traits that promoted plant nutrition. Traits associated with functionally equivalent symbionts stimulated plant growth, nutrition, or stress protection. Functionally equivalent symbionts included those symbionts that promoted different aspects of the same plant function (e.g. fungi and bacteria that enhanced the acquisition of phosphorus and nitrogen respectively) (see Table S1). In case studies involving tripartite plant symbioses with AF and bacteria ($n = 155$), traits conferred by bacteria to their plant hosts were further classified as either traits of resistance (i.e. production of antimicrobials, siderophores and induced plant resistance) or tolerance (i.e. nutrient uptake, production of plant-growth promoting hormones) against AF. Thus, each bacterium listed in the case studies involving plant-AF-bacteria associations was classified as exhibiting a combination of resistance & tolerance traits, only resistance traits, only tolerance traits, or unknown trait/s against AF (see Table S1).

We performed independent meta-analyses on data associated with plants interacting with (i) MF+bacteria ($n = 202$) and (ii) AF+bacteria ($n = 155$). Using data from (i), we evaluated the effect of bacteria on the performance on MF-associated plants. We also assessed whether the performance gain conferred by bacteria to MF-associated plants depended on the interaction between fungal–bacterial traits (i.e. symbionts conferring functionally distinct vs. functionally equivalent traits). Furthermore, we evaluated whether plant performance gains depended on the specific functions promoted by symbionts (i.e. functionally distinct group: growth & nutrition vs. nutrition & stress protection vs. growth & nutrition & stress protection; functionally equivalent group: growth vs. nutrition vs. stress protection). Furthermore, we evaluated the effect of MF and bacteria on the performance of symbiont-free plants ($n = 173$; $n = 157$). Using data from (ii), we evaluated the effect of bacteria on performance of AF-associated plants. Furthermore, we evaluated whether the performance gain conferred by bacteria to AF-associated plants depended on the type of protection trait conferred by the bacterium against AF (i.e. resistance and tolerance traits vs. only resistance traits vs. only tolerance traits). We also assessed the effect of AF and bacteria on the performance of symbiont-free plants ($n = 112$; $n = 46$). In these analyses, a $p < 0.05$ indicated that the mean effect size value at least in one categorical group did not overlap the 95% confidence interval (CI) effect size of another categorical group (Cumming & Finch, 2005) (effect size calculation details in the [supplementary material](#)).

We performed additional statistical analysis to determine whether the performance gain conferred by bacteria

to MF-associated plants varied with the presence of abiotic or biotic stresses. For this, we fitted a GLM to test the relationship between performance gains conferred by bacteria to MF-associated plants in the presence and absence of an abiotic or biotic stress. In this GLM, we also included data regarding the relationship between performance gains of plants conferred by bacteria in the presence and absence of AF (AF is the biotic stress). To compile the required case studies, we reviewed the entire database (Table S1) and identified studies that included the presence and absence of any abiotic or biotic stress in their experimental designs and information regarding the specific trait/s conferred by bacteria to their host plants. We retrieved 81 case studies from our database that fitted these criteria. Abiotic stresses ($n = 39$) included soil salinity, soil contamination, water restriction or nutrient scarcity, while biotic stresses ($n = 42$) included the presence of fungal pathogens (= AF; $n = 40$) or parasitic nematodes. In all these case studies, bacteria provided one or several mechanisms of stress protection to their host plants (e.g. ACC, antioxidants, antimicrobials) (see Table S1). In those study cases that included the presence/absence of biotic stresses ($n = 42$), we fitted an additional GLM to test whether the performance gain conferred by bacteria to MF-associated plants depended on the type of stress-protective traits added by the bacterium (i.e. resistance & tolerance traits vs. only resistance traits vs. only tolerance traits). The performance gain conferred by bacteria to plants associated with fungi (either MF or AF) in the presence of a specific stress (abiotic or biotic) was calculated by comparing the performance of symbiotic plants with and without bacteria while in the presence of the stress. The performance gain conferred by bacteria to plants in the absence of stress was determined by comparing the performance of MF-associated plants or AF-free plants (when AF were the cause of the stress) with and without bacteria in the absence of the stress. The visual inspection of data distribution associated with plant performance gain relationships uncovered the presence of one study that could influence the GLM results (the study at the upper right quadrant in Figure 4a,b). This study, while it did not show any technical abnormalities, was an outlier according to the bagplot method (Rousseeuw et al., 1999) (Figure S1). We performed GLM analyses with and without this particular study but found no differences between the outcomes (Tables S2 and S3).

RESULTS

Bacteria significantly enhanced the performance of plants associated with MF (mean effect size = 1.79; 95% CI = 1.21 to 2.36). The performance gain conferred by bacteria to MF-associated plants depended on the interaction between fungal–bacterial functional traits ($Q_m = 9.67$, $p = 0.008$). Plants simultaneously interacting

with fungi and bacteria that promoted distinct plant functions increased plant performance by a greater degree than symbionts enhancing equivalent plant functions (functionally distinct vs. functionally equivalent symbionts: mean effect sizes = 2.20 and 1.41; 95% CI = 1.56 to 2.84 and 0.70 to 2.10, respectively) (Figure 2). The performance gain in plants associated with functionally distinct symbionts was independent of the specific combination of plant functions that were promoted by fungi and bacteria ($Q_m = 0.55$, $p = 0.758$) (growth & nutrition vs. nutrition & stress protection vs. growth & nutrition & stress protection: mean effect sizes = 1.47, 1.88 and 1.66; 95% CI = 0.56 to 2.38, 1.11 to 2.65 and 0.91 to 2.41, respectively). Similarly, the performance gain in plants associated with functionally equivalent symbionts was independent on the specific plant functions that were promoted by fungi and bacteria ($Q_m = 0.51$, $p = 0.771$) (growth vs. nutrition vs. stress protection: mean effect sizes = 1.47, 3.05 and 0.51; 95% CI = -8.68 to 11.62, 0.99 to 5.11 and -6.65 to 7.66, respectively) (Figure 2). As expected, MF and bacteria significantly increased the performance of their host plants (MF and bacterial mean effect sizes = 2.42 and 1.72; 95% CI = 1.78 to 3.06 and 1.25 to 2.19) (Figure 2). Rosenthal's fail-safe numbers were higher than the reference numbers, indicating that the statistical inferences were robust (bacterial effects on MF-associated plants: $40,720 > 5 \times 202 + 10$; MF effects on symbiont-free plants: $60,633 > 5 \times 173 + 10$; bacterial effects on symbiont-free plants: $31,501 > 5 \times 157 + 10$).

The plant interaction with bacteria alleviated the negative effects of AF on plant performance (mean effect size = 3.64; 95% CI = 2.75 to 4.53) (Figure 3). Contrary to our prediction, the degree of alleviation provided

by bacteria against the negative effects imparted by AF on plants did not depend on the type of protective trait conferred by the bacterium ($Q_m = 0.63$, $p = 0.889$). Bacteria that conferred both resistance and tolerance traits against AF increased plant performance to a similar level as when bacteria provided only resistance or tolerance traits (resistance and tolerance vs. only resistance, vs. only tolerance traits against AF: mean effect sizes = 3.88, 3.54 and 3.55; 95% CI = 2.78 to 4.97, 2.59 to 4.49 and 2.29 to 4.81, respectively) (Figure 3). As expected, AF and bacteria reduced and increased the performance of their host plants respectively (AF and bacterial mean effect sizes = -3.14 and 2.36; 95% CI = -4.20 to -2.08 and 0.90 to 3.83) (Figure 3). Rosenthal's fail-safe number were higher than the reference numbers, indicating that the statistical inferences were robust (bacterial effects on AF-associated plants: $53,972 > 5 \times 155 + 10$; AF effects on symbiont-free plants: $20,996 > 5 \times 112 + 10$; bacterial effects on symbiont-free plants: $3303 > 5 \times 46 + 10$).

The relationship between the performance gains conferred by bacteria to plants associated with fungi (either MF or AF) in the presence and absence of stresses varied between the type of stress experienced by symbiotic plants (plant performance gain conferred by bacteria in absence of stress \times stress type: $F(1,77) = 20.84$, $p < 0.001$; stress type: $F(1,77) = 8.08$, $p = 0.006$; plant performance gain conferred by bacteria in absence of stress: $F(1,77) = 346.13$, $p < 0.001$). The relationships were positive in both abiotic and biotic stress situations, but greater than the 1:1 expectation with biotic stress (slope parameters in abiotic and biotic stress = 0.43 ± 0.15 and 1.61 ± 0.11 , $F[1,37; 1,40] = 8.96$ and 212.60 , $p = 0.005$ and < 0.001 , respectively) (Figure 4a). This indicates that

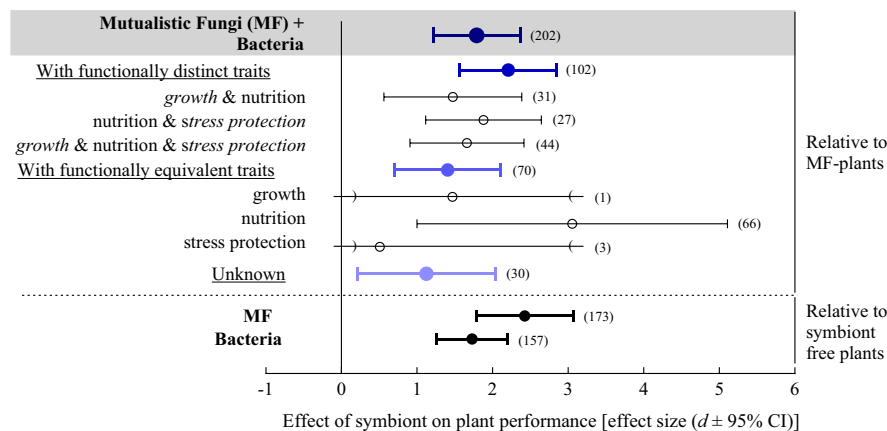


FIGURE 2 The effect of bacteria on the performance of plants associated with Mutualistic Fungi (MF). The overall effect of bacteria on plants associated with MF was categorised into three subgroups depending on the interaction between fungal and bacterial traits (i.e. functionally distinct group = bacteria that add traits promoting dissimilar plant functions than fungi, functionally equivalent group = bacteria that add traits promoting identical/similar plant functions than fungi, while the third group included symbiotic associations with an unknown relationship). The first two subgroups were further categorised according to the specific plant functions that bacteria and fungi promoted. Those plant functions that were exclusively promoted by bacteria are italicised. The effects of just MF and bacteria on performance of symbiont-free plants are also shown. An effect size with a positive value (95% confidence interval (CI) not overlapping zero) indicates a positive or beneficial effect of bacteria (relative to MF-plants or symbiont-free plants) or MF (relative to symbiont-free plants) on the performance of host plants. For simplicity, the 95% CI of 'growth' and 'stress protection' categories are not fully shown. We refer to plant performance as measures of fitness including biomass, survival and seed production. Values in parentheses indicate the number of studies analysed.

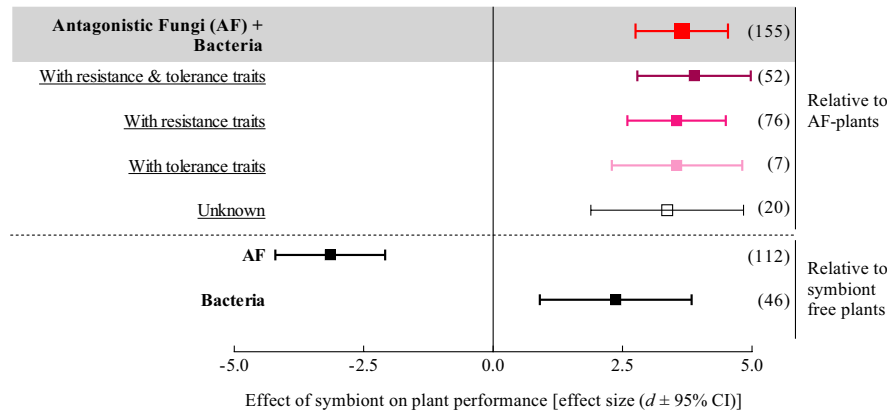


FIGURE 3 The effect of bacteria on the performance of plants associated with Antagonistic Fungi (AF). The overall effect of bacteria on plants associated with AF was categorised into four subgroups depending on the types of protective traits provided by bacteria to their host plant against AF (i.e. resistance and tolerance traits, only resistance traits, only tolerance traits and unknown). The effects of just AF and bacteria on performance of symbiont-free are also shown. An effect size with a positive value (95% confidence interval (CI) not overlapping zero) indicates a positive or beneficial effect of bacteria (relative to AF-plants or symbiont-free plants) or AF (relative to symbiont-free plants) on the performance of their host plants, whereas a negative value indicates the opposite. We refer to plant performance as measures of fitness including biomass and disease resistance. Values in parentheses indicate the number of studies analysed.

bacteria increased the performance of plants associated with fungi to a greater degree in the presence of biotic (but not in abiotic) stress compared to situations where the stress was absent. The relationship between plant performance gains in the presence/absence of biotic stress was affected by the type of stress-protective traits added by bacteria (plant performance gain conferred by bacteria in absence of stress \times type of stress-protective trait: $F(1,36) = 27.66$, $p < 0.001$; plant performance gain conferred by bacteria in absence of stress: $F(1,36) = 491.96$, $p < 0.001$; type of stress-protective trait: $F(1,36) = 0.61$, $p = 0.547$). The relationship was positive and greater than the 1:1 expectation when bacteria conferred resistance traits against biotic stressors (slope parameters in bacteria with traits of resistance & tolerance, only resistance and only tolerance: 0.16 ± 0.49 , 1.81 ± 0.05 and 0.15 ± 0.52 , F -values [1,4; 1,27; 1,5] = 0.10, 1363 and 0.08, $p = 0.785$, < 0.001 and $= 0.789$, respectively) (Figure 4b). This indicates that only bacteria that conferred resistance traits to their MF-associated plants led to a greater gain in plant performance in the presence of biotic stresses compared to those plants in the absence of any stress.

DISCUSSION

The factors regulating the performance gain conferred by bacteria to plants interacting with fungi have been scarcely studied. Our meta-analysis strongly advances the notion that the performance gain conferred by bacteria to MF-associated plants was greater when symbionts added traits that enhanced distinct plant functions compared to symbionts that promoted identical/similar functions. In addition, the performance gain in plants associated with functionally distinct or functionally equivalent symbionts was independent of the specific

plant functions that were promoted by fungi and bacteria (growth & nutrition vs. nutrition & stress protection vs. growth & nutrition & stress protection or growth vs. nutrition vs. stress protection). As expected, the negative effects of AF on their host plants were alleviated by bacteria. However, contrary to our prediction, the degree of alleviation conferred by bacteria to their host plants against AF was independent of the type of protection traits added by the bacterium (i.e. traits of resistance & tolerance vs. resistance vs. tolerance). Finally, our results confirmed that the degree of performance gain conferred by bacteria to plants associated with fungi (either MF or AF) was dependent on the abiotic/biotic environment. Bacteria that conferred stress protective mechanisms to their plant hosts led to a greater gain in plant performance in the presence of biotic stress compared to symbiotic plants in the absence of any stress. This was not the case for abiotic stress. In plants that experienced biotic stress, bacteria solely exhibiting resistance traits led to a greater gain in performance for their plant hosts than bacteria that either added resistance & tolerance or only tolerance traits in the presence/absence of the stress.

Bacteria can increase the fitness of plants when the host is simultaneously associated with MF (Larimer et al., 2010) and our meta-analysis confirmed this. More intriguingly, bacteria with traits that promoted plant functions that were distinct from those traits conferred by fungi increased plant performance to a greater degree compared to bacteria that conferred traits that promoted identical/similar plant functions than fungi. First, this outcome was expected since the benefit/cost relationship may be higher in plants harbouring functionally distinct symbionts compared to plants hosting functionally equivalent ones (Larimer et al., 2010). Second, functionally distinct symbionts can exert synergistic effects on the fitness of their host plants (i.e. net beneficial

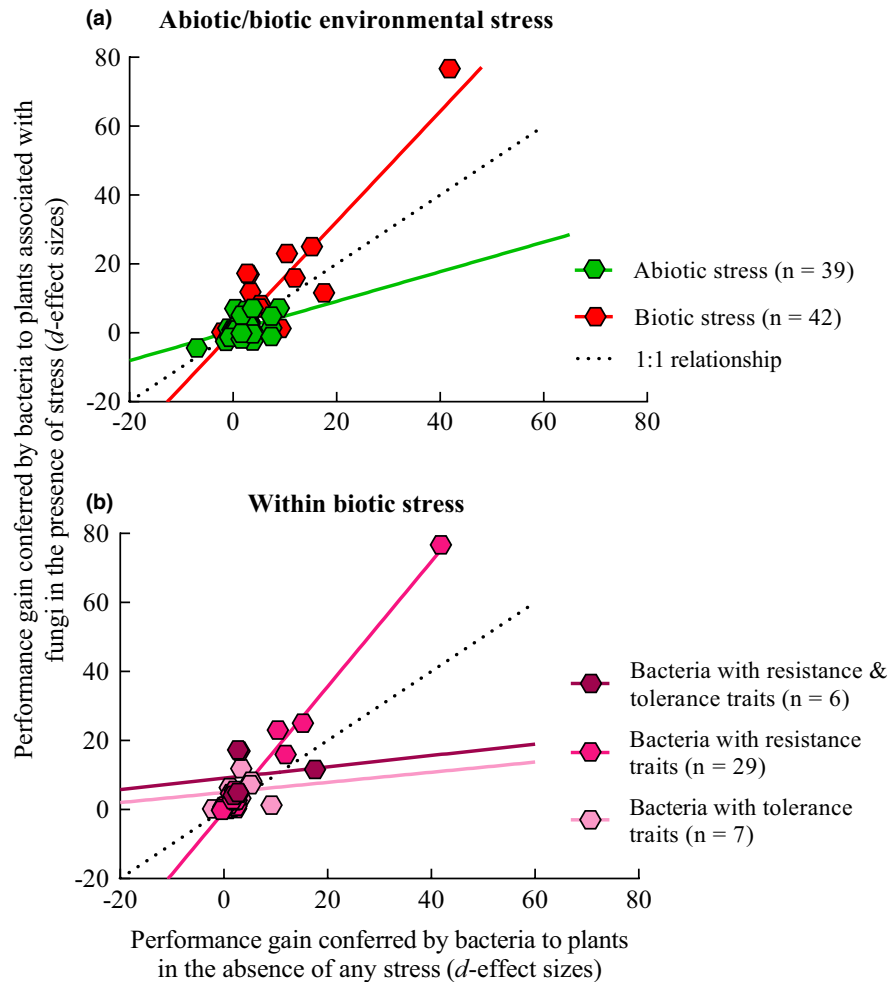


FIGURE 4 Relationships between performance gains conferred by bacteria to plants associated with fungi (either mutualistic or antagonistic) in the presence and absence of environmental stresses. (a) Abiotic/biotic environmental stresses: The effect of bacteria on plant performance gain was categorised into the type of stress (i.e. abiotic or biotic stress). (b) Within biotic stress: The effect of bacteria on the plant performance gain was categorised into three subgroups depending on the types of protective traits conferred by bacteria to their host plant against biotic stresses (i.e. resistance and tolerance traits, only resistance traits, only tolerance traits). Each dot represents a single study (for details see studies listed in Table S1). The discontinuous black line is a reference that indicates the 1:1 relationship between plant performance gains (the proportional effect conferred by bacteria on their plant hosts, which are associated with fungi in the presence and absence of abiotic/biotic stresses). The continuous lines represent the linear models inferred from the GLM analyses (abiotic: $y = 0.43 \pm 0.24 \times X + 0.56 \pm 0.84$; biotic: $y = 1.60 \pm 0.25 \times X + 0.31 \pm 1.11$; with resistance and tolerance traits: $y = 0.16 \pm 0.49 \times X + 9.10 \pm 3.79$; with resistance traits: $y = 1.81 \pm 0.04 \times X - 0.56 \pm 0.43$; with tolerance traits: $y = 0.14 \pm 0.51 \times X + 4.96 \pm 2.51$).

effect greater than the additive expectation) (Afkhami et al., 2014; Gamalero et al., 2004, 2008; Jäderlund et al., 2008; Pérez-de-Luque et al., 2017). Third, antagonistic effects can occur between functionally equivalent symbionts due to competition for plant resources, and this may reduce the performance gain conferred by bacteria on MF-associated plants (i.e. net beneficial effects lesser than additive expectations) (Afkhami et al., 2014, 2020; Surendirakumar et al., 2019). However, even when antagonistic associations were expected in plants simultaneously associated with functionally equivalent fungi and bacteria, the plant performance was increased by the presence of these symbionts (i.e. positive effect size in plants with functionally equivalent symbionts). This fascinating outcome suggests that the identity of traits conferred by functionally equivalent symbionts is important

in determining plant performance gain. For instance, sometimes mycorrhizal fungi and rhizobacteria exert synergistic effects on plant fitness, even when both symbionts enhance the same plant function (e.g. the acquisition of nutrients). This seems to be associated with that these symbionts confer traits that enhance the plant's acquisition of distinct nutrients (Afkhami et al., 2020). In fact, within the group of functionally equivalent symbionts, most case studies (ca. 67%) included microbes conferring traits that enhanced different aspects of the same plant function (47 out of 70 case studies). Our results also showed that the gain in plant performance conferred by bacteria was not optimised by any plant function or combination of plant functions promoted by fungi and bacteria. However, this result should be interpreted with caution as three out of six groups within

these analyses included bacteria with stress-protective traits, thus the contribution that these symbionts confer to plant performance is highly context-dependent and plant performance gain may be greater in those groups under situations of stress compared to situations where the stress is absent.

Bacteria can efficiently protect their plant hosts against attacks from single and multiple eukaryotic phytopathogens (Durán et al., 2018; Kobayashi & Crouch, 2009). In agreement with this, our finding showed that bacteria alleviated the negative effects on plant performance caused by AF. Our results did not support the hypothesis that bacteria conferring a combination of resistance and tolerance protective traits against AF would alleviate, to a greater degree, the negative effects imparted by AF than bacteria conferring a single type of protection trait (i.e. resistance *or* tolerance). This outcome might be related to the differences in the relative contribution of resistance and tolerance traits conferred by bacteria to plant performance. Comparing results of the effect sizes from the categories of 'with resistance & tolerance traits' and 'with resistance traits' (=no differences), it seems that the relative contribution of tolerance traits (in the plant alleviation of AF negative effects) was minor compared to the protection conferred by resistance traits. Examples of the minor contribution of bacterial tolerance traits to the protection against AF included the similar reduction in fungal disease symptoms in cucumber (*Cucumis sativus*) plants caused by either *Bacillus* strains that simultaneously produced anti-fungal and growth-promoting compounds (resistance + tolerance mechanisms, respectively) and the bacterial strain CZB5 that provided only anti-fungal compounds (Lin et al., 2014). The low contribution of the bacterial stress-tolerant traits in the plant alleviation against AF could be explained by reduced levels of bacterial stress-tolerant compounds within plant tissues due to the cost of producing stress-resistant metabolites (e.g. Peyraud et al., 2016). Additionally, the existence of potential trade-offs in the production of resistance and tolerance compounds could also have limited the accumulation of stress-tolerant compounds (Ferencsi, 2016) (e.g. Matilla et al., 2018). It is fair to mention that a contrasting conclusion emerges from the comparison between the effect sizes associated with the categories 'with resistance & tolerance traits' and 'with tolerance traits'. However, the low number of studies associated with tolerance traits ($n = 7$) and the lack of mechanistic explanations to elucidate this pattern weaken this conclusion.

The performance gain of plants conferred by bacteria associated with fungi depends on environmental conditions (i.e. abiotic/biotic stresses) (Acuña-Rodríguez et al., 2020; Porter et al., 2020) and our analysis advocates this notion. Our meta-analyses indicated that in the presence of either stress type (abiotic or biotic), symbiotic plants gained performance due to association with bacteria (recall that all bacteria included in the GLM analyses possessed traits that protect plants against stresses). This outcome may reflect the fact that in situations of stress,

the benefits conferred by bacteria to plants (=stress protection traits) outweighed the costs of harbouring symbionts (Bronstein, 1994). Remarkably, the relationship between plant performance gains conferred by bacteria was greater than the 1:1 expectation in the presence/absence of biotic stress, but not in the presence of an abiotic stress. In 83% of the studies related to biotic stress, bacteria conferred resistance traits, for example antimicrobial compounds, that protected their plant hosts by negatively affecting the fitness of biotic stressors (here, fungal phytopathogens and nematodes). Contrary to this, in all studies related to abiotic stress, bacteria conferred traits that did not directly affect the stressors but promoted plant responses to the stress (e.g. production of growth-promoting hormones, antioxidants). Thus, the higher plant performance gain in the presence/absence of biotic compared to abiotic stress might be explained by the action of the resistance traits added by bacteria against biotic stressors. This hypothesis was confirmed in the GLM analysis with biotic stress. Symbiotic plants gained a greater degree of performance gain in the presence of biotic stress compared to plants that lacked stress in situations where bacteria conferred stress-resistance, but not when bacteria conferred resistance & tolerance or only tolerance traits. This finding suggests that in situations of biotic stress, plants may benefit by recruiting bacteria that confer stress resistance traits rather than those that can confer stress tolerance traits. There are reports confirming this (e.g. Jousset et al., 2010; Liu et al., 2021). Notable was the fact that the plant performance gain conferred by bacteria was lower in abiotic stress situations than in the absence of any stress. In most studies related to abiotic stress (ca. 80%), bacteria conferred traits that promoted plant growth and/or plant nutrient acquisition. It was expected that the contribution of these bacterial traits to the plant performance gain was less in stress situations than when the stress was absent, as these traits do not directly counteract the stressors. Therefore, the negative effect of the stress on plants might have discounted part of the gain in plant performance conferred by the symbionts. Most of the studies within our analysis associated with biotic stresses (40 out of 42) included tri-partite plant associations with AF and bacteria (here AF was the stress), which were slightly different from the studies associated with abiotic stresses where plants were associated with MF and bacteria. Whereas a hypothetical inclusion of MF in the case studies associated with biotic stress could modify the predicted relationship between plant performance gains, it is likely that this modification would have a minor impact on the results. Our findings suggest that a major regulator of the relationship between performance gains in the presence/absence of biotic stress is the type of stress-protective trait conferred by bacteria. While MF could modify the magnitude of the benefit related to the bacterial trait (due to antagonism or synergism), the presence of a fungus cannot alter the type of trait conferred by the bacterium (e.g. to change from resistance to tolerance traits).

In conclusion, our study showed that the performance gain conferred by bacteria to plants associated with fungi was modulated by the interaction between fungal–bacterial traits and the abiotic/biotic environments experienced by these symbiotic plants. Our analysis suggests that plants experience optimal performance when they are associated with functionally distinct symbionts. However, there was not a specific combination of plant functions promoted by these symbionts that optimised the performance of plant hosts. Our results also showed that bacteria, that conferred stress-protective traits to plants associated with fungi (either MF or AF), increased plant performance to a greater degree in the presence of biotic, but not abiotic, stress compared to situations where the stress was absent. Furthermore, plant performance gain in the presence/absence of biotic stress was greater when bacteria conferred only resistance traits compared to resistance & tolerance or only tolerance traits. Considering that plants can regulate the presence and functionality of their microbial symbionts (Bastías et al., 2018; Liu et al., 2011, 2020, 2021), further research should evaluate whether plants possess specific mechanisms to stimulate the presence of functionally distinct symbionts or sanction the presence of functionally equivalent ones. Our findings highlight that bacteria exert significant beneficial effects on plants within tripartite associations and that to predict the effects of these symbionts on the performance of plants associated with fungi, it is essential to determine the interaction between symbionts' functional traits and the relationship between bacterial traits and environmental conditions.

AUTHOR CONTRIBUTION

All authors conceived the idea and wrote the manuscript. DAB and ERA collected data. DAB analysed data.

ACKNOWLEDGEMENTS

We thank David Hume and three anonymous reviewers for critical revision of the manuscript. This work was supported by the New Zealand Ministry of Business, Innovation and Employment (MBIE) through the Endeavour Fund, contract number LVLX1702. Open access publishing facilitated by AgResearch Ltd, as part of the Wiley - AgResearch Ltd agreement via the Council of Australian University Librarians.

FUNDING INFORMATION

Ministry of Business, Innovation and Employment, Grant/Award Number: LVLX1702

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/ele.14073>.

DATA AVAILABILITY STATEMENT

The data supporting these results are available in the Figshare Repository: [10.6084/m9.figshare.20126474.v1](https://doi.org/10.6084/m9.figshare.20126474.v1)

ORCID

Daniel A. Bastías  <https://orcid.org/0000-0002-0522-5538>

Emma R. Applegate  <https://orcid.org/0000-0003-3284-0066>

Linda J. Johnson  <https://orcid.org/0000-0002-3779-0902>

Stuart D. Card  <https://orcid.org/0000-0001-7140-7471>

REFERENCES

- Acuña-Rodríguez, I.S., Newsham, K.K., Gundel, P.E., Torres-Díaz, C. & Molina-Montenegro, M.A. (2020) Functional roles of microbial symbionts in plant cold tolerance. *Ecology Letters*, 23, 1034–1048.
- Afkhami, M.E., Almeida, B.K., Hernandez, D.J., Kiesewetter, K.N. & Revillini, D.P. (2020) Tripartite mutualisms as models for understanding plant–microbial interactions. *Current Opinion in Plant Biology*, 56, 28–36.
- Afkhami, M.E., Rudgers, J.A. & Stachowicz, J.J. (2014) Multiple mutualist effects: conflict and synergy in multispecies mutualisms. *Ecology*, 95, 833–844.
- Balestrini, R. (2021) Grand challenges in fungi–plant interactions. *Frontiers in Fungal Biology*, 2, 750003.
- Bangera, M.G. & Thomashow, L.S. (1999) Identification and characterization of a gene cluster for synthesis of the polyketide antibiotic 2,4-Diacetylphloroglucinol from *Pseudomonas fluorescens* Q2-87. *Journal of Bacteriology*, 181, 3155–3163.
- Bastías, D.A., Johnson, L.J. & Card, S.D. (2020) Symbiotic bacteria of plant-associated fungi: friends or foes? *Current Opinion in Plant Biology*, 56, 1–8.
- Bastías, D.A., Martínez-Ghersa, M.A., Newman, J.A., Card, S.D., Mace, W.J. & Gundel, P.E. (2018) The plant hormone salicylic acid interacts with the mechanism of anti-herbivory conferred by fungal endophytes in grasses. *Plant, Cell & Environment*, 41, 395–405.
- Bonfante, P., Venice, F. & Lanfranco, L. (2019) The mycobiota: fungi take their place between plants and bacteria. *Current Opinion in Microbiology*, 49, 18–25.
- Bronstein, J.L. (1994) Our current understanding of mutualism. *The Quarterly Review of Biology*, 69, 31–51.
- Cumming, G. & Finch, S. (2005) Inferencia by eye: confidence intervals and how to read pictures of data. *The American Psychologist*, 60, 170–180.
- Doehlemann, G., Ökmen, B., Zhu, W. & Sharon, A. (2017) Plant pathogenic fungi. *Microbiology Spectrum*, 5, FUNK-0023-2016.
- Durán, P., Thiergart, T., Garrido-Oter, R., Agler, M., Kemen, E., Schulze-Lefert, P. et al. (2018) Microbial interkingdom interactions in roots promote *Arabidopsis* survival. *Cell*, 175, 973–983.e14.
- Ferenci, T. (2016) Trade-off mechanisms shaping the diversity of bacteria. *Trends in Microbiology*, 24, 209–223.
- Frey-Klett, P., Burlinson, P., Deveau, A., Barret, M., Tarkka, M. & Sarniguet, A. (2011) Bacterial–fungal interactions: hyphens between agricultural, clinical, environmental, and food microbiologists. *Microbiology and Molecular Biology Reviews*, 75, 583–609.
- Gamalero, E., Berta, G., Massa, N., Glick, B.R. & Lingua, G. (2008) Synergistic interactions between the ACC deaminase-producing bacterium *Pseudomonas putida* UW4 and the AM fungus *Gigaspora rosea* positively affect cucumber plant growth. *FEMS Microbiology Ecology*, 64, 459–467.
- Gamalero, E., Fracchia, L., Cavaletto, M., Garbaye, J., Frey-Klett, P., Varese, G.C. et al. (2003) Characterization of functional traits of two fluorescent pseudomonads isolated from basidiomes of ectomycorrhizal fungi. *Soil Biology and Biochemistry*, 35, 55–65.

- Gamaleri, E., Trotta, A., Massa, N., Copetta, A., Martinotti, M.G. & Berta, G. (2004) Impact of two fluorescent pseudomonads and an arbuscular mycorrhizal fungus on tomato plant growth, root architecture and P acquisition. *Mycorrhiza*, 14, 185–192.
- Glick, B.R. (2012) Plant growth-promoting bacteria: mechanisms and applications. *Scientifica*, 2012, 963401, 1, 15.
- Hashem, A., Abd_Allah, E.F., Alqarawi, A.A., Radhakrishnan, R. & Kumar, A. (2017) Plant defense approach of *Bacillus subtilis* (BERA 71) against *Macrophomina phaseolina* (Tassi) Goid in mung bean. *Journal of Plant Interactions*, 12, 390–401.
- Hol, W.H.G., Bezemer, T.M. & Biere, A. (2013) Getting the ecology into interactions between plants and the plant growth-promoting bacterium *Pseudomonas fluorescens*. *Frontiers in Plant Science*, 4, 81.
- Jäderlund, L., Arthurson, V., Granhall, U. & Jansson, J.K. (2008) Specific interactions between arbuscular mycorrhizal fungi and plant growth-promoting bacteria: as revealed by different combinations. *FEMS Microbiology Letters*, 287, 174–180.
- Jousset, A., Rochat, L., Lanoue, A., Bonkowski, M., Keel, C. & Scheu, S. (2010) Plants respond to pathogen infection by enhancing the antifungal gene expression of root-associated bacteria. *Molecular Plant-Microbe Interactions*, 24, 352–358.
- Khan, N., Martínez-Hidalgo, P., Ice, T.A., Maymon, M., Humm, E.A., Nejat, N. et al. (2018) Antifungal activity of *Bacillus* species against *Fusarium* and analysis of the potential mechanisms used in biocontrol. *Frontiers in Microbiology*, 9, 2363.
- Kim, A.-Y., Shahzad, R., Kang, S.-M., Khan, A.L., Lee, S., Park, Y.-G. et al. (2017) *Paenibacillus terrae* AY-38 resistance against *Botrytis cinerea* in *Solanum lycopersicum* L. plants through defence hormones regulation. *Journal of Plant Interactions*, 12, 244–253.
- Kobayashi, D.Y. & Crouch, J.A. (2009) Bacterial/fungal interactions: from pathogens to mutualistic endosymbionts. *Annual Review of Phytopathology*, 47, 63–82.
- Larimer, A.L., Bever, J.D. & Clay, K. (2010) The interactive effects of plant microbial symbionts: a review and meta-analysis. *Symbiosis*, 51, 139–148.
- Larimer, A.L., Clay, K. & Bever, J.D. (2014) Synergism and context dependency of interactions between arbuscular mycorrhizal fungi and rhizobia with a prairie legume. *Ecology*, 95, 1045–1054.
- Lin, Y., Du, D., Si, C., Zhao, Q., Li, Z. & Li, P. (2014) Potential biocontrol *Bacillus* sp. strains isolated by an improved method from vinegar waste compost exhibit antibiosis against fungal pathogens and promote growth of cucumbers. *Biological Control*, 71, 7–15.
- Liu, H., Brettell, L.E., Qiu, Z. & Singh, B.K. (2020) Microbiome-mediated stress resistance in plants. *Trends in Plant Science*, 25, 733–743.
- Liu, H., Li, J., Carvalhais, L.C., Percy, C.D., Prakash Verma, J., Schenk, P.M. et al. (2021) Evidence for the plant recruitment of beneficial microbes to suppress soil-borne pathogens. *The New Phytologist*, 229, 2873–2885.
- Liu, Q., Parsons, A.J., Xue, H., Fraser, K., Ryan, G.D., Newman, J.A. et al. (2011) Competition between foliar *Neotyphodium lolii* endophytes and mycorrhizal *Glomus* spp. fungi in *Lolium perenne* depends on resource supply and host carbohydrate content. *Functional Ecology*, 25, 910–920.
- Martínez-Hidalgo, P., García, J.M. & Pozo, M.J. (2015) Induced systemic resistance against *Botrytis cinerea* by Micromonospora strains isolated from root nodules. *Frontiers in Microbiology*, 6, 922.
- Matilla, M.A., Daddaoua, A., Chini, A., Morel, B. & Krell, T. (2018) An auxin controls bacterial antibiotics production. *Nucleic Acids Research*, 46, 11229–11238.
- Minaxi, Saxena, J., Chandra, S. & Nain, L. (2013) Synergistic effect of phosphate solubilizing rhizobacteria and arbuscular mycorrhiza on growth and yield of wheat plants. *Journal of Soil Science and Plant Nutrition*, 13, 511–525.
- Partida-Martínez, L. & Heil, M. (2011) The microbe-free plant: fact or artifact? *Frontiers in Plant Science*, 2, 100.
- Pérez-de-Luque, A., Tille, S., Johnson, I., Pascual-Pardo, D., Ton, J. & Cameron, D.D. (2017) The interactive effects of arbuscular mycorrhiza and plant growth-promoting rhizobacteria synergistically enhance host plant defences against pathogens. *Scientific Reports*, 7, 16409.
- Peyraud, R., Cottret, L., Marmiesse, L., Gouzy, J. & Genin, S. (2016) A Resource allocation trade-off between virulence and proliferation drives metabolic versatility in the plant pathogen *Ralstonia solanacearum*. *PLoS Pathogens*, 12, e1005939.
- Porras-Alfaro, A. & Bayman, P. (2011) Hidden fungi, emergent properties: endophytes and microbiomes. *Annual Review of Phytopathology*, 49, 291–315.
- Porter, S.S., Bantay, R., Friel, C.A., Garoutte, A., Gdanetz, K., Ibarreta, K. et al. (2020) Beneficial microbes ameliorate abiotic and biotic sources of stress on plants. *Functional Ecology*, 34, 2075–2086.
- Rousseeuw, P.J., Ruts, I. & Tukey, J.W. (1999) The bagplot: a bivariate boxplot. *The American Statistician*, 53, 382–387.
- Roy, B.A. & Kirchner, J.W. (2000) Evolutionary dynamics of pathogen resistance and tolerance. *Evolution*, 54, 51–63.
- Selvakumar, G., Shagol, C.C., Kim, K., Han, S. & Sa, T. (2018) Spore associated bacteria regulates maize root K⁺/Na⁺ ion homeostasis to promote salinity tolerance during arbuscular mycorrhizal symbiosis. *BMC Plant Biology*, 18, 109.
- Siddiqui, Z.A. & Sayeed Akhtar, M. (2009) Effects of antagonistic fungi, plant growth-promoting rhizobacteria, and arbuscular mycorrhizal fungi alone and in combination on the reproduction of *Meloidogyne incognita* and growth of tomato. *Journal of General Plant Pathology*, 75, 144–153.
- Sun, G., Yao, T., Feng, C., Chen, L., Li, J. & Wang, L. (2017) Identification and biocontrol potential of antagonistic bacteria strains against *Sclerotinia sclerotiorum* and their growth-promoting effects on *Brassica napus*. *Biological Control*, 104, 35–43.
- Surendirakumar, K., Pandey, R.R. & Muthukumar, T. (2019) Influence of indigenous arbuscular mycorrhizal fungus and bacterial bioinoculants on growth and yield of *Capsicum chinense* cultivated in non-sterilized soil. *The Journal of Agricultural Science*, 157, 31–44.
- Vivas, A., Barea, J.M., Biró, B. & Azcón, R. (2006) Effectiveness of autochthonous bacterium and mycorrhizal fungus on *Trifolium* growth, symbiotic development and soil enzymatic activities in Zn contaminated soil. *Journal of Applied Microbiology*, 100, 587–598.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Bastías, D.A., Applegate, E.R., Johnson, L.J. & Card, S.D. (2022) Factors controlling the effects of mutualistic bacteria on plants associated with fungi. *Ecology Letters*, 25, 1879–1888. Available from: <https://doi.org/10.1111/ele.14073>