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Elevated air temperature shifts the interactions between plants and endophytic fungal entomopathogens in an agroecosystem

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ABSTRACT

The possible effects of climate change on species interactions remain a very complex and challenging subject in community ecology. Here, we comparatively examine the interactions between maize (*Zea mays*) and an endophytic fungal entomopathogen (*Beauveria bassiana*) in a typical agroecosystem under both ambient and elevated air temperatures. We found that under ambient temperature certain key biological characteristics in maize (i.e., height, relative growth rate, biomass, and defense enzymes) and *B. bassiana* (i.e., conidia yield, germination rate of conidia, and virulence) were positively related to each other. Under elevated air temperature, however, we only detected positive effects of maize on *B. bassiana* (i.e., conidia yield, germination rate of conidia, and virulence), but little effect of *B. bassiana* on maize. These observations suggest that elevated air temperature could shift the interactions between plants and the endophytic fungal entomopathogens, possibly even from mutualism to commensalism. Both the nature and strength of species interactions are important for better understanding and predicting structure and stability of ecological communities in agroecosystems under climate change.

1. Introduction

Species interactions can play an important role in the structure of ecological communities (Thébault and Fontaine, 2010; Zhong et al., 2014). A species may show a changing relationship with coexisting species in both complex natural ecosystems and/or relatively simple agroecosystems (Cardinale et al., 2003; Tylianakis et al., 2008). In general, species interactions can be influenced by the species' innate traits, especially in the context of climate warming (Boukal et al., 2019). Until recently, a growing number of studies have focused on the effects of climate warming on the strength of species interactions (Barton et al., 2009; Sentis et al., 2017). Yet, to what extent mutualistic interactions between species can be affected by elevated temperature remains elusive. In agroecosystems, although species interactions involving crops could be simpler than in natural ecosystems, certain species interactions may help maintain or even promote stability and sustainability, thus possibly enhancing crop production (Cardinale et al., 2003). With ongoing climate warming, these interactions may have been more influential than previously recorded (Olesen et al., 2011; Murrell, 2017). Nevertheless, whether and how climate warming may alter the nature of previously established species interactions in agroecosystems (e.g. from one nature to another) remains largely unknown.

Previous studies on species interactions in agroecosystems have mainly focused on the antagonistic relationships between plants and insect pests (Batra, 1982; Médiène et al., 2011). Recently, indirect plant-natural enemy interactions have been studied in efforts to improve the quality and safety of crop products (Pomari-Fernandes et al., 2018). As a natural enemy, fungal entomopathogens are well-known for killing insects by penetrating through the host cuticle and causing nutrient depletion (Pedrini et al., 2007), resulting in an indirect but positive effect on plants. Indeed,

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growing evidence shows that endophytic fungal entomopathogens play a role in promoting plant growth and resistance to environmental stresses (Vega et al., 2009; Krell et al., 2018; Vega, 2018). There is also evidence that plants not only take up essential nutrients through fungal entomopathogens, but also provide photosynthate to the fungus (Behie et al., 2012, 2017). However, a clear understanding of the possible feedback effects of plants on fungal entomopathogens is still lacking.

Climate warming is one of the key environmental drivers with far-reaching consequences (Tylianakis et al., 2008; Rasmussen et al., 2020), and the responses of plants and endophytes to elevated air temperature have been documented (Fargues et al., 1997; Davidson et al., 2003; Barnabás et al., 2008). Changes in temperature affect nearly all aspects of plant physiology, chemistry, life history traits, development, and growth (Sánchez et al., 2014; Asemaninejad et al., 2018; Orians et al., 2019), and may consequently affect interactions with other species such as their associated microbes (Pieterse and Dicke, 2007). It is clear that some endophytes avoid stress through plant symbiosis (Márquez et al., 2007). Conversely, alterations in temperature can further influence the interactions between plants and microbes by influencing microbes. For example, microbes associated with plants (e.g. arbuscular mycorrhizal fungi) may respond to elevated air temperature with enhanced growth and plant colonization, and consequently ameliorating temperature stress in some plants (Bunn et al., 2009; Kivlin et al., 2013).

Most studies examining the effects of elevated air temperature on species interactions have focused on the reciprocal feedbacks between plants and their associated beneficial microbes (Compant et al., 2010; Mohan et al., 2014), but ignored the effects on microbes (e.g. fungal entomopathogens) associated with insects, though the colonization of entomopathogenic fungi in plants has been well documented (Vega et al., 2009; Vega, 2018). This leaves an important knowledge gap on how elevated air temperature may alter species interactions between plants and endophytes. Here, we explore the responses of *Zea mays*, an important crop worldwide, and *Beauveria bassiana* (Ascomycota: Hypocreales), an endophytic fungal entomopathogen, to elevated air temperature in an agroecosystem. We address the following two questions: (1) What nature of plant-fungal entomopathogens interaction is established (i.e., mutualism, commensalism, or amensalism) under ambient temperature; and (2) Do elevated air temperature alter plant-fungal entomopathogens interactions?

2. Materials and methods

2.1. Study site

This experiment was conducted at the Gongzhuling Experimental Station of the Institute of Plant Protection, Jilin Academy of Agricultural Sciences, China (43°30′ N, 124°47′ E; 225 m above sea level). The site has a temperate continental monsoon climate with mean annual temperature and mean monthly temperature from May to October (growing season) of 6.7 °C and 15.4 °C, respectively, and the mean annual precipitation is 535 mm. The annual accumulated temperature is approximately 2400 °C, and mean frost-free period was 144 days/year during the past 10 years (2006–2015).

2.2. Study organisms

Our experiment included two key interactive species in an agroecosystem: a crop (*Zea mays*), and a fungal entomopathogen (*Beauveria bassiana*).

2.3. The plant

Z. mays, as one of the world's main crops for human consumption and animal fodder (Fig. S1A), accounts for more than one-third of China's cereal production (FAO, 2016). We chose the maize single hybrid KN1 in this study because it is a major cultivar in China's commercial production and has low resistance to insect pests (e.g. *Ostrinia furnacalis*).

2.4. The fungal entomopathogens

B. bassiana strain BbofDH1-5 (D1-5) (GenBank accession number: PRJNA178080) was isolated from a dead *O. furnacalis* (Lepidoptera: Pyralidae) larva at the Institute of Plant Protection, Jilin Academy of Agricultural Sciences in 2008. The strain was deposited in Agriculture Culture Collection of China, and preservation number is ACCC No. 32726. The fungus was grown on potato dextrose agar (PDA, Hopebio Spectrum Instruments Co., Ltd., China) for 15 days at 26 °C in the dark. Conidia were harvested by scraping with a sterile spatula, and then kept at 4 °C in dark storage before use.

2.5. Experimental design

The experiment was conducted from May to September 2015 using a randomized complete block design. There were four treatments and each had eight replicates: (1) maize inoculation with Tween-80 solution and plants grown at ambient temperature (Control); (2) maize inoculation with *B. bassiana* suspension and plants grown at ambient temperature (Bb); (3) maize inoculation with Tween-80 solution and plants grown at elevated air temperature (W); and (4) maize inoculation with *B. bassiana* suspension and plants grown at elevated air temperature (W + Bb).

Previous reports show that the temperature has increased at a rate of 0.019 °C/yr since 1909 at the experimental area (Zheng et al., 2018), and thus elevated air temperature of 2.0 ± 0.5 °C compared to ambient temperature was applied in this experiment. Elevated air temperature was achieved in eight octagonal Open Top Chambers (OTCs) with rigid frames (4.2 m in diameter, and 3.0 m in height) and transparent glass walls (Fig. S1C). Two rings of breather pipes (16 cm diameter) were installed inside the OTC, and the air inlet was placed at the side near the gas cylinders' room, and each pipe had two air vents toward the middle of the OTC. To avoid overheating, a cooling system was installed in the OTC. The air was continuously distributed from the blowers into the chambers through a water curtain cooling system into perforated polyethylene ducts inside the chamber base-wall at 10 cm above the level of the soil. Additionally, eight rigid-frames of regular octagonal covered with transparent polyethylene (4.2 m in diameter, and 3.0 m in height) were used for the ambient temperature treatment. Previous testing has showed that there was no difference in light transmission between transparent polyethylene and glass (Pieters et al., 1997). Air temperature (both ambient and elevated air temperature) was monitored and recorded at 1 h intervals during the whole experiment, showing that temperature in OTCs (elevated air temperature) was ca. 2.0 °C higher than that in net rooms (ambient) during our experiment (Fig. S2).

In an attempt to establish *B. bassiana* as an endophyte in *Z. mays*, we combined two treatments: seed immersing and soil drench inoculation. Soil (about 2.86% organic matter, 0.15% total nitrogen, 0.08% total phosphorus with a pH of 5.68) was taken from the field near the experimental station. Prior to planting, the soil was autoclaved twice for 2 h (leaving one day between autoclaving), and it was aerated and mixed to avoid gases toxic to microbiota and plants (Trevors, 1996). Then 23 kg soil was placed into each plastic pot (32 cm in diameter, and 36 cm in height). Maize seeds were

immersed in a *B. bassiana* conidia suspension (1×10^8 conidia ml^{-1} in 0.05% Tween-80) for 12 h (see Supplementary data, Appendix 1 for details). For soil drench inoculations, 20 ml of *B. bassiana* conidia suspension (1×10^8 conidia ml^{-1} in 0.05% Tween-80) was applied 7 and 12 days after sowing. For the two treatments not inoculated with *B. bassiana*, the seeds were immersed in 0.05% Tween-80, or the soil was drenched with the same amount of 0.05% Tween-80. A small amount of *B. bassiana* in uninoculated soil was observed, but the population of *B. bassiana* in inoculated treatments was significantly higher (Fig. S3).

2.6. Maize planting and endophytism

Three maize seeds were sown per pot, with 16 pots per replicate for each of four treatments. In each pot, 12.7 g of fertilizer (NPK 28-14-12) was added for seedling survival and growth. The seedlings were then thinned to one after emergence. All plants were watered as needed.

The colonization of *B. bassiana* in maize leaves was assessed by plating surface-sterilized leaf segments on PDA 14 days after seedling emergence (Tefera and Vidal, 2009). For each plant, nine 1 cm × 1 cm segments of the 3rd entire fully developed leaflet were taken from each of the four treatments. These segments were surface-sterilized with 1% sodium hypochlorite for 3 min, followed by 2 min in 75% ethanol, rinsed in sterile water three times, and then placed on sterile tissue paper in a laminar flow cabinet. To evaluate the efficacy of the surface sterilization, the number of colonies of microbes in 50 μl of the water that had been used to rinse the tissues after surface sterilization was analyzed by plating on PDA for 20 days at 26 °C in the dark. No microorganisms were detected. These nine surface-sterilized segments per plant were incubated on PDA for 20 days at 26 °C in the dark. *B. bassiana* can be identified by characteristic white dense mycelia becoming cream to pale yellow at the edge. When in doubt, the specimen was mounted in a drop of water and inspected under a microscope, looking for globose conidia and zigzag-shaped conidiophores, characteristic of the species (Fernandes et al., 2006; Parsa et al., 2013) (Fig. S1B). Colonization rates were calculated as follows: colonization rate = 100% × (the number of *B. bassiana* colonized plants/total number of plants).

2.7. Effects of endophytes on maize

To examine the effects of endophytes on plants, we classified the maize development into five stages: bell mouth stage (S1), early jointing stage (S2), middle jointing stage (S3), tasseling stage (S4); and milk-ripe stage (S5). Maize height during S1−S5 and maize biomass during S5 were measured. The height of all maize plants per pot was measured from the soil surface to the tip of the stem. Maize shoot and root biomass were determined by drying in a forced-air oven for 48 h at 80 °C and subsequent weighing. Shoot biomass included maize leaves and stems and total plant biomass was the sum of shoot and root biomass. To evaluate the maize resistance against various pathogens and pests, the activity of two key enzymes in the plant-defense pathways, phenylalanine ammonia lyase (PAL) and polyphenol oxidase (PPO), were measured (Rivero et al., 2001; Lavania et al., 2006). Six maize plants at the jointing stage of maize were randomly selected from each replicate of treatments. An approximately 9 cm^2 segment (10 cm from leaf tip) from the 3rd unfolded leaf of each plant was chosen, and the mixture of six sampled plants was kept at −80 °C until analysis (Yingsanga et al., 2008). PAL was analyzed using the method described by Zucker (1968), and PPO was analyzed according to the method by Murr and Morris (1974). One unit of PAL or PPO activity was defined as the amount of enzyme causing an increase of 0.01 in absorbance per hour at 290 nm and 410 nm, respectively.

2.8. Effects of maize on Beauveria bassiana

To examine the effects of maize on *B. bassiana*, the growth and virulence of *B. bassiana* from three strains were assessed: the original strain *B. bassiana* D1-5 (FO), the strain isolated from maize leaves at ambient temperature (FA), and the strain isolated from maize leaves at elevated air temperature (FW). The isolated strains (FA and FW) were obtained during investigation of colonization rate. Specifically, a single colony of FA or FW was inoculated and cultured on PDA media, and each of these strains was subcultured twice. For each strain, six single spores were randomly selected for culture and sporulation in PDA (26 °C in the dark). Conidia yield was counted in a hemocytometer (Shanghai Qiujing Biochemical Reagent Instrument Co., Ltd., China) as the conidia number in 0.1 g conidia powder which had been carefully scraped from the medium at 10 days post plating and suspended in 10 ml of 0.05% Tween-80 (Soundarapandian and Chandra, 2007). Conidia were cultured on Sabouraud Dextrose Yeast broth (SDY) (Hopebio Spectrum Instruments Co., Ltd., China) and grown at 26 °C for 16−18 h. For each strain, at least 300 spores were counted from several fields of view and were considered germinated if the germ tube was longer than the conidia width. The germination rate of conidia was considered as the ratio of the number of conidia with a germ tube to total observed number of conidia (Hywel-Jones et al., 1990). The decussation method (Ma et al., 2013) (two diameters of the colony were measured vertically and averaged) was used to measure the diameter of the colony on 4, 6, 8, and 10 days post-plating, and the colony diameter was calculated as the average diameter of the colony. The virulence of each *B. bassiana* strain was determined by dipping 3rd instar Asian corn borer (*O. furnacalis*) larvae into a *B. bassiana* suspension (1×10^8 conidia ml^{-1} in 0.05% Tween-80) for 2 s, and then transferring these larvae into 24-well tissue culture plates with an artificial diet. For the controls, insects were dipped in 0.05% Tween-80 solution. We examined 24 insects in each replicate, with five replicates for each *B. bassiana* strain. Survival rate was recorded daily for 10 days, and was calculated as the ratio of the number of living larvae to the total number of insects (Kaplan and Meier, 1958).

2.9. Statistical analyses

Data were tested for normality assumptions using *qqplot* before analysis. Levene's homogeneity test and Shapiro-Wilk normality test were set at the 0.05 significance level. For plants, two-way ANOVA was used to examine the main and interactive effects of *B. bassiana* and elevated air temperature on plant height, biomass, and activity of the two enzymes. Data were further analyzed using one-way ANOVA within the four treatments if the interaction between the two was significant. For *B. bassiana*, we used one-way ANOVA to test the difference in conidia yield, germination rate of conidia, and average diameter of the colony between the three treatments; *B. bassiana* virulence was estimated by the Kaplan-Meier method. Multiple comparisons of mean values were made using Tukey's test ($P < 0.05$). All statistical analyses were performed using SPSS 17.0 (SPSS Inc., 2008).

3. Results

3.1. Colonization rates of B. bassiana in maize

The colonization rate of *B. bassiana* in uninoculated plants was 3.3% at ambient temperature and 4.2% at elevated air temperature.

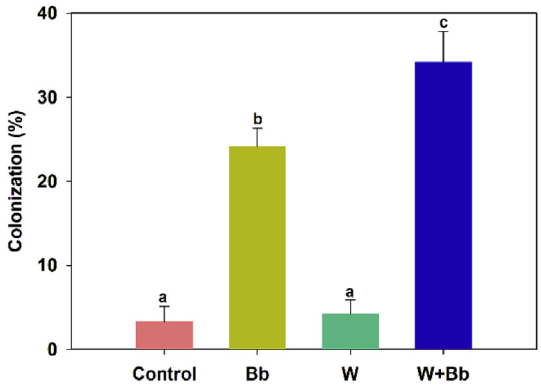

Fig. 1. Maize (*Zea mays*) colonization by *Beauveria bassiana* (endophytic) in four treatments: Control: maize inoculation with Tween-80 solution at ambient temperature; Bb: maize inoculation with *B. bassiana* suspension with Tween-80 at ambient temperature; W: maize inoculation with Tween-80 solution at elevated air temperature; and W + Bb: maize inoculation with *B. bassiana* suspension with Tween-80 at elevated air temperature. Values are means ± SE. Different letters above bars indicate significant differences between four treatments ($P < 0.05$).

In contrast, the colonization rate of *B. bassiana* in inoculated plants at ambient temperature was 24.1% and 34.1% at elevated air temperature (Fig. 1). Maize colonization by *B. bassiana* was significantly higher in *B. bassiana*-inoculated treatments than that in uninoculated treatments at both ambient and elevated air temperatures.

3.2. Effects of B. bassiana on maize

There was a significant interactive effect of *B. bassiana* and elevated air temperature on plant height during various growth stages and mean plant height (Table S1). The main effect of elevated air temperature on plant height was significant while *B. bassiana* only significantly affected plant height during the S1−S3 growth stages (Table S1). Plant height under W and W + Bb treatments was significantly taller than those in the Control and Bb treatments during S1−S4 stages, and plant height was significantly greater in the W treatment than in the Control treatment at S5 stage (Fig. 2A). Mean plant height under Bb, W and W + Bb treatments was significantly higher by 8.2%, 28.4%, and 25.4%, respectively, than that in the Control (Fig. 2B).

We observed a significant interactive effect of *B. bassiana* and elevated air temperature on shoot biomass and total plant biomass. The effect of elevated air temperature on total plant biomass and root biomass was also significant (Table S1). Total plant biomass in Bb was significantly higher by 12.9% than that in W + Bb (Fig. 2C). In comparison to the Control, shoot biomass in Bb and W was higher by 8.81% and 6.93%, respectively (Fig. S4A). The root biomass in Bb was 24.95% higher than that in W + Bb (Fig. S4B).

The interactive effect between *B. bassiana* and elevated air temperature on PAL activity, effect of elevated air temperature on PPO activity, and effect of *B. bassiana* on PAL activity in plants were all significant (Table S1). PAL activity in Bb, W, and W + Bb treatments significantly increased by 29.0%, 10.8%, and 21.8%, respectively (Fig. 3A). PPO activity in W and W + Bb treatments significantly increased by 10.9%, and 12.8%, respectively (Fig. 3B).

3.3. Effects of maize on B. bassiana

Conidia yield of *B. bassiana* isolated from plants under ambient (from FA) and elevated air temperature (from FW) increased by 80.2% and 111.2%, respectively, compared to that of FO (Fig. 4A). The germination rate of conidia from strain FA and FW significantly increased by 44.5% and 38.3%, respectively, compared to that of conidia from strain FO (Fig. 4B). The diameter of the colony of FA was significantly greater than that of strain FO at each time interval, and average diameter of the colony of FA was increased by 11.5% compared to that of strain FO (Fig. 4C, Fig. S5). There was a significant effect on cumulative survival rate between FO, FA and FW on larva 4 days post inoculation (Fig. 4D).

4. Discussion

Our results show that species interactions in a typical agroecosystem may have remarkable responses to climate warming, as also shown by Fuhrer et al. (2003) and Chidawanyika et al. (2019) for other ecosystems. Specifically, maize and *B. bassiana* exerted a positive effect on each other under ambient temperature. However, at elevated air temperature, there was a positive effect of maize on *B. bassiana*, but little effect of *B. bassiana* on maize. These findings suggest that elevated air temperature could reshape the pattern of species interactions between plants and fungal entomopathogens in agroecosystems. These findings thus further the understanding of interactions between crop and fungal entomopathogens in the context of climate warming.

4.1. Interactions between plant and fungal entomopathogens under ambient temperature

We found that both plants and the fungal entomopathogens have strong effects on each other under ambient temperature (Fig. 5A). *B. bassiana* directly increased maize height during the early stages (S1−S3) and the shoot biomass in milk-ripe stage (S5), which is consistent with other studies showing that fungal entomopathogens can endophytically colonize and promote plant growth, both in monocots (e.g. wheat, maize and banana) and in dicots (e.g. tomato, cotton and potato) (Vega, 2008, 2018; Ownley et al., 2010). Meanwhile, higher plant height during early stages may result in an increase in plant shoot biomass (Yin et al., 2012). This might be because *Beauveria*, like *Metarhizium*, may have the capacity to transfer nitrogen to plants (Behie and Bidochka, 2014; Basu et al., 2018), improving plant growth. Another possible mechanism for promoting plant growth is that, as a plant endophyte, *B. bassiana* might produce phytohormones, secondary metabolites that are synthesized via diverse metabolic pathways (Chanclud and Morel, 2016; Schulz et al., 2019). For example, Priyadharsini and Muthukumar (2017) showed that a dark septate endophyte fungus mediates plant growth through phosphate solubilization and phytohormone production. However, to ascertain whether *B. bassiana* indeed improves plant growth by promoting nutrient element absorption and/or by producing phytohormones needs further exploration.

A possible beneficial effect of endophytic fungal entomopathogens on plants is the enhanced resistance against biotic and abiotic stress (Hardoim et al., 2015), for example, when confronted by pests and pathogens (Vega, 2008, 2018). Endophytes may in general play a mutualistic role within their hosts by increasing the concentration of defense metabolites potentially active against pathogens (Schulz et al., 1999). Both PAL and PPO are involved in the synthesis of defense metabolites (Han et al., 2009) and are usually involved in the main lines of cell acclimation in plants against pests and pathogens (Rivero et al., 2001). Our results show that the activities of these two important enzymes in maize, PAL and PPO, significantly increased following *B. bassiana* treatments at the jointing stage of maize, two enzymes which usually increase the host's defense ability in response to biotic and abiotic stress (Rivero et al., 2001; Singh et al., 2013).

On the other hand, fungal entomopathogens may benefit from growth in plants, as observed after isolation from plants. In contrast to the reported positive effects of fungal entomopathogens on plants

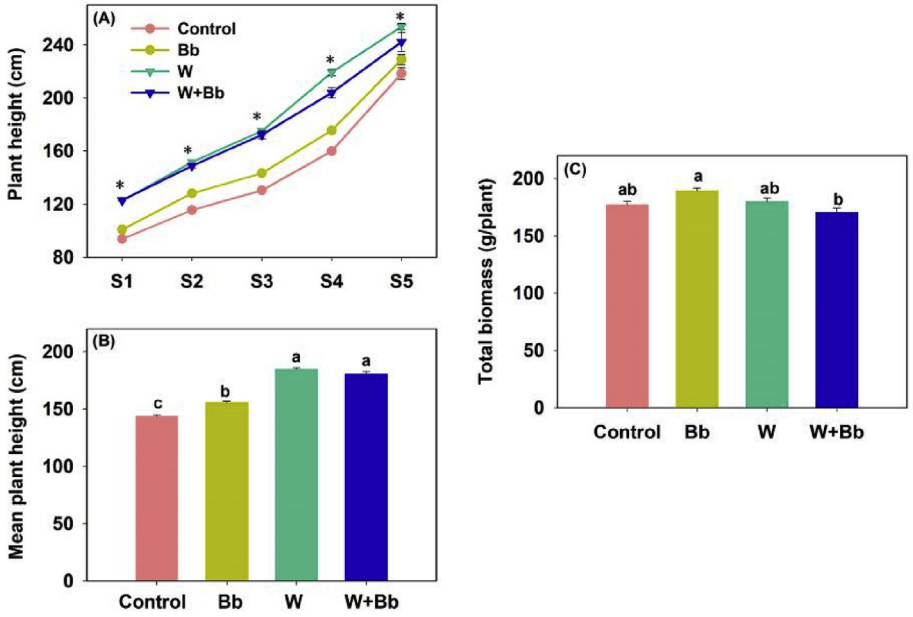

Fig. 2. Effects of *Beauveria bassiana* (Bb) and elevated air temperature (W) on maize (*Zea mays*) height during five growth stages (A); mean plant height (B); and total biomass (C). S1: bell mouth stage; S2: early jointing stage; S3: middle jointing stage; S4: tasseling stage; S5: milk-ripe stage. Values are means ± SE. Different letters above bars indicate significant differences between the four treatments at $P < 0.05$.

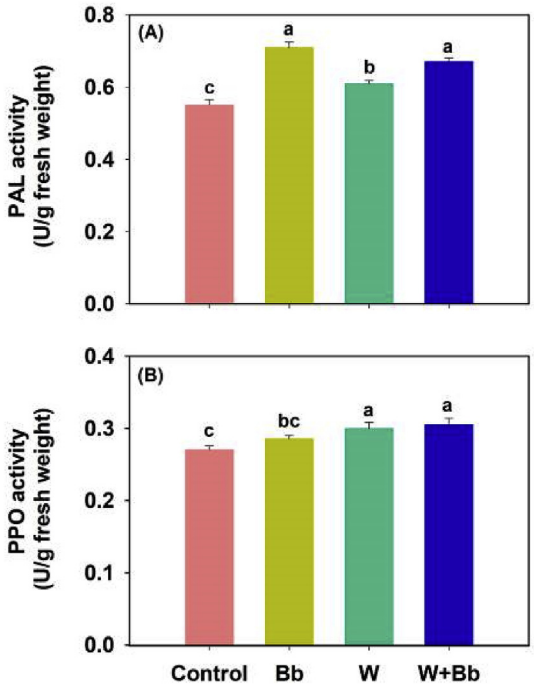

Fig. 3. Effects of *Beauveria bassiana* (Bb) and elevated air temperature (W) on activities of defense enzymes in maize (*Zea mays*): (A) phenylalanine ammonium lyase (PAL); and (B) polyphenol oxidase (PPO). Values are means ± SE. Different letters above bars indicate significant differences between the four treatments at $P < 0.05$.

(Jaber and Enkerli, 2017), few studies have identified the effects of plants on fungal entomopathogens (Behie et al., 2017). Our results show that conidia yield, germination rate, and colony diameter of *B. bassiana* isolated from maize significantly increased compared to the original strain, suggesting that there was a positive effect of maize on *B. bassiana*. Previous research showed that good growth of endophytes is positively correlated with symbiotic growth within their hosts (Fellbaum et al., 2012). *B. bassiana* may also obtain assimilates from plants they colonize (Behie et al., 2017), as other endophytic fungi do (Mack and Rudgers, 2008). In addition, better conidial traits such as conidia yield and germination rate may also be beneficial in increasing virulence of strains (Shah et al., 2005).

4.2. Interactions between plant and fungal entomopathogens under elevated air temperature

Elevated air temperature is a primary factor affecting the rate of plant growth and development (Hatfield and Prueger, 2015). Elevated air temperature directly influences plant traits through physiological adaptation, which may in turn alter the interactive network with co-existing species (Atkin et al., 2000; Jamieson et al., 2012). Our results seem to confirm this conclusion. Elevated air temperature of ca. 2.0 °C increased maize height, shoot biomass, and defense enzyme activities in maize, although no effect on total maize biomass was found in this study. These complex responses of plants are similar to previous findings that climate warming has no effect on aboveground biomass and grain yield, though the number of grains per year declines (Ferris et al., 1998; Winkler et al., 2019). Maize height significantly increased under elevated air temperature in our experiment, which may be related to the physiological response (e.g. photosynthetic and respiratory activity) of a C4 plant when warming occurs (Bokhorst et al., 2010), and thus result in a phenotypic modulation of C4 plants (Nicotra et al., 2010). Many studies have shown that the activities of plant defense enzymes are sensitive to temperature change (Ali et al., 2005; Zhu et al., 2010), and the increase in both PAL and PPO may result from cell acclimation to stress (Rivero et al., 2001). Our results indicate that elevated air temperature may induce higher activities in PAL and PPO.

Indeed, the growth characteristics of fungal entomopathogens are likely to be relatively stable under changing environmental conditions (Rangel et al., 2010), with *B. bassiana* growing at a wide optimum temperature range from 20 °C to 30 °C. However, germination is delayed when temperature is above 35 °C or falls below 15 °C (Fargues et al., 1997; Ekesi et al., 1999). In our study, the mean ambient temperature was 25 ± 0.5 °C, and air temperature

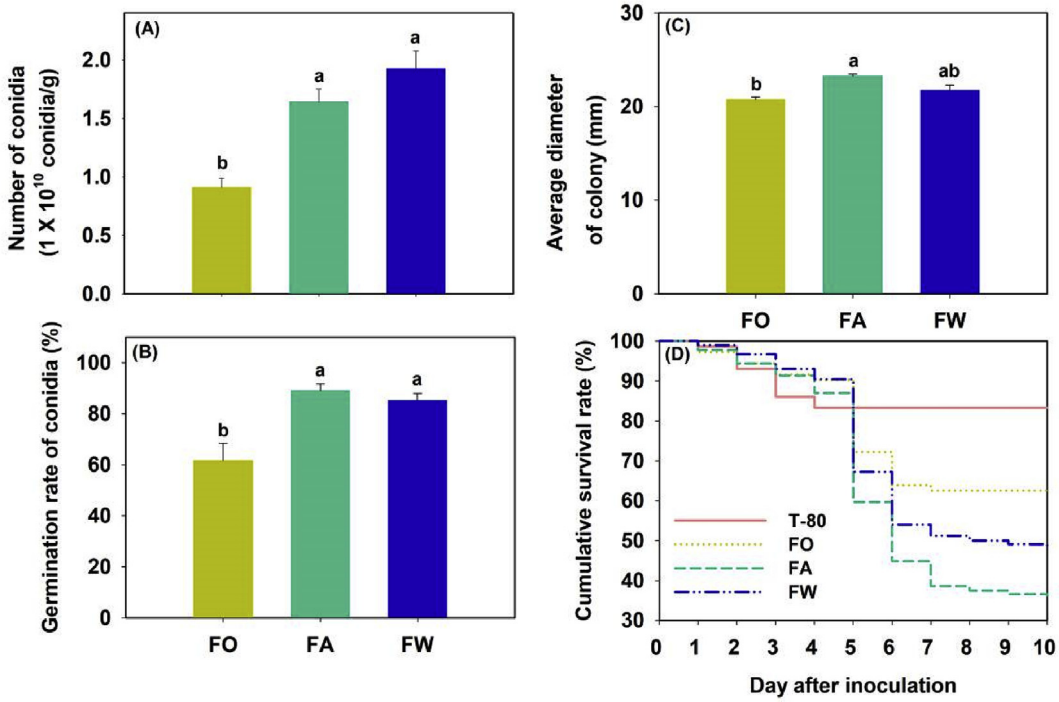

Fig. 4. Number (A) and germination rate (B) of *Beauveria bassiana* conidia, average diameter of colony (C), and cumulative survival rate of the Asian corn borer (D). FO: the original *B. bassiana* D1-5 strain; FA: *B. bassiana* isolated from maize leaves growing at ambient temperature; FW: *B. bassiana* isolated from maize leaves growing at elevated air temperature; T-80: Tween-80 (Control). Different letters above bars indicate significant differences between the three treatments at $P < 0.05$.

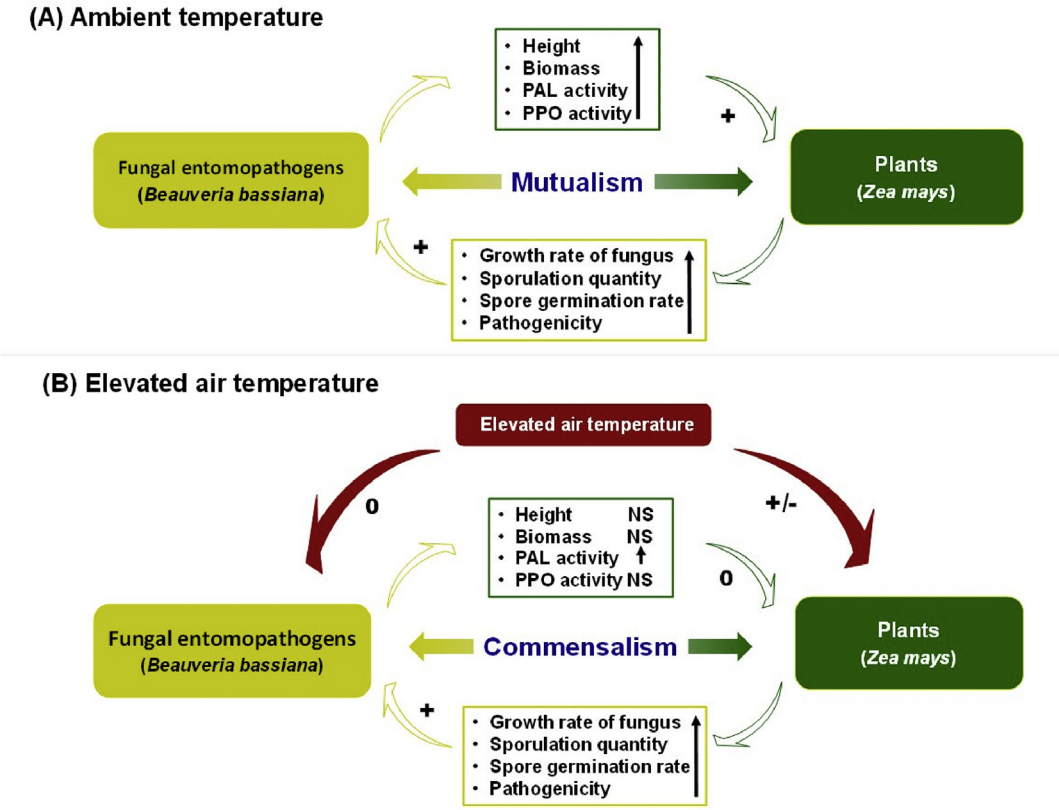

Fig. 5. Concept framework on the interactions between plant (*Zea mays*) and fungal entomopathogens (*Beauveria bassiana*) under (A) ambient temperature and (B) elevated air temperature. "+": positive effect, "-": negative effect, "0": no effect, up arrow indicates treatment-enhanced effects, and "NS" indicates no significant effect.

was increased by 2 ± 0.5 °C in the warming treatment. That means that both temperatures are suitable for the growth of *B. bassiana*, and the biological characteristics of *B. bassiana* were probably unchanged at elevated air temperature of approximately 2 °C. The effect of elevated air temperature on plants was significant in our study and is speculated to have no effects on fungal entomopathogens. Thus, the improved growth of plants in W + Bb treatments may be primarily due to the elevated air temperature, rather than to the influences of the entomopathogen or to interactive effects.

Conversely, significant effects of plants on *B. bassiana* under elevated air temperature were detected (Fig. 5B). Some research suggests that the growth and reproduction of fungal entomopathogens depend on the nutrients provided by plants (Behie et al., 2017). A growing body of evidence suggests that an enhancement in plant biomass production can stimulate more carbon allocation, and subsequently cascade to microbes, especially to those that parasitize plants, thereby helping plants to support greater microbial populations (Zhang et al., 2005; Rajkumar et al., 2013). Although the mechanisms responsible for the beneficial effects of plants on fungal entomopathogens remain to be investigated, our results indeed confirm this effect under elevated air temperature.

5. Conclusion

It is critical to understand species interactions that may regulate the stability of agroecosystems and ecological communities (Márquez et al., 2007; Delmas et al., 2019). Although previous studies have focused on the strength of species interactions (Kiers et al., 2010; Sentis et al., 2017), how interactions may shift from one nature to another (e.g. shifts between mutualism, commensalism, and amensalism), has received much less attention, particularly in the context of climate change (He et al., 2013; Olsen et al., 2016). Schulz and Boyle (2005) confirm that endophytic fungi have the ability to adapt to changing environmental conditions, consequently influencing the interactions with other species. In this study, we found that elevated air temperature has altered the nature of interactions between plants and fungal entomopathogens in an agroecosystem. Such species interactions may influence the status of food webs, further affecting crop production. Therefore, we highlight the importance of understanding the nature of species interactions and their strength to better realize and predict novel and changing structural stability of agroecosystems.

Author's contributions

H.Z., D.W., L.S., and Q.L. designed the experiment; L.S., W.X., and Z.Z. performed the experiment; H.Z., L.S., and L.W. conducted data analyses; H.Z., L.S., D.W., and Q.G. wrote the manuscript.

Data availability statement

Full data sets are available at (Dryad, Figshare, Hal).

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Supplementary data

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