seeds

MDPI

Article

Effects of *Marquandomyces marquandii* SGSF043 on Maize Growth Promotion and Soil Enzyme Activity

Xu Zheng [1,2,3,†], Bo Zhang [2,3,4,†], Feng Shi [2,3], Yuanlong Chen [2,3] and Xiumei Zhao [1,*]

[1] Qiqihar Branch of Heilongjiang Academy of Agricultural Sciences, Qiqihar 161006, China; ztlovezx@163.com
[2] Engineering Research Center of Agricultural Microbiology Technology, Ministry of Education & Heilongjiang Provincial Key Laboratory of Ecological Restoration and Resource Utilization for Cold Region & Key Laboratory of Microbiology, College of Heilongjiang Province & School of Life Sciences, Heilongjiang University, Harbin 150080, China; wbl970512@163.com (B.Z.); sf18846435880@163.com (F.S.); 2221630@s.hlju.edu.cn (Y.C.)
[3] Jiaxiang Industrial Technology Research Institute of Heilongjiang University, Jiaxiang 272400, China
[4] Heilongjiang Academy of Agricultural Sciences, Heilongjiang Academy of Black Soil Conservation and Utilization, Harbin 150086, China
* Correspondence: zxm0452@126.com
† These authors contributed equally to the paper.

Abstract: In order to further clarify the growth-promoting effect of the non-core *Metarhizium* sp. *Marquandomyces marquandii* on plants, *M. marquandii* SGSF043, which was obtained via pre-screening in the laboratory, was selected as a test strain and the seed soaking method was adopted. The effects of a fermentation broth obtained from this strain on the seed germination, seedling growth, and rhizosphere soil enzyme activity of maize were studied. The results were as follows: In seed germination tests, *M. marquandii* SGSF043 fermentation liquid had a certain inhibitory effect on corn seed germination, and the germination rate was only 15%. When the fermentation solution was diluted 10 times, the germination rate reached 97%. After the germination test, the growth of maize plumules was promoted in the groups treated with 10-times and 1000-times dilutions. In the field community experiment, based on the comprehensive evaluation of seedling biomass indicators, the solution diluted 100 times had the best growth-promoting effect. The aboveground fresh weight was increased by 127.13% compared with the control group. The results show that *M. marquandii* SGSF043 has the potential to promote the growth of maize and improve the soil environment, which provides a theoretical basis for the research on and the application of *M. marquandii* in farmland.

Keywords: *Metarhizium*; *Marquandomyces marquandii*; maize; seed germination; seedling growth

Citation: Zheng, X.; Zhang, B.; Shi, F.; Chen, Y.; Zhao, X. Effects of *Marquandomyces marquandii* SGSF043 on Maize Growth Promotion and Soil Enzyme Activity. *Seeds* **2024**, *3*, 203–215. https://doi.org/10.3390/seeds3020016

Academic Editor: Athanasios Koukounaras

Received: 24 January 2024
Revised: 29 March 2024
Accepted: 4 April 2024
Published: 16 April 2024

1. Introduction

In recent years, due to the increase in maize yields and planting areas, the use of chemical fertilizers has risen year by year, exacerbating soil stagnation, acidification, salinization, and, in combination with reduced use of organic fertilizers, has led to crop yield reductions, increased resistance of phytophagous pests, increased production costs, and environmental pollution, among other problems [1,2]. Implementing actions and strategies for sustainable food production systems, such as integrated pest management (IPM) and the development of organic agriculture, which involve the use of microorganisms and their metabolites to control pest and disease damage to crops, has the potential to increase plant "immunity" to abiotic stresses and promote crop growth. Such actions and strategies utilize large-scale insect sex pheromone traps, spray equipment, automatic robotic weed mowers, microporous polyethylene bags or fine mesh bags for insect protection, and physical barriers (pathogen control mulch/mulch) [3–6]. Among biocontrol bacteria, Rai et al. isolated and identified pseudomonas RS-9 from a healthy tomato plant with bacterial wilt, which can inhibit the incidence of bacterial wilt and promote the height

and dry weight of tomato plants and is a promising strain for the biological control of tomato bacterial wilt [7]. In recent years, *Metarhizum* sp. are widely known as entomopathogenic fungi and are widely distributed throughout the world, usually surviving in nature as plant inter-root fungi [8], plant endophytes [9], insect pathogens [10], and soil fungi. In 1883, Sorokin officially named the first species of *Metarhizium Metarhizium anisopliae* [11], after which research on *Metarhizium* fungi as insect pathogens and biopesticides began. Based on previous studies, in addition to increasing plant resistance to pathogenic strains [12] and having strong insecticidal activity, it has been reported in recent years that *Pseudomonas aeruginosa* can colonize different plant tissues to promote the growth of the host plant and have a positive impact on the growth indicators of the plant. The treatment of seeds with microorganisms has been used to protect plants against plant pathogens and promote better growth of plants. Kenia Almeida Diniz et al. used a variety of biocontrol bacteria (fungi) to conduct germination tests on pepper seeds and explored the effects of polymers and biocontrol bacteria (fungi) on pepper seed germination [13]. Enrique González Pérez et al. [14] have shown that *Metarhizium anisoplia* can colonize the roots of tomato, maize, *arabidopsis* thaliana, peanut, soybean, cotton, coffee, and other plants, and that it has a certain promotional effect on biological indexes, such as plant seedling height and root length, and above- and belowground parts of plants [15,16]. Liao et al. showed that, compared with the control group, the amount of leaf crown formation, stem length, average stem leaf biomass, and cob biomass were significantly increased by the treatment of maize seeds with a *Metarhizium* fungicide. And these main effects on maize yield occurred during early nutritive growth [17]. Plant photosynthesis is the basic unit of material accumulation and physiological metabolism in the process of plant growth, and it is also an important means of analyzing the influence of environmental factors on plant growth. It has been shown that endophytic fungi have an extremely strong ability to promote photosynthesis in plants by increasing the supply of carbon dioxide needed for photosynthesis, thus improving plant photosynthesis [18]. It has been shown that the association between *Metarhizium* spp. and plants and insects is mainly based on nutrition [19]. *Metarhizium* spp. can transfer nitrogen produced by insects to plants for plant growth by parasitizing and killing insects [9]. Plants provide carbon for *Metarhizium* spp. [20]. Through photosynthesis and vigorous growth, plants can provide a more stable habitat for *Metarhizium* spp., while *Metarhizium* spp., due to their parasitic nature, can further protect the healthy growth of plants. *M. marquandii* belongs to the genus of non-core *Metarhizium* spp., which are widely present in soil. Some studies have shown that the secondary metabolites of *M. marquandii* not only have antimicrobial and antitumor activities [21], but also have the potential to be used in the removal of environmental pollutants (heavy metal pollutants, pesticide residues, etc.), the control of crop pests and diseases, and the promotion of plant growth [22,23]. However, only a few studies on the plant growth-promoting effects of this strain have been reported.

Soil enzymes are mainly derived from the cellular secretions of animals, plants, and microorganisms in the soil and the decomposition of their residues. They are important participants in the soil material cycle and energy transformation and have a close relationship with the fertility level of the soil. They can characterize the dynamic equilibrium of the soil–microorganism–plant system to a certain extent [24]. There is an intrinsic link between soil enzymes and plant growth. The application of biotrophic agents can significantly improve the inter-root soil conditions of crops and create a suitable environment for soil enzyme production, and the increase in soil enzyme activity can also promote crop growth and development [25]. Studies have shown that fungal fertilizers can regulate the micro-ecological environment of the plant root system and the activity of soil alkaline phosphatase and soil urease, enhance crop resilience, improve the ability of crops to absorb nutrients, and realize the effects of increasing yield and efficiency. However, it has also been shown that there was no significant increase in soil enzyme activities due to the influence of environmental factors [26–28].

In this project, a strain of *M. marquandii* was isolated from the understory apomictic material in Da Hinggan Ling Range in a preliminary study and was identified morphologically and by 16sRNA and named *M. marquandii* SGSF043. Few studies have been reported on the growth promotion of field crops by *M. marquandii*. In this study, the maize seed of "Meiyu" were soaked with a fermentation broth of *M. marquandii*. SGSF043 to study the effect of this strain on the germination of maize seeds and the growth of seedlings, and, at the same time, the inter-root soil enzyme activity of maize seedlings was measured to clarify the effect of *M. marquandii*. on the growth and development of maize and the role of the soil environment in the inter-root zone of seedlings. The results of the study are intended to provide a basis for further exploring the potential advantages of *Metarhizium* as a microbial fertilizer for crops.

2. Materials and Methods

2.1. Experimental Strain

M. marquandii SGSF043. was isolated from litterfall in the Nanweng River National Nature Reserve in Da Hinggan Ling Range of Heilongjiang Province, China. The main plant species are *Tilia amurensis* Rupr., *Quercus mongolica* Fisch. *exTurcz*, and *Fraxinus mandshurica* Rupr. The litter was divided into undecomposed, semi-decomposed, and decomposed layers, from top to bottom, and the samples were collected in layers. The samples were brought back to the laboratory and placed in a cool place, then dried naturally for isolation and purification. The above strain was kept in the Mycological Collection Center of Wuhan University (CCTCC no. M2020555).

Source of Experimental Maize: The "Meiyu" sweet and glutinous maize variety, with a harvest period of 75–80 days, was purchased from a seed market and is a common maize variety in Harbin.

2.2. Media Formulation and Fermentation Cultivation

Potato Dextrose Broth Medium (PDB): A quantity of 25.0 g of PDB dry powder was added to 1 L of distilled water and heated until dissolved. The pH of the PDB liquid medium was adjusted to 8.0 with NaOH, and then the medium was autoclaved at 121 °C for 20 min (Figure 1, right).

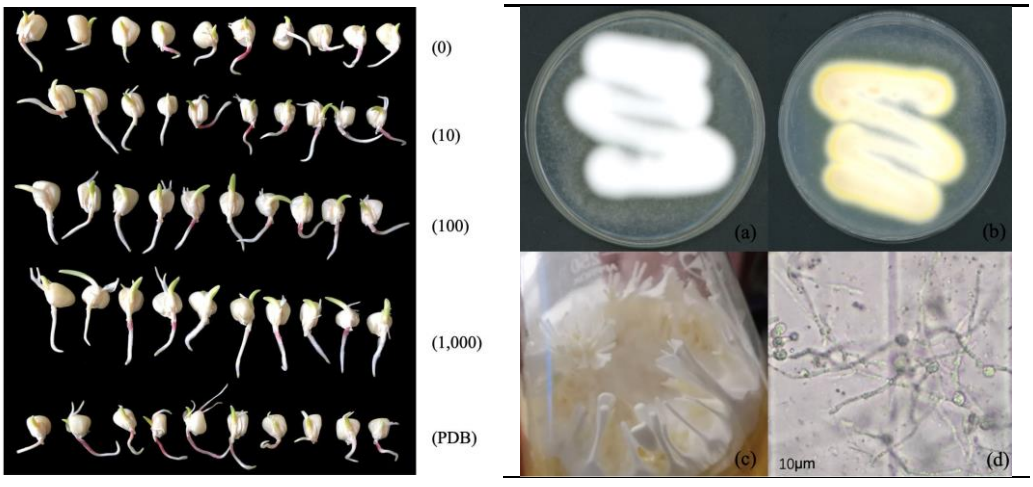

Figure 1. Germination morphology of maize seeds and mycelium and spore morphological structure of *M. marquandii* SGSF043 in different treatment groups. (**left**) Effect of *M. marquandii* SGSF043 on germination of maize (two days after germination). 0, *M. marquandii* SGSF043 fermentation liquid; 10, 10-times dilution of *M. marquandii* SGSF043; 100, 100-times dilution of *M. marquandii* SGSF043; 1000, 1000-times dilution of *M. marquandii* SGSF043; PDB, blank control. (**right**) Photo of *M. marquandii* SGSF043. (**a**,**b**) Colony morphology of *M. marquandii* SGSF043 in solid medium. (**c**) Photo of fruiting body of SGSF043 liquid medium. (**d**) Mycelium and spore morphology of *M. marquandii* SGSF043.

Culture conditions for the fermentation broth of *M. marquandii* SGSF043: The activated *M. marquandii* SGSF043 was transferred to PDB liquid medium (150 mL/bottle) and placed in a constant-temperature shaker at 25 °C, 180 r·min^{-1}, in dark conditions for 14 d. The cultured fungal fermentation broth was filtered through sterile filter paper to obtain the spore-containing fermentation broth, in which the effective amount of viable fungus was measured to be 4.12 × 10^9 CFU/mL.

2.3. Experimental Design

The experiment was conducted in August 2022 in the greenhouse of Heilongjiang University. Maize seeds were selected to be sterilized by 70% alcohol for 30 s and rinsed with sterile water 5 times. Before germination, samples of 30 maize seeds were soaked in different treatments of fungal solution for 24 h, and the soaked seeds were put into 28 °C incubators for germination. A total of five treatments were set up in the experiment: the fungus filtrate was diluted with sterile water 0 (fermentation solution), 10, 100, and 1000 times and PDB medium was used as a blank control, and three replications of each treatment were performed.

2.4. Effect of Soaking Seeds in Fermentation Solution on Germination of Maize Seeds

The samples of 30 maize seeds were immersed in different treatments of the fungal solution for 24 h before germination and subsequently placed in glass Petri dishes with sterilized filter paper for germination.

The germination was recorded as starting from the time when the radicle and endosperm sprouted, and the germination of the maize seeds was observed every day for 2 days; the germination potential (%), the germination rate (%), and the germination index were calculated. At the end of the seed germination test, the radicle length and radicle thickness of young maize shoots were determined using a straightedge [29].

$$\text{Germination potential (\%) GP/\%} = \frac{A_{2d}}{A_t} \times 100 \quad (1)$$

In the formula, "GP" stands for the germination potential (%), "A_{2d}" is the number of germinated seeds in the first two days, and "A_t" is the total number of test seeds.

$$\text{Germination rate (\%) GE/\%} = \frac{A_c}{A_t} \times 100 \quad (2)$$

In the formula, "GE" is the germination rate (%), "A_c" is the number of fully germinated seeds, and "A_t" is the total number of test seeds.

$$\text{Germination index GI} = \sum (\mathrm{G_t}/\mathrm{D_t}) \quad (3)$$

In the formula, "GI" is the germination index, "G_t" is the amount of germination on day t, and "D_t" is the corresponding number of days to germination.

2.5. Determination of Biomass in Maize Seedlings

Seeds were treated with fungus solution before sowing in field plot trials, and samples were taken 20 d after maize sowing; five seedlings were selected for each treatment, the roots of the plants were cleaned with tap water, and the surface water was blotted with filter paper to measure the plant height, the root length, the ground stem thickness, the aboveground fresh weight, and the belowground fresh weight.

2.6. Determination of Photosynthetic Indexes and Chlorophyll SPAD Content in Maize Seedlings

On the 25th day after seeding (when significant differences in growth indicators began to be found), the photosynthetic gas exchange parameters were determined using an LI–6400XT, LI–COR Corporate, Lincoln, NE, USA). From 9 am to 11 am on 2 September 2022, plants with consistent growth were selected in each treatment to determine the relevant

indexes, and each treatment was measured three times. The measured indexes included the net photosynthetic rate (*A*), the transpiration rate (*E*), the intercellular CO_2 concentration (*Ci*), and the stomatal conductance (GH_2O).

The SPAD values of maize leaves were measured using a chlorophyll meter (FK-YL01, Shandong Fangke Instrument Co., Ltd., Weifang, China) in the middle of the functional maize leaves, and each treatment was measured six times.

2.7. Determination of Inter-Root Soil Enzyme Activities in Maize Seedlings

A Soil Alkaline Phosphatase Kit (Solarbio Life Sciences, BC0285, Jiangsu, China), a Soil Neutral Protease Kit (Solarbio Life Sciences, BC0275, Jiangsu, China), and a Soil urease kit (Solarbio Life Sciences, BC0125, Jiangsu, China) were used to determine the enzyme activity in the rhizosphere soil of the maize seedlings according to the manufacturers' instructions.

2.8. Statistical Analysis

Microsoft Excel 2021 and SPSS 27 were used for data processing and statistical analysis, and GraphPad Prism, Version 9.1.1(223) software package was used for plotting. The significance of differences between groups was tested at the 0.05 level by the LSD test in a one-way ANOVA (one-way analysis of variance).

3. Results

3.1. Effect of Fermentation Solution Soaking on Germination of Maize Seeds

The data in Tables 1 and 2 show that the fermentation broth (Filtrate Stock, 0) treatment had an inhibitory effect on seed germination. Among all the dilution treatments, the highest seed germination rate of 97% was recorded for the group treated with the 10-times dilution of the solution and was significantly higher than the germination rates of the other treatment groups, with a germination time of 1 d ($p < 0.05$). The differences in the germination potential and germination indexes between the 10-times dilution and the 1000-times dilution treatment groups were not significant ($p > 0.05$), and the values were higher than those recorded for the 100-times dilution treatment group; nor were there significant differences in the germination potential or the germination indexes between the treatment groups and the control (PDB liquid medium) group ($p > 0.05$).

Table 1. Effect of fermentation solution on germination rate of maize seeds.

Treatment	Dilution Times	Germination Days/d	Germinating Energy/%	Germination Percentage/%	Germination Index
M. Marquandii SGSF043	0	2	37 ± 0.10 c	15 ± 1.90 c	5.50 ± 0.10 c
	10	1	97 ± 0.10 a	97 ± 0.69 a	14.53 ± 0.06 a
	100	2	94 ± 0.24 b	94 ± 1.39 b	14.00 ± 0.00 b
	1000	2	97 ± 0.10 a	94 ± 0.84 b	14.50 ± 0.10 a
Control (PDB)	-	1	97 ± 0.15 a	94 ± 2.12 b	14.47 ± 0.06 a

The results are presented as the means ± standard deviations of three replicates. a–c represent significant differences between different treatment groups ($p < 0.05$).

As can be seen from the group diagram of some samples 2 d after soaking shown in Figure 1, among all the dilution multiples, the embryo buds and embryonic axes of seeds in the groups treated with the 100-times fermentation solution and the 1000-times fermentation solution had the best growth, and with the increase in the dilution multiples of the fungal solution, the number of white lateral roots around the main roots of the seeds increased gradually in the form of a regular radial shape. Combined with the data in Table 2, it can be seen that the radicle coarses, the plumule lengths, the plumule coarses, and the hypocotyl lengths of maize seeds in the 1000-times dilution treatment group increased compared to the other treatment groups. They increased by 13.8%, 32.5%, 10.43%, and

27.1%, compared with the control group, respectively. The greatest effects were on the germ lengths and the embryonic axis lengths of the maize seeds.

Table 2. Effect of fermentation broth on the growth of maize embryos.

Treatment	Dilution Times	Radicle Length (cm)	Radicle Coarse (cm)	Plumule Length (cm)	Plumule Coarse (cm)	Hypocotyl Length (cm)
M. Marquandii SGSF043	0	0.46 ± 1.41 c	0.19 ± 0.56 c	0.98 ± 1.46 d	0.54 ± 0.76 c	0.42 ± 1.10 c
	10	12.16 ± 3.48 a	1.68 ± 0.64 ab	5.17 ± 1.81 ab	1.83 ± 0.64 ab	3.10 ± 1.24 b
	100	8.17 ± 4.36 b	1.37 ± 0.78 b	4.58 ± 2.76 bc	1.59 ± 0.77 b	3.13 ± 1.71 b
	1000	11.38 ± 4.51 a	1.74 ± 0.66 a	5.56 ± 1.73 a	2.02 ± 0.48 a	4.07 ± 1.24 a
Control (PDB)	—	11.37 ± 1.11 a	1.52 ± 0.41 ab	4.20 ± 1.39 c	1.83 ± 0.43 ab	3.2 ± 0.75 b

The results are presented as the means ± standard deviations of three replicates. a–c, and d represent significant differences between different treatment groups ($p < 0.05$).

3.2. Effect on Maize Seedling Biomass

A box-and-line plot was chosen to statistically graph the degree of data dispersion for each indicator of maize seedling biomass. Figure 2 shows the effect of *M. marquandii* on the biomass of maize seedlings, indicating that different dilutions of the fermentation filtrate of the test strain can promote the growth of maize seedlings. Different dilution concentrations had different effects on the seedling morphology of the maize plants.

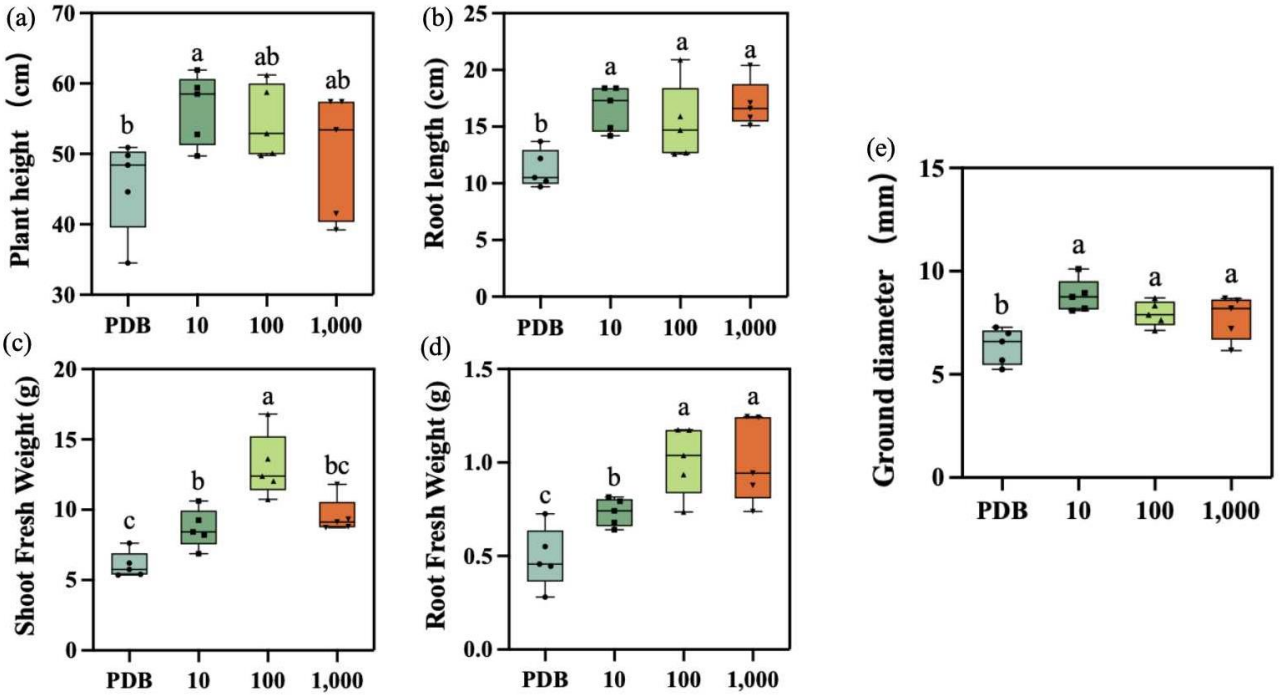

Figure 2. Effect of maize seedling biomass. (**a**) Plant height. (**b**) Root length. (**c**) Shoot fresh weight. (**d**) Root fresh weight. (**e**) Ground diameter. The results are presented as the means ± standard deviations of three replicates. Different letters indicate the differences between the different treatment groups ($p < 0.05$).

In Figure 2a, it can be seen that the difference in plant height between the 10-times dilution treatment group and the control group was significant ($p < 0.05$) The relatively large dispersion of the 1000-times dilution treatment group's plant height data indicates that the data fluctuated within the group.

The median of the 10-times dilution treatment group was 58.5 cm, that of the 100-times dilution group was 52.9 cm, that of the 1000-times dilution group was 53.4 cm, and that the

control group was 48.4 cm. The heights of the fungal dilution treatment groups increased by 20.90%, 9.30%, and 10.33% compared with the control group, respectively.

In Figure 2b, it can be seen that the root lengths of the plants in all the dilution treatment groups differed significantly ($p < 0.05$) from those of the control group. The 100-times solution root length data were more discrete, suggesting that the data fluctuated within the group. The median of the 10-times dilution group was 17.3 cm, the median of the 100-times dilution group was 14.7 cm, the median of the 1000-times dilution group was 16.6 cm, and the median of the control group was 10.2 cm. The heights of the maize seedlings in each bacterial dilution group were higher than those of the control group; they were 69.61%, 30.61%, and 62.75% higher, respectively.

In Figure 2c, it can be seen that the differences between the aboveground fresh weight of the 100-times dilution treatment group and those of the other treatment groups were significant ($p < 0.05$). The median of the 10-times dilution group was 8.425 g, that of the 100-times dilution group was 12.04 g, that of the 1000-times dilution group was 8.791 g, and that of the control treatment group was 5.356 g. The aboveground fresh weight of maize in each dilution treatment group was increased by 57.3%, 125.8%, and 64.13% compared with the control group, respectively.

In Figure 2d, the difference between the underground fresh weight of the 100-times dilution treatment group and that of the 1000-times dilution treatment group was not significant ($p > 0.05$). The median of the 10-times dilution group was 0.742 g, that of the 100-times dilution group was 1.038 g, that of the 1000-times dilution group was 0.943 g, and that of the control treatment group was 0.457 g. The aboveground fresh weights of the maize in the treatment groups for each bacterial dilution were 62.36%, 127.13%, and 106.35% greater than that of the control group, respectively.

From Figure 2e, it can be seen that the maize seedling ground diameter thickness for each dilution treatment group was higher than that of the control treatment group, and the data were stable within the group. The median of the 10-times dilution group was 8.76 mm, that of the 100-times dilution group was 7.89 mm, that of the 1000-times dilution group was 8.2 mm, and that of the control treatment group was 6.6 mm, and the treatments of each dilution increased the diameter of the ground roughness by 32.73%, 19.55%, and 24.24%, compared with the control group, respectively.

3.3. Effect on Photosynthetic Indexes and Chlorophyll SPAD in Maize Seedlings

Treatments of different dilutions of the fermentation filtrate of *M. marquandii* had an effect on the photosynthetic properties of the maize seedling leaves. Different dilutions of the fermentation solution increased the net photosynthetic rates (A) of maize seedlings (Figure 3a), which differed significantly ($p < 0.05$) compared with those of the treatment group soaked in sterilized PDB liquid medium, and the data for the group treated with the 10-times dilution of the fermentation solution were stable compared with those for the 100-times dilution treatment group and the 1000-times dilution treatment group.

The transpiration rate (E) (Figure 3b) and stomatal conductance (GH_2O) (Figure 3d) of the plants in the 100-times dilution treatment group differed significantly ($p < 0.05$) from those of the control treatment group. The transpiration rates of the plants corresponding to the 10-times dilution, 100-times dilution, and 1000-times dilution treatments were the most significant at 3.04 mmol/($m^2 \cdot s$), 3.48 mmol/($m^2 \cdot s$), and 2.5 mmol/($m^2 \cdot s$), respectively, compared to 1.66 mmol/($m^2 \cdot s$) for the control treatment group. The highest leaf stomatal conductance of 132.6 mmol/($m^2 \cdot s$) was recorded for the 100-times dilution treatment group.

The control treatment group plants had the highest interstitial carbon dioxide concentration (Ci) of 121 μmol/($m^2 \cdot s$), and the 1000-times dilution treatment group had the lowest interstitial carbon dioxide concentration (Ci) of 57.4 μmol/($m^2 \cdot s$). The interstitial carbon dioxide concentrations (Cis) of the plants in the 10-times dilution and the 100-times dilution treatment groups were 73.2 μmol/($m^2 \cdot s$) and 85.4 μmol/($m^2 \cdot s$), respectively.

The highest chlorophyll SPAD value of 41.12 was recorded for maize seedlings in the 100-times dilution treatment group; values of 40.94 were recorded for the 10-times dilution

treatment group, 38.62 for the 1000-times dilution treatment group, and 38.92 for the control treatment group.

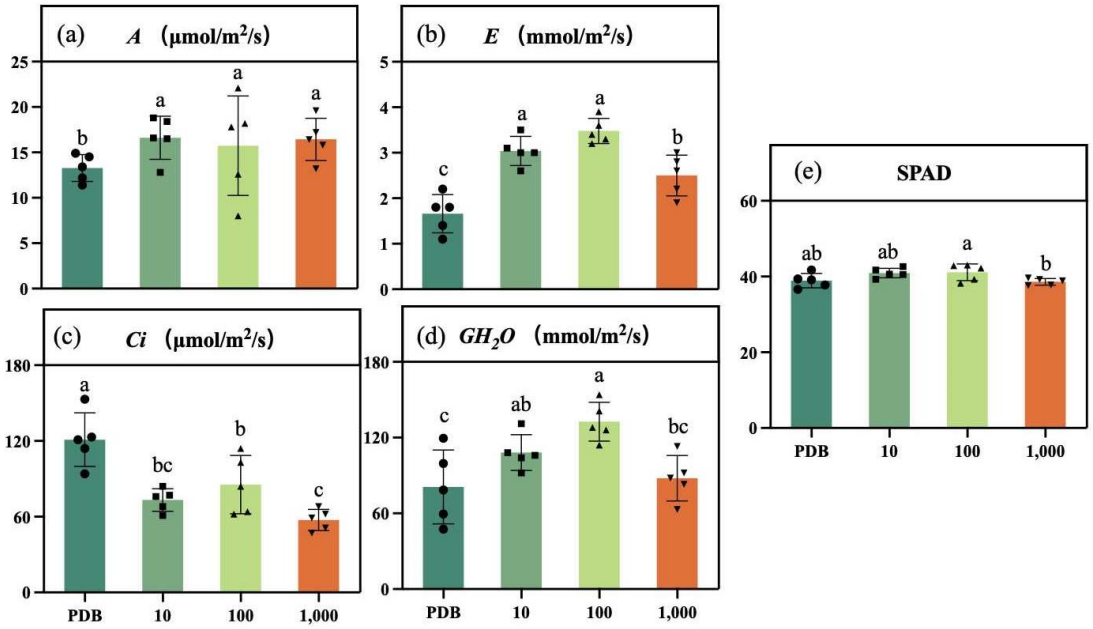

Figure 3. Effect of maize seedling biomass. (**a**) *A*. (**b**) *E*. (**c**) *Ci*. (**d**) GH_2O. (**e**) SPAD. The results are presented as the means ± standard deviations of three replicates. Different letters indicate the differences between different treatment groups ($p < 0.05$). The circles in the bar chart represent the sampling points of each photosynthetic parameter in the PDB treatment group. The square represents the sampling points of each photosynthetic parameter in the *M. marquandii* SGSF043 fermentation solution diluted 10 times. The equilateral triangle represents the sampling points of each photosynthetic parameter in the *M. marquandii* SGSF043 fermentation solution diluted 100 times. The inverted triangle represents the sampling point of photosynthetic parameters in the *M. marquandii* SGSF043 fermentation solution diluted 1000 times.

3.4. Inter-Root Soil Enzyme Activities in Maize Seedlings

Soil alkaline phosphatase (Figure 4a), soil neutral protease (Figure 4b), and soil urease (Figure 4c) were measured in the inter-root soil of the maize seedlings in the different treatment groups. The differences in soil alkaline phosphatase and soil urease between treatments were not significant ($p > 0.05$). Soil neutral protease (Figure 4b) was significantly ($p < 0.05$) higher in the 10-times dilution treatment group than in the other three treatment groups. There were significant differences among the treatments ($p < 0.05$).

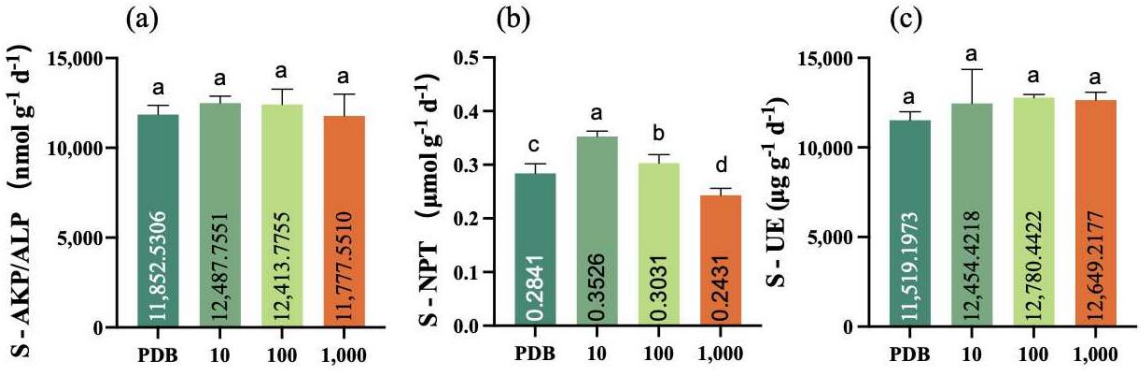

Figure 4. Inter-root soil enzyme activities in maize seedlings. (**a**) S–AKP/ALP. (**b**) S–NPT. (**c**) S–UE. The results are presented as the means ± standard deviations of three replicates. Different letters indicate the differences between different treatment groups ($p < 0.05$).

4. Discussion

The experimental study was undertaken using *M. marquandii* SGSF043, which had been isolated and identified in forest litter. By diluting the *M. marquandii* SGSF043 fungal fermentation broth at different multiples in the growth stabilization period of maize and soaking the seeds, it was found that the seed germination rate of the undiluted fungal fermentation solution was only 15%, which indicated that the fermentation broth of *M. marquandii* SGSF043 had an inhibitory effect on the germination of maize seeds. The fermentation solution diluted with sterile water began to show a promoting effect on seed germination, the highest seed germination rate being found in the group treated with the 10-times dilution of the bacterial solution, and the germination time was 1 d. After the seed germination test was completed, we continued to observe the seed germination and found that the radicles and plumules of seeds in the groups treated with the 10-times dilution fermentation solution and the 1000-times dilution fermentation solution exhibited the best growth, and the seeds treated with the 1000-times dilution fermentation solution had the most root hairs on the lateral roots, which had a regular radial shape. Moreover, studies have shown that foreign microbes can influence root configurations and that the growth of taproots, lateral roots, and root hairs are susceptible to influence by inter-root zone colonization, which is basically consistent with the results of this study. According to the current research results, there are also studies that indicate that microorganisms can produce potential biological control agents to resist plant pathogenic fungi or produce flavonoids to promote plant growth. Next, we may explore the growth-promoting and disease resistance mechanisms of this strain using the secondary metabolites of *M. marquandii* [30–33].

During the seedling growth period, different dilutions of the fermentation broth of *Marquandomyces marquandii* promoted increases in various indicators, including maize seedling height, root length, ground diameter, and aboveground and underground fresh weight, and had different effects on the root morphology of the maize seedlings. The growth indicators of the bacterial fermentation broth treatment groups were better than those of the control group. Regarding the seeds that were soaked in the fermentation broth and planted in the soil, the 100-times dilution treatment group was the best in terms of plant growth and development. The growth promotion of plants is first manifested in the changes in roots. Roots are not only absorption organs, but also organs that sense environmental changes. The growth and development of plant roots are closely related to the environment [34]. In the fermentation broth treatment groups, the root lengths of the seedlings were longer than those of the control group. The fresh weights of maize stems and roots in the 100-times dilution solution group were significantly higher than those of the other treatment groups. With a change in environment, the seeds, after the same treatments, were cultured in Petri dishes and soil. As the environment became more complex, the impact of *M. marquandii* on plants changed accordingly. For example, the 1000-times dilution treatment in the Petri dish promoted the growth of seed germs and radicles. The best growth and development indexes of seedlings in the soil were observed in the 100-times dilution treatment group, and the required concentration of *M. marquandii* increased. Therefore, it is presumed that *M. marquandii* SGSF-043 may achieve growth-promoting effects in maize by regulating the expression of genes related to lateral root formation, auxin synthesis, and transportation, though further research is needed through molecular research methods.

Endogenous growth-promoting bacteria (fungi) have positive effects on the photosynthesis of plants. These microorganisms can promote plant photosynthesis through various mechanisms, such as producing hormones to promote plant growth and development and increasing leaf areas and photosynthetic rates. By helping plants absorb nutrients, they improve photosynthesis efficiency. They also maintain photosynthesis in plants under stress and reduce damage to photosynthetic organs. Promoting root elongation and growth and increasing root surface area improves the plant's ability to absorb water and nutrients and provides more raw materials for photosynthesis [35]. In this experiment, we found that the leaf A (net photosynthetic rate), E (transpiration rate), and GH_2O (stomatal conductance)

values for each of the fermentation solution treatment groups were higher than those of the control group, and the maize soaked in the *M. marquandii* SGSF043 fermentation broth exhibited improved photosynthetic capacity due to an increase in its photosynthetic gas exchange capacity and water uptake, which results are consistent with the findings of the previous study mentioned which found that when host endophytic fungi infested plant roots, the fungus could consume the photosynthesized products in the host's body to make photosynthesis increase [36]. The interesting finding is that the *Ci* (intercellular carbon dioxide concentration) values for each of the fermentation solution treatment groups were lower than those for the blank control treatment group. It is hypothesized, in conjunction with the results of the experiments corresponding to the other photosynthetic indexes, that, probably, when photosynthesis is accelerated, the plant absorbs more carbon dioxide for photosynthesis from the environment and from other cells, which makes the intra- and intercellular carbon dioxide concentration lower, the plant absorbing carbon dioxide and water and converting them into chemical energy (glucose and oxygen) under the action of light. On the other hand, the cause of the decrease in intercellular CO_2 concentration could also be the accelerated rate of CO_2 consumption by the plant, resulting in a slower rate of CO_2 production. In the respiration of plants, plant chlorophyll content is one of the main indicators of the photosynthetic capacity of plants [37]. In the results of this study, the chlorophyll contents of the plants in the fermentation solution treatment groups did not differ much from the chlorophyll content of the control group, which is slightly different from the results of previous studies.

The addition of beneficial microorganisms to the soil around the roots of plants can directly or indirectly, and to varying degrees, increase soil enzyme activity, this being closely related to the organic matter content and other factors. Soil enzyme activities can reflect the type and strength of biochemical reactions in soil [38]. In this experiment, there were no significant differences in soil alkaline phosphatase and soil urease contents between the fermentation liquid treatment groups and the control group. The contents of soil alkaline phosphatase in the 10-times dilution liquid and the 100-times dilution liquid treatment groups increased by 635.22 nmol g^{-1} d^{-1} and 561.24 nmol g^{-1} d^{-1}, respectively, while that in the 1000-times dilution liquid treatment group decreased by 74.98 nmol g^{-1} d^{-1} compared with the control group. The contents of soil urease in each fermentation liquid treatment group increased by 935.22 μg g^{-1} d^{-1}, 1261.24μg g^{-1} d^{-1}, and 1130.02 μg g^{-1} d^{-1} compared with the control group, respectively. The soil neutral protease content was significantly higher in the 10-times dilution treatment group than in the other treatment groups. The content of neutral protease in soil was significantly lower than that in the PDB control group when the dilution ratio was increased to 1000, which may have been due to the decrease in the spore content of bacteria when the dilution ratio was increased. PDB has the function of recruiting beneficial (harmful) microbial flora, so the content of neutral protease is high. Inorganic nitrogen is released through the mineralization of organic nitrogen, which is an important source of nitrogen for plant growth. The soil inorganic nitrogen content characterizes the soil's ability to supply nitrogen. It has been shown that the highest neutral protease activity was found in soil with ammonium nitrogen alone, while the neutral protease activity was enhanced with the increase in the ammonium nitrogen percentage [39]. It can be hypothesized that *M. marquandii* may produce inorganic ammonium nitrogen or recruit beneficial microorganisms that can produce ammonium nitrogen, thus directly or indirectly promoting the uptake and utilization of nutrients in the soil by plants, and the mechanisms related to the plant-promoting potential of *M. marquandii* will be investigated in more detail in the future.

5. Conclusions

The fermentation broth of *M. marquandii* SGSF-043 can promote the germination of maize seeds and increase the biomass of maize seedlings at a suitable concentration. In the maize seed germination tests, the seed germination effect of the 10-times dilution of the bacterial fermentation broth was the best one. After the seed germination test, as the seed

germs continued to grow, the radicle lengths, radicle coarses, plumule lengths, plumule coarses, and hypocotyl lengths of the seeds were measured. It was found that there was no significant difference in seed growth between the 10-times dilution treatment group and the control group, and the 100-times dilution and 1000-times dilution treatments had the best effects. The 1000-times dilution treatment had the most obvious effect on the elongation and growth of lateral roots around the seed main root, which increased by 13.8%, 32.5%, 10.43%, and 27.1% compared with the control group. According to the field plot test, there were no significant differences in chlorophyll content among the treatments. According to the measurement data for maize growth and development, photosynthesis, and rhizosphere soil-related enzyme activities, it can be shown that the best effect regarding the promotion of maize growth and development was achieved with the fermentation broth of *M. marquandii* diluted 100 times. This shows that *M. marquandii* has the potential for development and utilization and that it can be further studied and applied.

Author Contributions: Conceptualization, B.Z. and F.S.; methodology, F.S.; software, Y.C.; validation, F.S. and B.Z.; formal analysis, X.Z. (Xu Zheng) and Y.C.; investigation, X.Z. (Xiumei Zhao); resources, X.Z. (Xiumei Zhao); data curation, B.Z.; writing—original draft preparation, X.Z. (Xu Zheng) and B.Z.; writing—review and editing, X.Z. (Xu Zheng) and B.Z.; visualization, B.Z. and F.S.; supervision, X.Z. (Xiumei Zhao); project administration, X.Z. (Xu Zheng). X.Z. (Xu Zheng) and B.Z. have contributed equally to this work. All authors have read and agreed to the published version of the manuscript.

Funding: This research was carried out with the financial assistance of a grant from Heilongjiang Province for the funding of the scientific research project (CZKYF2021-2-C016); Xu Zheng was supported by an experimental condition from the Qiqihar Branch of Heilongjiang Academy of Agricultural Sciences; the Engineering Research Center of Agricultural Microbiology Technology, Ministry of Education & Heilongjiang Provincial Key Laboratory of Ecological Restoration and Resource Utilization for Cold Region & Key Laboratory of Microbiology, College of Heilongjiang Province & School of Life Sciences, Heilongjiang University. Jiaxiang Industral Technology Research Institute of Heilongjiang University.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on reasonable request from the corresponding author.

Acknowledgments: We would like to thank the laboratory of Fuqiang Song and Fengshan Yang of Heilongjiang University for their experimental equipment and technical support.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Zhao, L.; Xu, Y.; Chang, J.; Li, M.; Zhang, Y.; Dang, Y.; Wang, M.; Cheng, Y.; Zhang, B. Screening, resistance and growth-promoting effect of endophytic bacteria with ACC deaminase activity isolated from soybean nodules. *Acta Microbiol. Sin.* **2016**, *56*, 1009–1021.
2. Xu, J.; Sun, T.; Li, S. Application of microbial fertilizers in agricultural production of China. *Crops* **2016**, *1*, 1–6.
3. Grasswitz, T.R. Integrated pest management (IPM) for small-scale farms in developed economies: Challenges and opportunities. *Insects* **2019**, *10*, 179. [CrossRef]
4. Ab Rahman, S.F.S.; Singh, E.; Pieterse, C.M.; Schenk, P.M. Emerging microbial biocontrol strategies for plant pathogens. *Plant Sci.* **2018**, *267*, 102–111. [CrossRef] [PubMed]
5. Ghorbanpour, M.; Omidvari, M.; Abbaszadeh-Dahaji, P.; Omidvar, R.; Kariman, K. Mechanisms underlying the protective effects of beneficial fungi against plant diseases. *Biol. Control Theory Appl. Pest Manag.* **2018**, *117*, 147–157. [CrossRef]
6. Raad, M.; Glare, T.R.; Brochero, H.L.; Müller, C.; Rostás, M. Transcriptional Reprogramming of *Arabidopsis thaliana* Defence Pathways by the Entomopathogen *Beauveria bassiana* Correlates with Resistance against a Fungal Pathogen but Not Against Insects. *Front. Microbiol.* **2019**, *10*, 438959. [CrossRef]
7. Rai, R.; Srinivasamurthy, R.; Dash, P.K.; Gupta, P. Isolation, characterization and evaluation of the biocontrol potential of *Pseudomonas protegens* RS-9 against *Ralstonia solanacearum* in Tomato. *Indian J. Exp. Biol.* **2017**, *55*, 595–603.
8. Abd-Elsalam, K.A.; Murugan, K. (Eds.) Index. In *Bio-Based Nanoemulsions for Agri-Food Applications*; Elsevier: Amsterdam, The Netherlands, 2022; pp. 451–458.

9. Behie, S.W.; Zelisko, P.M.; Bidochka, M.J. Endophytic Insect-Parasitic Fungi Translocate Nitrogen Directly from Insects to Plants. *Science* **2012**, *336*, 1576–1577. [CrossRef]
10. Vega, F.E.; Goettel, M.S.; Blackwell, M.; Chandler, D.; Jackson, M.A.; Keller, S.; Koike, M.; Maniania, N.K.; Monzon, A.; Ownley, B.H.; et al. Fungal entomopathogens: New insights on their ecology. *Fungal Ecol.* **2009**, *2*, 149–159. [CrossRef]
11. Sorokin, N. Plant parasites of man and animals as causes of infectious diseases. *J. Mil. Med.* **1883**, *2*, 268–291.
12. Sasan, R.K.; Bidochka, M.J. The insect-pathogenic fungus *Metarhizium robertsii* (Clavicipitaceae) is also an endophyte that stimulates plant root development. *Am. J. Bot.* **2012**, *99*, 101–107. [CrossRef] [PubMed]
13. Diniz, K.A.; Silva, P.d.A.; Oliveira, J.A.; Emiliorelli Evangelista, J.R. Sweet pepper seed responses to inoculation with microorganisms and coating with micronutrients, aminoacids and plant growth regulators. *Sci. Agric.* **2009**, *66*, 293–297. [CrossRef]
14. Pérez, E.G.; Amaro, M.A.O.; Elihú, B.; Pablo, D.S.; Francisco, J.B.J. The entomopathogenic fungus *Metarhizium anisopliae* enhances *Arabidopsis*, tomato, and maize plant growth. *Plant Physiol. Biochem.* **2022**, *176*, 34–43. [CrossRef] [PubMed]
15. Baron, N.C.; Pollo, A.d.S.; Rigobelo, E.C. *Purpureocillium lilacinum* and *Metarhizium marquandii* as plant growth-promoting fungi. *PeerJ* **2020**, *8*, e9005. [CrossRef]
16. Elena, G.J.; Beatriz, P.J.; Alejandro, P.; Lecuona, R. *Metarhizium anisopliae* (Metschnikoff) Sorokin promotes growth and has endophytic activity in tomato plants. *Adv. Biol. Res.* **2011**, *5*, 22–27.
17. Liao, X.G.; O'Brien, T.R.; Fang, W.G.; St Leger, R.J. The plant beneficial effects of *Metarhizium* species correlate with their association with roots. *Appl. Microbiol. Biotechnol.* **2014**, *98*, 7089–7096. [CrossRef]
18. Bangari, M.P.S.; Nataraja, K.N. Can endophytes minimize photosynthetic limitation? *Trends Plant Sci.* **2023**, *29*, 403–405. [CrossRef]
19. Shagufta; Noor-un-Nisa; Jamali, F.A.; Ahmad, W.; Ul-Allah, S.; Wahocho, N.A.; Jamali, M.F.; Shah, S.A. Comparative Effect of Varieties and Types of Containers on Seed Germination and Seedling Growth of Geranium (*Palergonium graveolens*). *Seeds* **2023**, *2*, 165–176. [CrossRef]
20. Barelli, L.; Moonjely, S.; Behie, S.W.; Bidochka, M.J. Fungi with multifunctional lifestyles: Endophytic insect pathogenic fungi. *Plant Mol. Biol.* **2016**, *90*, 657–664. [CrossRef] [PubMed]
21. Behie, S.W.; Moreira, C.C.; Sementchoukova, I.; Barelli, L.; Zelisko, P.M.; Bidochka, M.J. Carbon translocation from a plant to an insect-pathogenic endophytic fungus. *Nat. Commun.* **2017**, *8*, 14245. [CrossRef]
22. Filipello Marchisio, V.; Curetti, D.; Cassinelli, C.; Bordese, C. Keratinolytic and keratinophilic fungi in the soils of Papua New Guinea. *Mycopathologia* **1991**, *115*, 113–119. [CrossRef] [PubMed]
23. Mongkolsamrit, S.; Khonsanit, A.; Thanakitpipattana, D.; Tasanathai, K.; Luangsa-Ard, J. Revisiting *Metarhizium* and the description of new species from Thailand. *Stud. Mycol.* **2020**, *95*, 171–251. [CrossRef] [PubMed]
24. Bais, H.P.; Vepachedu, R.; Gilroy, S.; Callaway, R.M.; Vivanco, J.M. Allelopathy and exotic plant invasion: From molecules and genes to species interactions. *Science* **2003**, *5638*, 301. [CrossRef] [PubMed]
25. Song, D.; Xi, X.; Huang, S.; Liang, G.; Sun, J.; Zhou, W.; Wang, X. Short-Term Responses of Soil Respiration and C-Cycle Enzyme Activities to Additions of Biochar and Urea in a Calcareous Soil. *PLoS ONE* **2016**, *11*, e0161694. [CrossRef] [PubMed]
26. Li, D.; Hockaday, W.C.; Masiello, C.A.; Alvarez, P.J.J. Earthworm avoidance of biochar can be mitigated by wetting. *Soil Biol. Biochem.* **2011**, *43*, 1732–1737. [CrossRef]
27. Oleszczuk, P.; Jośko, I.; Futa, B.; Pasieczna-Patkowska, S.; Pałys, E.; Kraska, P. Effect of pesticides on microorganisms, enzymatic activity and plant in biochar-amended soil. *Geoderma Int. J. Soil Sci.* **2014**, *214–215*, 10–18. [CrossRef]
28. Xiao-Guang, J.; Chong-Sheng, G.; Guo-Hong, L. Effect of Long-Term Fertilization on Soil Enzyme Activities under Different Hydrothermal Conditions in Northeast China. *Agric. Sci. China* **2011**, *10*, 11.
29. Szewczyk, R.; Sobon, A.; Slaba, M.; Dlugonski, J. Mechanism study of alachlor biodegradation by *Paecilomyces marquandii* with proteomic and metabolomic methods. *J. Hazard. Mater.* **2015**, *291*, 52–64. [CrossRef] [PubMed]
30. Dash, P.K.; Gupta, P.; Panwar, B.S.; Rai, R. Isolation, cloning and characterization of *phl*B gene from an Indian strain of Gram negative soil bacteria *Pseudomonas fluorescens*. *Indian J. Exp. Biol.* **2020**, *58*, 412–419.
31. Wang, L.X.; Chen, M.X.; Lam, P.Y.; Dini-Andreote, F.; Dai, L.; Wei, Z. Multifaceted roles of flavonoids mediating plant-microbe interactions. *Microbiome* **2022**, *10*, 233. [CrossRef]
32. Tatematsu, K.; Kumagai, S.; Muto, H.; Sato, A.; Watahiki, M.K.; Harper, R.M.; Liscum, E.; Yamamoto, K.T. *MASSUGU2* Encodes Aux/IAA19, an Auxin-Regulated Protein That Functions Together with the Transcriptional Activator NPH4/ARF7 to Regulate Differential Growth Responses of Hypocotyl and Formation of Lateral Roots in *Arabidopsis thaliana*. *Plant Cell Online* **2004**, *16*, 379–393. [CrossRef]
33. Oldroyd, G.E.D. Speak, friend, and enter: Signalling systems that promote beneficial symbiotic associations in plants. *Nat. Rev. Microbiol.* **2013**, *11*, 252–263. [CrossRef] [PubMed]
34. Vitale, S.; Di Pietro, A.; Turra, D. Autocrine pheromone signalling regulates community behaviour in the fungal pathogen *Fusarium oxysporum*. *Nat. Microbiol.* **2019**, *4*, 1443–1449. [CrossRef]
35. Ma, Y.; Cao, M.; Shi, X.; Li, Z.; Luo, Y. Function of plant growth-promoting bacteria and its application in sustainable agriculture. *Acta Pedol. Sin.* **2023**, *606*, 1555–1568.
36. Eisenhardt, K.M.; Reuscher, C.M.; Klug, G. PcrX, an sRNA derived from the 3′–UTR of the Rhodobacter sphaeroides puf operon modulates expression of puf genes encoding proteins of the bacterial photosynthetic apparatus. *Mol. Microbiol.* **2018**, *110*, 325–334. [CrossRef] [PubMed]
37. Pitman, M.G.; Läuchli, A. Global impact of salinity and agricultural ecosystems. *Salin. Environ.-Plants-Mol.* **2002**, *3*, 20.

38. Tu, C.M. Effect of Four Experimental Insecticides on Enzyme Activities and Levels of Adenosine Triphosphate in Mineral and Organic Soils. *J. Environ. Sci. Health Part B* **1990**, *25*, 787–800. [CrossRef]
39. Wachendorf, C.; Lampe, C.; Taube, F.; Dittert, K. Nitrous oxide emissions and dynamics of soil nitrogen under 15N-labeled cow urine and dung patches on a sandy grassland soil. *J. Plant Nutr. Soil Sci.* **2008**, *171*, 171–180. [CrossRef]