water

MDPI

Article

Rice Physiological Response with *Bacillus subtilis* and *Saccharomyces cerevisiae* Inoculation into Soil under Reclaimed Water–Fresh Water Combined Irrigation

Hongfei Lu [1,2], Xuebin Qi [1,*], Shafeeq ur Rahman [1,2], Dongmei Qiao [1], Ping Li [1], Yang Han [1] and Zhijuan Zhao [1,3]

[1] Farmland Irrigation Research Institute, Chinese Academy of Agricultural Sciences, Xinxiang 453003, China; luhongfei@caas.cn (H.L.); malikshafeeq1559@gmail.com (S.u.R.); qiaodongmei78@163.com (D.Q.); firilp@163.com (P.L.); 13940585693@163.com (Y.H.); zhaozhijuan7184@163.com (Z.Z.)
[2] Graduate School of Chinese Academy of Agricultural Sciences (GSCAAS), Beijing 100081, China
[3] Water Environment Factor Risk Assessment Laboratory of Agricultural Products Quality and Safety, Ministry of Agriculture, Xinxiang 453002, China
* Correspondence: qxb6301@sina.cn

Abstract: The increasing soil salinity levels under reclaimed water irrigation have a negative effect on plant growth. Greenhouse experiments were conducted in 2018 and 2019 under reclaimed water–fresh water combined irrigation. After transplanting (Day 1), rice was irrigated with clean water (tap water) for 10 days to facilitate rice root colonisation. Subsequently, rice was irrigated with reclaimed water for 50 days (Day 11 to 60), and then irrigated with clean water. *B. subtilis* and *S. cerevisiae* were mixed with clean water (tap water) and irrigated into soil at Day 61. *B. subtilis* (20 billion colony-forming units/g) and *S. cerevisiae* (20 billion colony-forming units/g) were mixed at the following proportions: 5 g and 0 (J1), 3.75 g and 1.25 g (J2), 2.5 g and 2.5 g (J3), 1.25 g and 3.75 g (J4), and 0 and 5 g (J5), respectively; rice treated with reclaimed water (CK) and clean water (J0) with no microorganisms applied were also used. We measured NO_3^--N and NH_4^+-N concentrations and electrical conductivity (EC) in the soil at 0–5, 5–15, and 15–25 cm layers; root activity; and malondialdehyde (MDA), soluble sugar, superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and glutamine synthetase (GS) activity in leaves at Day 71. *B. subtilis* and *S. cerevisiae* combination could promote rice physiological indices, and *B. subtilis* had a greater effect than *S. cerevisiae*. There are obvious differences in the physiological performance and soil N between 2018 and 2019 due to the EC of reclaimed water. Redundancy analysis revealed that soil NO_3^--N and the mass of *B. subtilis* applied were major factors influencing leaf physiological indices. Five grams of *B. subtilis* is recommended to facilitate rice growth after irrigation with reclaimed water. Our research provides a new agronomic measure for the safe utilisation of reclaimed water.

Keywords: *Bacillus subtilis*; *Saccharomyces cerevisiae*; rice; soil nitrogen; physiology

Citation: Lu, H.; Qi, X.; Rahman, S.u.; Qiao, D.; Li, P.; Han, Y.; Zhao, Z. Rice Physiological Response with *Bacillus subtilis* and *Saccharomyces cerevisiae* Inoculation into Soil under Reclaimed Water–Fresh Water Combined Irrigation. *Water* **2021**, *13*, 773. https://doi.org/10.3390/w13060773

Academic Editors: Pei Xu and Yanyan Zhang

Received: 1 February 2021
Accepted: 5 March 2021
Published: 12 March 2021

1. Introduction

In China, the exploitation of reclaimed water in agriculture is in its infancy, and extensive studies on its safe utilisation in agricultural activities, in addition to technological developments, are required. There have been breakthroughs in the fields of drip irrigation [1] and vegetable production [2] and flower growth [3] using reclaimed water, which promotes the growth of microbes involved in soil carbon (C) and nitrogen (N) transformation, and effects numerous changes in soil microbial communities [2], in addition to improving soil microbial biomass [4].

Reclaimed water contains sodium, calcium and hydrogen carbonate ions, in addition to N, P and potassium nutrients, among others, although it also poses soil salinisation risks [5] and could deteriorate soil physical and chemical properties [6]. However,

reclaimed water is not particularly safe, considering potential risks to agriculture, the environment and human health [7]. Furthermore, water reclaimed from municipal treatment plants could have higher levels of electrical conductivity (EC) and higher sodium adsorption ratio when compared to other freshwater resources [8].

Soil microbes improve soil physical and chemical processes and influence soil nutrient concentrations, soil aeration and soil structure. Plant growth-promoting bacteria facilitate plant development by enhancing plant growth or influencing plant metabolism directly through their metabolic activities [9]. *Bacillus* spp. are capable of forming long-lived, stress-tolerant spores and secreting metabolites that stimulate plant growth and prevent pathogen infection. *Bacillus* spp. also secrete exopolysaccharides and siderophores that inhibit the movement of toxic ions, facilitate ionic balance maintenance and water translocation in plant tissues, and inhibit pathogenic microbe growth [10]. *Bacillus* spp. are considered safe bacteria that produce substances that are beneficial for crops and that could be used in the production of industrial compounds [11]. *B. subtilis* GB03 regulates cell expansion and auxin synthesis in *Arabidopsis* [12], and *B. subtilis* FTC01 has been demonstrated to be a rich source of xylanolytic enzymes [13]. Yeast has been reported to be a rich source of phytohormones, vitamins, enzymes, amino acids and minerals [14]. Rhizosphere yeast promotes P nutrition in arbuscular-mycorrhizal maize, which is associated with enhanced specific root length and arbuscular mycorrhizal fungi (AMF) [15]. Soil yeast can promote rice growth [16]. Mohamed (2005) [17] observed that active foliar application of dry yeast improved plant growth and yield, and increased N concentrations in grain.

The positive effect of single microorganism has attracted much attention, but whether the combined application of two microorganisms can play the positive role of both has not been determined. Yeast (*Candida sake*, *Cryptococcus aerius* and *Williopsis californica*) and AMF (*Glomus intraradices* BEG140 and *Glomus mosseae* BEG95) resulted in increased shoot biomass [18] and improved crop resistance to environmental stress while decreasing malondialdehyde (MDA) [19]. A mixture of AM fungus and *B. subtilis* was previously found to increase the yield of geranium [20]. Inoculation of *Chloris guyana* with the *S. cerevisiae* alone did not affect plant growth [21]. It can be inferred that the mixed inoculation of the two microorganisms has the best ratio or might inhibit plant growth.

Rice cultivation requires a lot of water; consequently, the use of reclaimed water for irrigation could minimises shortages in fresh water resources, while reclaimed water contains high salt concentrations, which would increase soil salinity [22] and, in turn, salt stress and physiological disorders in plants [23]. Rice crops exhibit considerable genotypic variations in response to salt stress, with higher sensitivity during early seedling stages and reproductive stages [24]. The use of reclaimed water in rice irrigation would require investigation of appropriate agronomic strategies of alleviating the potential salt stress in the soil. *B. subtilis* facilitates the maintenance of ionic balance and secretes metabolites that stimulate plant growth, while *S. cerevisiae* improves soil nutrient supply. The combination of the two species does not enhance root activity and enzyme activity in the leaves, and there is a certain proportion of the two microorganisms that can improve the yield of rice.

Ammonium N (NH_4^+-N) and nitrate N (NO_3^--N) are the two major types of N fertilisers that are absorbed and utilised by crops. Under salt and drought stress, free radical accumulation in plant cells is too high; superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activities are high; and higher MDA concentrations are produced, which impair plant growth and development. Most of the soluble proteins in plants are enzymes involved in various metabolic processes, and the processes are closely linked to the plant enzymes. Soluble protein concentrations are critical indices that facilitate the understanding of the total metabolism activities of plants. Chlorophyll is the key pigment involved in plant photosynthesis, and chlorophyll concentrations can reflect N supply in the soil. Plants generate a greater concentration of soluble sugar to facilitate salt stress tolerance. In addition, root activity influences soil nutrient concentrations and water absorption by plants, which further influences plant growth. Soluble sugars provide energy and metabolic intermediates for plant growth.

Considering that the difference in EC in reclaimed water could change the effect of microorganisms on rice, pot experiments using rice as test material were conducted in 2018 and 2019, and the EC of reclaimed water is 1411 and 1723 $\mu S \cdot cm^{-1}$, respectively. After 50 days of irrigation with reclaimed water, different proportions of *B. subtilis* and *S. cerevisiae* were inoculated into the soil. The soil N and EC, root activity, enzyme activity, chlorophyll and malondialdehyde (MDA) of rice leaves were investigated. The objectives were (i) to reveal the positive physiological effects of *B. subtilis* and *S. cerevisiae* on rice and (ii) determine the role of soil EC and N in rice growth under reclaimed water–fresh water combined irrigation.

2. Materials and Methods

2.1. Site Descriptions

The experiments were carried out in the greenhouse at the Agricultural Soil and Water Environment Field Scientific Observation and Experiment Station of the Chinese Academy of Agricultural Sciences. The test site is located in Xinxiang City, Henan Province, where the annual average temperature is 14.1 °C, the frost-free period is 210 days, the number total sunshine hours is 2398.8 h, the annual average precipitation is 589 mm, and the annual average evaporation is 2000 mm.

2.2. Experimental Materials

The experimental rice variety was "Wugeng 519". The plastic buckets used for the tests had bottom diameters of 20.5 cm, upper diameters of 25 cm and depths of 28.5 cm. The test soil was a sandy loam, which was obtained from a wheat field near the test station. The saturated moisture content (by mass) of the soil was 38.92%, and the other soil physical properties are listed in Table 1. *B. subtilis* and *S. cerevisiae* were cultured by Shandong Sukehan Bioengineering Co., Ltd. (Weifang, China) at a 20 billion CFU/g concentrations. The reclaimed water, which comes from the sewage treatment plant, was stored in the refrigerator at 4 °C, and the water quality properties are listed in Table 2.

Table 1. Physical indices of soil.

Indices	2018	2019
Available phosphorus/$mg \cdot g^{-1}$	0.12	0.11
Available potassium/$mg \cdot g^{-1}$	0.18	0.21
Organic matter/$mg \cdot g^{-1}$	21.05	20.1
Na^+/$mg \cdot g^{-1}$	0.26	0.21
K^+/$mg \cdot g^{-1}$	0.034	0.044
EC/$\mu S \cdot cm^{-1}$	510	497
pH	8.94	8.56

Table 2. Water quality indices.

Indices	2018		2019	
	Reclaimed Water	Tap Water	Reclaimed Water	Tap Water
NO_3^--N/$mg \cdot L^{-1}$	21.72	11.17	19.94	10.25
NH_4^+-N/$mg \cdot L^{-1}$	11.02	0.826	9.74	0.839
pH	7.68	8.76	8.06	8.52
EC/$\mu S \cdot cm^{-1}$	1411	259	1723	241
K^+/$mg \cdot L^{-1}$	7.92	2.8	5.03	2.5
Na^+/$mg \cdot L^{-1}$	116.95	16.26	135.73	16.01

2.3. Experimental Designs

The experiments were carried out from May 2018 to October 2018 and from May 2019 to October 2019. The experimental design used reclaimed water and fresh water (tap water). The soil moisture was determined by weighing the pots using an electronic scale (20 kg) daily at 8:00 am. Each pot contained 11 kg dry soil in two years, and the urea, potassium

sulphate and potassium dihydrogen phosphate concentrations were 2.5 g, 1.0 g and 3.0 g in each pot, respectively.

The day of transplanting was considered the first day of the rice growth period, marked as S1. In 2018, the seedbed was prepared and soaked on 3 May, seeded on 5 May, loaded on 9 June, soaked on 12 June, transplanted on 14 June (S1, rice growth stage day 1) and harvested on 18 October (S127, rice growth stage day 127). In 2019, the seedbed was prepared and soaked on 17 April, and seeded on 29 April. Thereafter, the soil was loaded on 26 May, the was fertiliser applied, water was added to soak the soil on 31 May and the seedlings were transplanted on 2 June (S1). The plants were harvested on 8 October (S129, rice growth stage day 129).

Five *B. subtilis* and *S. cerevisiae* combination treatments (*wt/wt*) were established (5 g and 0, 3.75 g and 1.25 g, 2.5 g and 2.5 g, 1.25 g and 3.75 g, and 0 and 5 g, and referred to as J1, J2, J3, J4 and J5, respectively) (Table 3).

Table 3. Experimental design of each treatment.

Year	Date	CK	J0	J1	J2	J3	J4	J5
2018	9 June	Loading the bucket with dry soil	Loading the bucket with dry soil					
	12 June	Soaking the soil with tap water	Soaking the soil with tap water					
	14 June (S1)	Transplanting seedlings	Transplanting seedlings					
	S1–S10	Irrigation with tap water (FI)	Irrigation with tap water (FI)					
	S11–S60	Irrigation with reclaimed water (CI)	Irrigation with reclaimed water (CI)					
	13 August (S61)	No microorganisms	Inoculating microorganisms					
	S61–S127	Irrigation with reclaimed water (CI)	Irrigation with tap water (CI)					
	18 October (S127)	Harvest	Harvest					
2019	26 May	Loading the bucket with dry soil	Loading the bucket with dry soil					
	31 May	Soaking the soil with tap water	Soaking the soil with tap water					
	2 June (S1)	Transplanting seedlings	Transplanting seedlings					
	S1–S10	Irrigation with tap water (FI)	Irrigation with tap water (FI)					
	S11–S60	Irrigation with reclaimed water (CI)	Irrigation with reclaimed water (CI)					
	1 August (S61)	No microorganisms	Inoculating microorganisms					
	S61–S129	Irrigation with reclaimed water (CI)	Irrigation with tap water (CI)					
	8 October (S129)	Harvest	Harvest					

S1–S127 (S129), represent rice growth stages 1 to 127 in 2018 (129 in 2019) starting from the day of transplantation to harvest. At 60 days after transplanting, *Bacillus subtilis* (BS) and *Saccharomyces cerevisiae* (SC) were mixed in water proportionally and then irrigated into the soil. FI is flooded irrigation with 0–5 cm water depth; CI is controlled irrigation: no water layer will be established in other growth periods except for the 0–50 mm water layer after transplanting for 10 days; the upper limit of soil water control in the root layer is the saturated water content, and the lower limit is 60–80% of the saturated water content.

After irrigation with reclaimed water for 50 days (S11–S60), *B. subtilis* and *S. cerevisiae* were mixed in tap water (200 mL) and the water used to irrigate the soil. Tap water was used to irrigate J1, J2, J3, J4 and J5 treatments from S61 to harvest. Treatments with no *B. subtilis* and *S. cerevisiae* application in the case of the reclaimed water (control, CK) and tap water (J0) were also established. Each treatment had 3 replicates.

2.4. Test Indexes and Analysis Methods

The chlorophyll and MDA concentrations as well as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), glutamine synthetase (GS) activity, soluble sugar, soluble protein and root activity were measured at S71 in 2018 and 2019. For chlorophyll, 0.2 g of weighted fresh samples of leaves was soaked in 20 mL 95% ethanol until the colour of leaves disappeared completely, the extracting solution was measured at 470 nm, 649 nm and 665 nm. MDA, SOD, POD, CAT and GS activities were determined using a kit manufactured by Nanjing Jiancheng Bioengineering Institute (http://www.njjcbio.com/). Phosphate buffer (PBS, pH = 7.4) was used as homogenising medium, and the reaction liquid was measured with spectrophotometer. For MDA, 0.5 g of weighted fresh samples of leaves was milled with the help of a motor, 2 mL 10% TCA and a small amount of quartz sand were added and ground to homogenate, 3 mL TCA was added and then further ground. The homogenised sample was centrifuged at 12,000 rpm for 10 min. To 2 mL supernatant, 0.67% TBA was added, and then the solution was mixed and boiled for 15 min in a 100 °C water bath. The sample was cooled at room temperature and centrifuged again. The absorption values of samples were measured at 532 nm, 600 nm and 450 nm,

respectively [25]. For SOD, POD, CAT and GS, 0.5 g of fresh samples of leaves was milled with the help of a motor and pestle and standardised in 0.05 mol/l PBS under chilled condition; the standardised mixture was centrifuged at 12,000 rpm for 10 min at 4 °C after sieving through four layers of muslin cloth.

Soluble sugar concentrations were measured using anthrone colorimetry following the method by Jing et al. [26]. Weighted fresh leaves (0.2 g) were cut into pieces and put into a triangular flask, then 20 mL distilled water was added and the triangular flask was sealed with plastic film and extracted in boiling water for 30 min. The samples were cooled, filtered and then the volume was fixed to 100 mL for testing. We then transferred 0.5 mL of the solution to be tested into a 20 mL test tube, added 1.5 mL of distilled water, 0.5 mL of anthrone ethyl acetate 0.5 mL and 5 mL of concentrated sulphuric acid, fully vibrated and immediately put the test tube into boiling water, kept it warm for 1 min, took it out and cooled it to room temperature and then the samples were measured at 630 nm.

Soluble protein concentrations were measured using the Coomassie Brilliant Blue method based on the method by Bradford [27] using the standardised mixture for determination of enzyme activity in leaves. Root activity (Dehydrogenase activity) was measured using 2,3,5-triphenyltetrazolium chloride (TTC) based on the method by Bon, et al. [28]. Weighed fresh root tip samples (0.2 g) were put it into a 25 mL beaker, we then added 5 mL of 0.4% TTC solution and 5 mL of phosphate buffer (pH = 7.0), kept it in the dark at 37 °C for 1–3 h and then added 2 mL of 1 mol/L sulphuric acid. The sample was extracted with ethyl acetate and measured at 485 nm.

Three soil layers (0–5, 5–15 and 15–25 cm) were obtained at S71, the soil electricity conductivity (EC) of dry soil was measured using Lei-ci DDB-303A (INESA Scientific Instrument Co., Ltd., Shanghai, China) and the NO_3^--N and NH_4^+-N concentrations in fresh soil were determined using an Auto Analyzer 3 (Brown rupee company, Hamburg, Germany) [29]. We took the average value of three layers for correlation analysis. At harvest, the plant height was measured by ruler (the length from the soil to the top of panicle). The number of tillers included invalid tillers. All ears in each pot were stored in paper bags and dried at 80 °C for 24 h.

The figures were illustrated using MS Excel 2010 (Microsoft Corp, Redmond, WA, USA), and analysis of variance and correlation between EC, NO_3^--N and NH_4^+-N of soil and physiological indexes of rice (chlorophyll, MDA, soluble protein, soluble sugar, enzyme activity and root activity) was performed using IBM SPSS Statistics 19.0 (IBM Corp., Armonk, NY, USA). Redundancy analysis (RDA) was carried out using CANOCO 5.0 (Microcomputer Power, Ithaca, NY, USA); MDA, SOD, POD, CAT, GS, soluble sugar, soluble protein, chlorophyll concentrations and root activity were used as the species variables; NO_3^--N and NH_4^+-N in 0–5, 5–15, 15–25 cm soil layers, and the mass of *B. subtilis* and *S. cerevisiae* were used as the environment variables.

3. Results

3.1. Soil EC, NO_3^--N and NH_4^+-N

Ten days after the restoration of fresh water irrigation (Table 4), the soil EC (mean value of three layers of soil) of J0–J5 treatment decreased at S71, and J2 had the largest decrease rate, and the difference between J0–J5 and CK was significant in 2019 ($p < 0.05$). In 2018, soil EC of J2 and J4 treatments was also lower than J0, and in 2019, soil EC of J2–J5 was lower than J0, indicating that the application of microbial agents can further reduce soil EC. The reclaimed water irrigation with different EC significantly affected the soil EC of 0–5 cm ($F = 6.690$, $p < 0.05$), 15–25 cm ($F = 22.099$, $p < 0.01$) and the average EC of three layers ($F = 27.848$, $p < 0.01$).

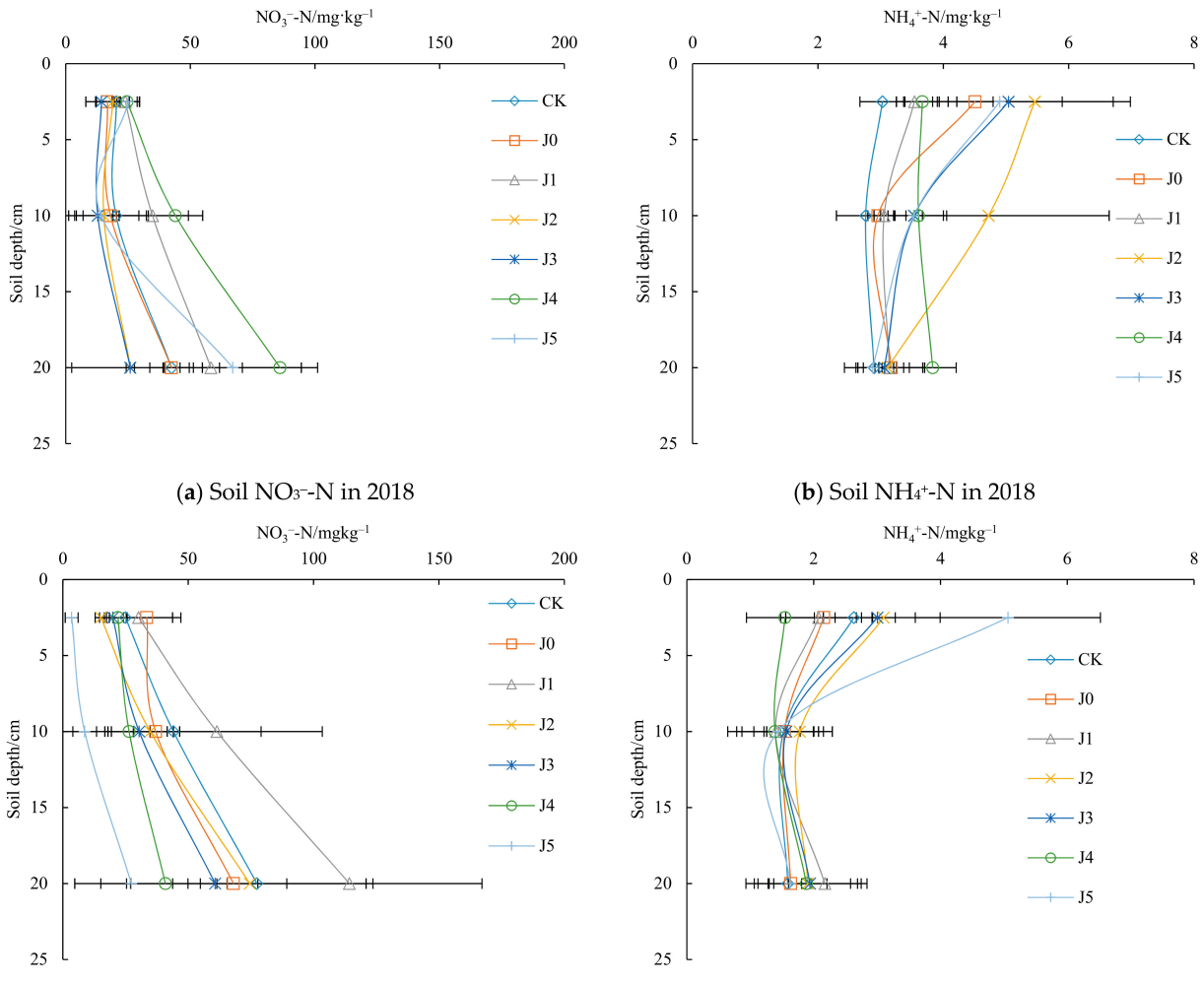
In 2018, the fresh water irrigation treatment (J0) and the *B. subtilis* and *S. cerevisiae* treatments (J1–J5) increased the NO_3^--N (Figure 1a) and NH_4^+-N (Figure 1d) concentrations in different soil layers, excluding NH_4^+–N in the 15–25 cm layer in the J5 treatment). Specifically, in the 0–5 cm soil layer, compared with CK, the NO_3^--N concentrations in the J0, J2 and J3 treatments decreased, while those in the J1, J4 and J5 treatments increased. In

the 5–15 cm soil layer, compared with CK, the NO_3^-–N concentrations in the J1 and J4 treatments increased by 72.50% and 118.04%, respectively, and the J4 treatment had significant differences with the CK, J0, J2, J3 and J5 treatments ($p < 0.05$). In the 15–25 cm layer, compared with CK, the NO_3^--N concentrations in the J4 and J5 treatments increased, and the concentrations in the J4 treatment were significant when compared with the concentrations in the CK ($p < 0.05$).

Table 4. Soil EC at S71 in 2018 and 2019 (μS/cm).

Year	Soil Layer	CK	J0	J1	J2	J3	J4	J5
2018	0–5 cm	808 ± 89 a	554 ± 84 a	572 ± 163 a	517 ± 74 a	684 ± 160 a	619 ± 191 a	573 ± 381 a
	5–15 cm	702 ± 131 ab	703 ± 111 ab	734 ± 178 ab	597 ± 9 ab	690 ± 95 ab	800 ± 312 a	465 ± 165 b
	15–25 cm	928 ± 45 a	786 ± 174 a	861 ± 88 a	709 ± 393 a	892 ± 29 a	987 ± 203 a	852 ± 159 a
2019	0–5 cm	768 ± 99 a	796 ± 112 a	691 ± 93 a	635 ± 263 a	844 ± 1 a	788 ± 76 a	726 ± 141 a
	5–15 cm	840 ± 206 a	904 ± 156 a	876 ± 248 a	813 ± 301 a	707 ± 45 a	604 ± 320 a	755 ± 132 a
	15–25 cm	1533 ± 301 a	1111 ± 206 ab	1382 ± 217 ab	944 ± 288 b	1220 ± 287 ab	929 ± 201 b	1081 ± 20 b

Note In the same year, different lowercase letters indicate significant differences among treatments ($p < 0.05$); Treatments: reclaimed water with no *Bacillus subtilis* and *Saccharomyces cerevisiae* (CK), tap water with no *B. subtilis* and *S. cerevisiae* (J0), tap water with *B. subtilis* 5 g and *S. cerevisiae* 0 g (J1), tap water with *B. subtilis* 3.75 g and *S. cerevisiae* 1.25 g (J2), tap water with *B. subtilis* 2.5 g and *S. cerevisiae* 2.5 g (J3), tap water with *B. subtilis* 1.25 g and *S. cerevisiae* 3.75 g (J4), tap water with *B. subtilis* 0 g and *S. cerevisiae* 5 g (J5). The same as below.



(**a**) Soil NO_3^--N in 2018

(**b**) Soil NH_4^+-N in 2018

(**c**) Soil NO_3^--N in 2019

(**d**) Soil NH_4^+-N in 2019

Figure 1. Soil nitrate nitrogen and ammonium nitrogen concentrations in 2018 and 2019. (**a**) Soil NO_3^--N data from S71 in 2018, (**b**) soil NH_4^+-N data from S71 in 2018, (**c**) soil NO_3^--N data from S71 in 2019 and (**d**) soil NH_4^+-N data from S71 in 2019. Values are the mean ± SD ($n = 3$).

NH_4^+–N increased following *B. subtilis* and *S. cerevisiae* application. In the 0–15 cm soil layer, compared with the CK, the NH_4^+-N concentrations in the J0, J1, J2, J3, J4 and J5 treatments increased. In the 15–25 cm soil layer, the NH_4^+-N concentrations in the J0, J1, J2, J3 and J4 treatments increased by 5.67–9.49%, while the concentrations in the J5 treatment decreased by 0.74%, and there were significant differences between the J4 treatment and the CK, J3 and J5 treatments ($p < 0.05$).

In 2019, the NO_3^--N (Figure 1c) and NH_4^+-N (Figure 1d) concentrations in the soil increased under J0 treatment, while the NO_3^--N concentrations in the J2, J3, J4 and J5 treatments in the soil decreased. In the 0–5 cm layer, the NO_3^--N concentrations in the J0 and J1 treatments increased by 34.14% and 20.97%, respectively, which were also significantly higher than the concentrations in the J2, J3, J4 and J5 treatments ($p < 0.05$). In the 5–15 cm soil layer, the concentrations in the J1 treatment were significantly higher than that in the J5 treatment ($p < 0.05$). In the 15–25 cm soil layer, the NO_3^--N concentrations in the J1 treatment increased by 47.61%, and J0, J2, J3, J4 and J5 decreased by 12.12%, 3.72%, 21.51%, 47.15% and 64.70%, respectively, compared to CK, and the J1 treatment was significantly higher than the J3, J4 and J5 treatments ($p < 0.05$).

Unlike in 2018, the NH_4^+-N concentrations in some of the treatments decreased in 2019. In the 0–5 cm layer, compared with CK, the NH_4^+-N concentrations decreased in the J0, J1 and J4 treatments, while the concentrations in the J2, J3 and J5 treatments increased, and the concentrations in the J5 treatment were the highest, and the differences between the J5 and the CK treatments were significant ($p < 0.05$). In the 5–15 cm soil layer, there were no significant differences between the J0–J5 treatments and CK; in the 15–25 cm layer, the NH_4^+-N concentrations increased by 2.52–37.11%, but the difference was not significant.

3.2. Plant Height, Tiller Number and Yield of Rice

As Table 5 shows, there are differences in plant height, tiller number and yield between 2018 and 2019. In 2018, the J0–J5 treatments increased the plant height of rice, in which the results of J0–J4 were significantly different to CK ($p < 0.05$), the J2 treatment increased by 15.61% and J0–J5 treatments significantly reduced the number of tillers by 19.44–32.78% ($p < 0.05$) and significantly increased the rice yield by 333.65–527.88% ($p < 0.05$), in which J2 treatment increased by 527.88%. In 2019, there was no significant difference in plant height between J0–J5 and CK, but significantly increased the number of tillers by 76.32–128.95% ($p < 0.05$), and the yield of J0 and J1 treatments increased by 251.85% and 311.11% ($p < 0.05$), respectively; there was no significant difference between other treatments and CK. On the whole, the J1 treatment can increase yield.

Table 5. Plant height, tiller number and yield at harvest in 2018 and 2019 ($n = 4$).

Indexes	Year	CK	J0	J1	J2	J3	J4	J5
Plant Height/cm	2018	66.63 ± 2.13 c	75.03 ± 6.5 ab	72.00 ± 4.07 ab	77.03 ± 4.11 a	76.18 ± 3.21 a	75.75 ± 4.5 ab	71.05 ± 6.04 bc
	2019	66.63 ± 4.11 ab	67.68 ± 5.27 ab	69.68 ± 0.74 a	61.75 ± 7.19 b	63.25 ± 3.48 ab	67.05 ± 6.94 ab	64.38 ± 2.53 ab
Tiller number per pot	2018	45.00 ± 2.58 a	34.00 ± 2.45 b	36.25 ± 4.79 b	30.25 ± 3.86 b	35.25 ± 6.02 b	36.00 ± 6.93 b	36.25 ± 6.70 b
	2019	19.00 ± 9.27 c	43.50 ± 2.65 a	38.50 ± 5.74 ab	35.25 ± 10.28 ab	33.50 ± 2.08 b	35.75 ± 4.65 ab	35.75 ± 3.86 ab
Yield/g·pot^{-1}	2018	1.04 ± 0.24 c	4.58 ± 1.26 b	5.18 ± 0.69 ab	6.53 ± 1.42 a	5.32 ± 1.01 ab	5.20 ± 1.41 ab	4.51 ± 1.58 b
	2019	0.54 ± 0.44 cd	1.90 ± 0.11a b	2.22 ± 0.96 a	0.18 ± 0.18 d	0.58 ± 0.23 cd	0.87 ± 0.68 cd	1.28 ± 0.53 bc

Note In the same year, different lowercase letters indicate significant differences among treatments ($p < 0.05$).

3.3. Chlorophyll Concentrations in Rice Leaves

Chlorophyll a (Chl a), chlorophyll b (Chl b), carotenoid and total chlorophyll (Chl a + Chl b) concentrations, in addition to the ratio of chlorophyll a to chlorophyll b (Chl a/b) in rice leaves at S71, are illustrated in Figure 2. In 2018 (Figure 2a), J3–J5 increase chlorophyll concentrations, but the difference in J0–J5 treatments with CK on Chlorophyll concentrations was not significant. In 2019 (Figure 2b), there was significant difference between J1, J2 and J5 treatments with CK on Chl a +b ($p < 0.05$), which increased by 34.59%, 41.44% and 29.11%, respectively.

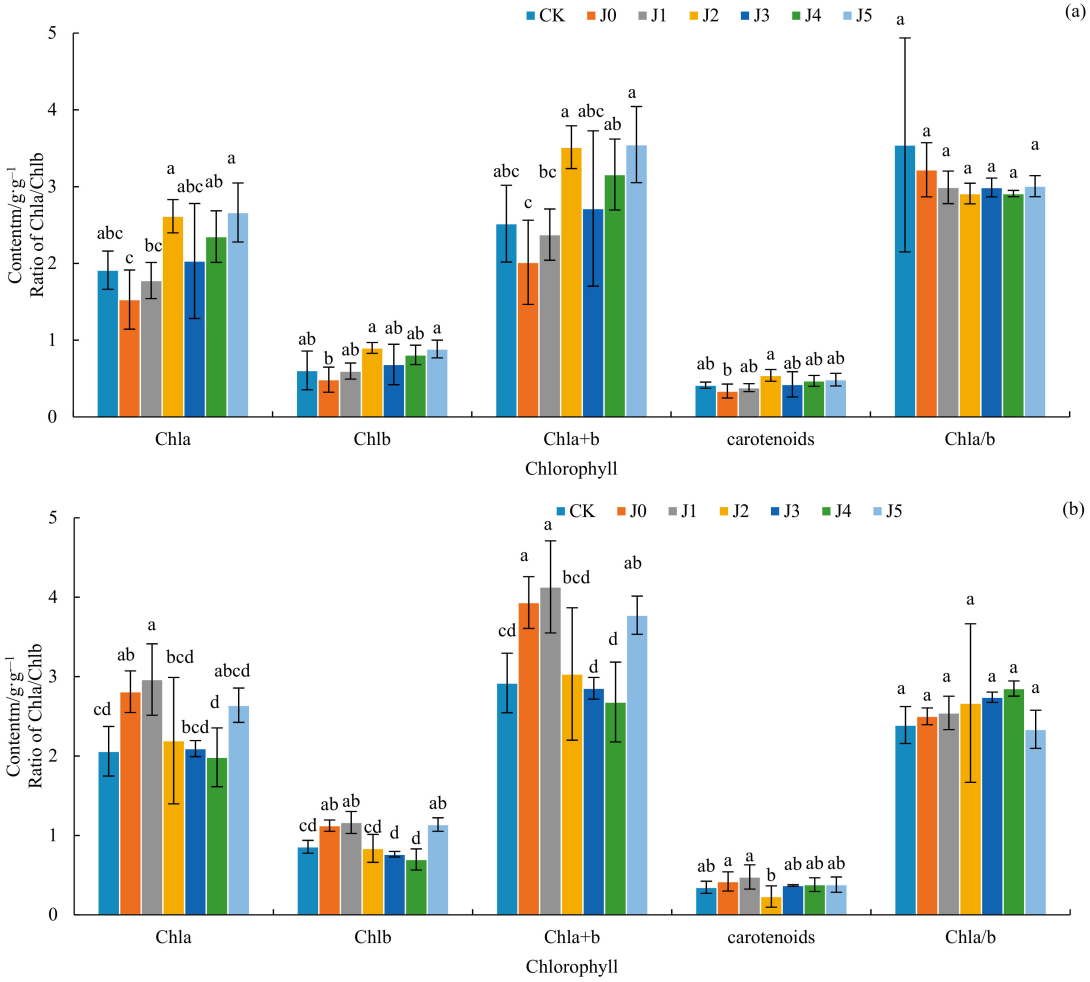


Figure 2. Chlorophyll of the second leaf after application of microorganisms at S71 in2018 (**a**) and 2019 (**b**); different lowercase letters indicate significant differences among treatments ($p < 0.05$).

3.4. Root Activity and Soluble Sugars and MDA Concentrations

In 2018, the root activity (Dehydrogenase) (Figure 3a) in J0, J1, J2, J3, J4 and J5 treatments increased when compared with the root activity in the CK. The differences in root activity between the J4, J4 and J5 treatments and CK were significant ($p < 0.05$), and the root activity in the J4 and J5 treatments was significantly higher than in the J0 treatment ($p < 0.05$). In 2019, the root activity in the J0, J2, J3, J4 and J5 treatments decreased when compared to the activity in the CK, while that in the J1 treatment increased by 13.89% with no significant difference. In addition, root activity in the J1 treatment was significantly higher than in the J3 treatment ($p < 0.05$).

In 2018, compared with CK, the soluble sugar concentrations of J0–J5 treatments had no significant differences with CK (Figure 3b), but the J1 and J5 treatments had significant differences with the J0 treatments ($p < 0.05$). In 2019, the soluble sugar concentrations in the J1 and J2 treatments increased by 131.21% and 112.68% ($p < 0.05$), respectively, and J1 was significantly higher than J0 ($p < 0.05$). The above results showed that the inoculation of *B. subtilis* and *S. cerevisiae* increased the soluble sugar concentrations in leaves.

In 2018, the MDA concentrations in the J0, J2, J3 and J4 treatments decreased when compared with the concentrations in the CK (Figure 3c), and there were significant differences between J4 and CK ($p < 0.05$). In 2019, the MDA concentrations in the J0, J1, J2, J3 and J4 treatments decreased, and the concentrations in the J4 treatment were significantly different from the concentration in the CK, J1 and J5 treatments.

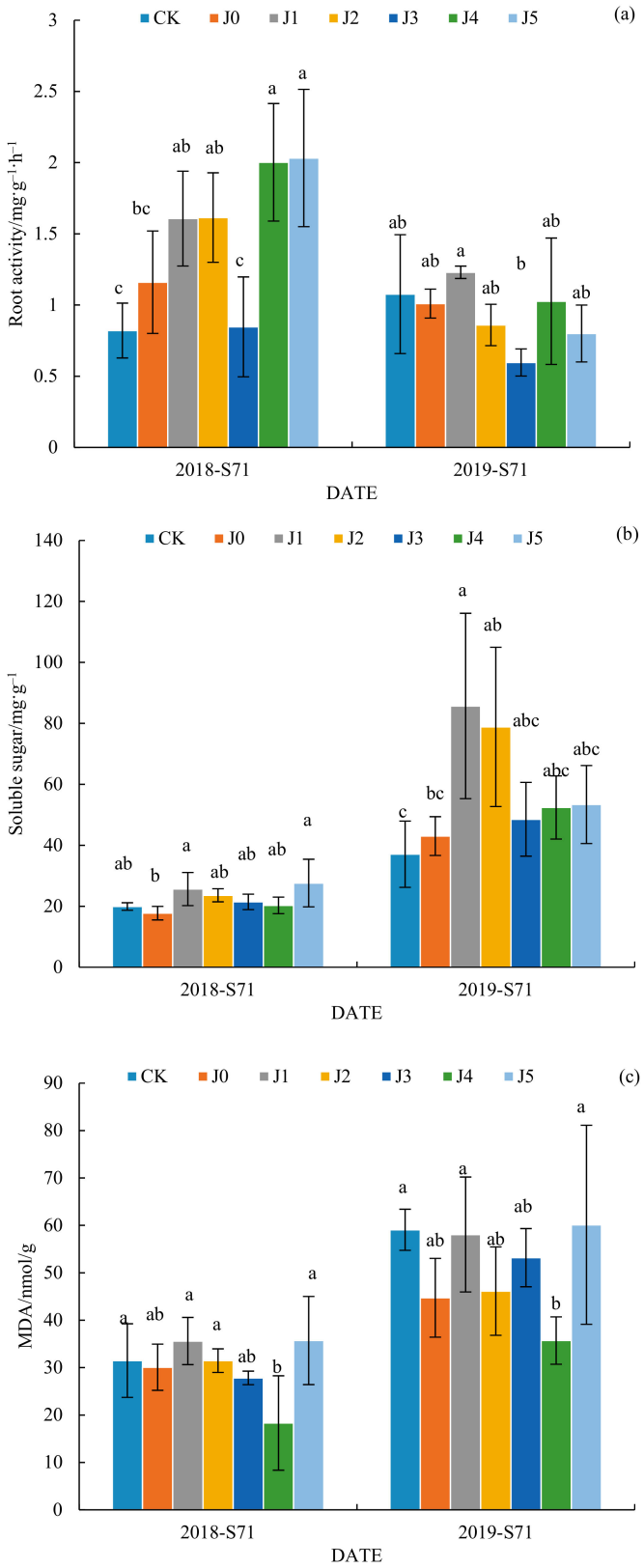


Figure 3. Rice root activity (**a**), soluble sugar (**b**), malondialdehyde (MDA) (**c**) of leaf after the application of microorganisms at S71; different lowercase letters indicate significant differences among treatments ($p < 0.05$).

3.5. Leaf Enzyme Activity and Protein Levels

The enzyme activity and protein quantity in rice leaves in 2018 are listed in Table 6. SOD activity in the J0, J1, J3, J2, J4 and J5 treatments increased when compared with the activity in the CK; the differences between the J2 and J3 treatments and CK were significant ($p < 0.05$); and the concentrations in the J3 treatment were significantly higher than those in the J0, J1, J4 and J5 treatments ($p < 0.05$). Compared with CK, the CAT activities of the J0, J1, J3, J2, J4 and J5 treatments increased, and the differences between the J2 and J3 treatments and the CK were significant ($p < 0.05$). In addition, the activity in the J3 treatment was significantly higher than the activity in the J0, J1, J2, J4 and J5 treatments.

Table 6. Enzyme activity and protein concentrations in the second leaf at S71 in 2018 and 2019.

Index		CK	J0	J1	J2	J3	J4	J5
SOD	2018	40.23 ± 4.56 c	42.61 ± 6.72 bc	47.40 ± 6.40 bc	59.96 ± 15.09 ab	75.40 ± 4.74 a	40.81 ± 3.53 c	46.74 ± 14.96 bc
	2019	51.45 ± 5.24 ab	45.87 ± 2.36 abc	44.74 ± 9.67 abc	47.33 ± 0.98 abc	50.18 ± 3.69 abc	52.22 ± 8.63 a	39.58 ± 6.32 c
CAT	2018	38.83 ± 3.97 c	40.52 ± 4.17 bc	47.75 ± 6.14 bc	55.11 ± 12.99 b	71.25 ± 4.81 a	41.11 ± 2.90 bc	44.13 ± 12.68 bc
	2019	29.52 ± 0.50 a	35.81 ± 1.97 a	19.05 ± 4.52 b	31.70 ± 84.70 a	19.74 ± 4.41 b	15.82 ± 1.07 b	16.95 ± 7.16 b
POD	2018	52.75 ± 8.07 ab	95.46 ± 70.26 a	24.74 ± 14.48 b	43.67 ± 19.39 ab	62.23 ± 38.45 ab	25.07 ± 10.24 b	35.87 ± 21.10 ab
	2019	90.46 ± 5.53 a	96.82 ± 5.06 a	90.89 ± 15.90 a	91.36 ± 7.90 a	100.37 ± 10.33 a	99.32 ± 2.76 a	77.04 ± 6.25 a
GS	2018	2.15 ± 0.29 a	2.25 ± 0.62 a	2.34 ± 0.05 a	1.45 ± 0.88 a	1.96 ± 1.23 a	2.15 ± 0.24 a	1.22 ± 0.57 a
	2019	1.70 ± 0.17 ab	1.76 ± 0.16 a	1.50 ± 0.13 abc	1.70 ± 0.22 ab	1.17 ± 0.10 c	1.12 ± 0.09 c	1.09 ± 0.36 c
Protein	2018	85.15 ± 4.86 a	86.77 ± 11.23 a	79.65 ± 16.77 ab	60.26 ± 12.34 bc	44.54 ± 5.29 c	85.50 ± 8.06 a	75.29 ± 14.70 ab
	2019	30.23 ± 2.01 c	30.32 ± 4.97 c	50.52 ± 16.79 a	37.86 ± 2.01 bc	46.10 ± 2.38 ab	45.63 ± 4.67 ab	42.60 ± 2.61 ab

Note In the same year, different lowercase letters indicate significant differences among treatments ($p < 0.05$).

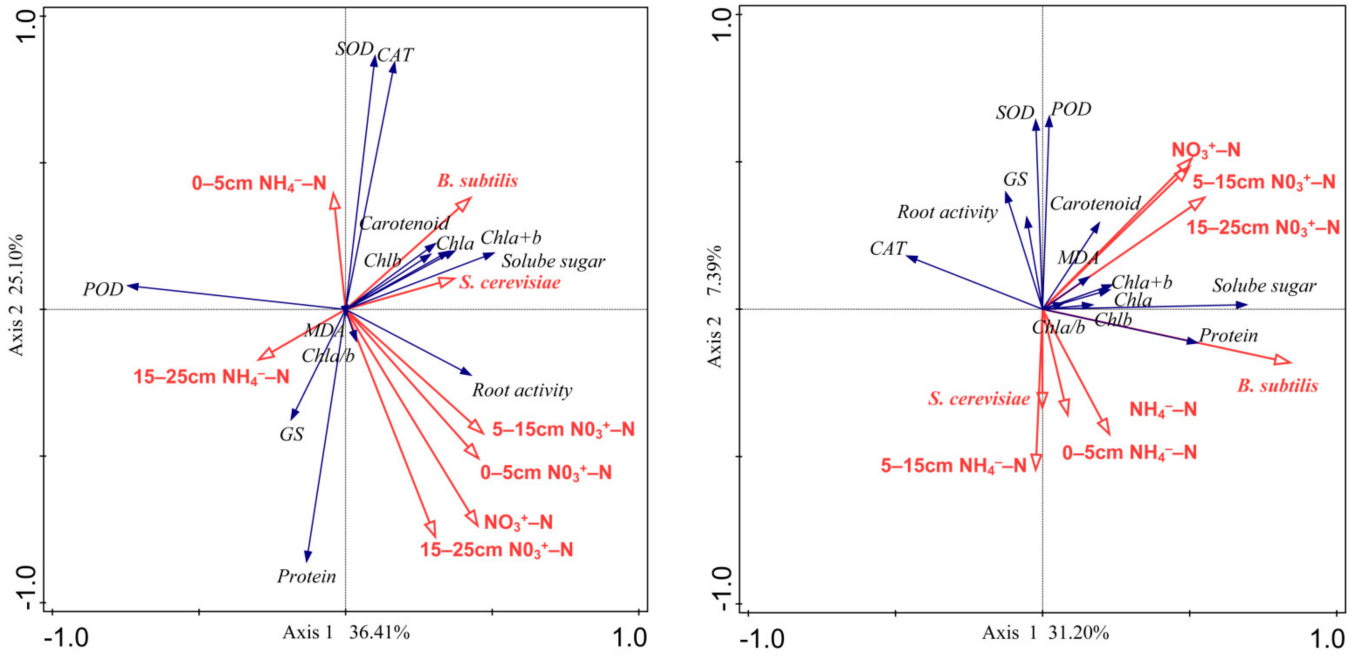
Compared with CK, the POD and GS activity in the J0–J5 treatments had no significant difference with CK. However, the POD activity in the J0 treatment was significantly higher than in the J1 and J4 treatments ($p < 0.05$). The protein concentrations in the J1, J2, J3 and J5 treatments were lower than the concentrations in the CK treatment, and J2 and J3 treatments significantly decreased by 29.23% and 47.69% ($p < 0.05$), respectively.

In 2019, SOD activity of J5 was significantly decreased by 23.07% compared to CK, which was also significantly different from J4. CAT activity in the J0 and J2 treatments increased by 21.31% and 7.66%, respectively, although the differences were not significant, whereas that in the J1, J3, J4 and J5 treatments decreased ($p < 0.05$). The POD activity in the J0–J5 treatments had no significant difference with CK. The GS activity in the J3, J4 and J5 treatments were significantly different from CK. The soluble protein concentrations in the J0, J1, J2, J3, J4 and J5 treatments increased, and the differences between J1, J3, J4 and J5 and CK were significant ($p < 0.05$). Fresh water irrigation facilitated rice physiological recovery, while continuous use of reclaimed water for irrigation caused damage. *B. subtilis* and *S. cerevisiae* improved protein levels in the leaves.

3.6. The Relevance between Physiological Indices, Soil Nitrogen and the Mass of B. subtilis and S. cerevisiae

Redundancy analyses accurately described the effects of soil N and microorganisms on the composition of physiological index (*pseudo-F* = 2.5 and $p = 0.038$ in 2018, *pseudo-F* = 2.5 and $p = 0.038$ in 2019). In 2018 (Figure 4a), at S71, based on the amount of *B. subtilis* and *S. cerevisiae* applied, NO_3^--N was positively correlated with axis 1 and 15–25 cm NH_4^+-N was negatively correlated with axis 1; the application amount of *B. subtilis* and *S. cerevisiae* at 0–5 cm NH_4^+-N was positively correlated with axis 2, while NO_3^--N and 15–25 cm NH_4^+-N were negatively correlated with axis 2. The eigenvalues of the first two axes were 0.3641 and 0.2510, respectively, and the correlation coefficients between physiological indices and environmental factors were 0.7627 and 0.8563, respectively. The first two axes explained 61.51% of the degree variation degree in the physiological indices. The degrees of variation in the physiological indices explained by NO_3^--N, 15–25 cm NH_4^+-N and the amount of *B. subtilis* and *S. cerevisiae* applied were 21.1% ($p = 0.014$), 10.8% ($p = 0.074$), 11.9%

(p = 0.034) and 11.6% (p = 0.03), respectively. Other indexes had little effect on physiological indexes.



(**a**) 2018 (**b**) 2019

Figure 4. Redundancy analyses between leaf physiological indices and environmental factors. Response data are compositional and have a gradient 0.9 *SD* and 0.6 *SD* units long in 2018 and 2019, respectively. (**a**) All Axes: *Pseudo-F* = 2.5, p = 0.038. NO_3^--N: 21.1%, *Pseudo-F* = 5.1, p = 0.014; 15–25 cm NH_4^+-N: 10.8%, *Pseudo-F* = 2.8, p = 0.074; *B. subtilis*: 11.9%, *Pseudo-F* = 3.6, p = 0.034; *S. cerevisiae*: 11.6%, *Pseudo-F* = 4.1, p = 0.03; 5–15 cm NO_3^--N: 3.9%, *Pseudo-F* = 1.4, p = 0.208; 0–5 cm NH_4^+-N: 1.5%, *Pseudo-F* = 0.5, p = 0.512; 15–25 cm NO_3^--N: 1.0%, *Pseudo-F* = 0.4, p = 0.65; 0–5 cm NO_3^--N: 0.5%, *Pseudo-F* = 0.2, p = 0.872. (**b**) All Axes: *Pseudo-F* = 2.5, p = 0.038. *B. subtilis:* 22.8%, *Pseudo-F* = 7.4, p = 0.002; NO_3^--N: 5.3%, *Pseudo-F* =1.8, p = 0.128; *S. cerevisiae*: 6.9%, *Pseudo-F* = 2.4, p = 0.064; 5–15 cm NH_4^+-N: 4.6%, *Pseudo-F* = 1.7, p = 0.156; 0–5 cm NH_4^+-N: 3.6%, *Pseudo-F* = 1.4, p = 0.224; 5–15 cm NO_3^--N: 2.0%, *Pseudo-F* = 0.7, p = 0.55; 15–25 cm NO_3^--N: 1.5%, *Pseudo-F* = 0.5, p = 0.634; NH_4^+-N: 0.5%, *Pseudo-F* = 0.2, p = 0.956.

In 2019, at S71 (Figure 4b), the amount of *B. subtilis* applied, 5–15 cm and 15–25 cm NO_3^--N, soil average NO_3^--N and soil average NH_4^+-N were positively correlated with axis 1; 5–15 cm, 15–25 cm NO_3^-–N and soil average NO_3^-–N were positively correlated with axis 2; the amount of *B. subtilis* applied, soil average NH_4^+-N, 0–5 cm NH_4^+-N, 5–15 cm NH_4^+-N and the amount of *S. cerevisiae* applied were negatively correlated with axis 2. The eigenvalues of the first two axes were 0.3120 and 0.0739, respectively. The correlation coefficients of physiological indices and environmental factors were 0.7457 and 0.7026, respectively, and the first two axes explained 38.59% of the variation. In addition, the applied amount of *B. subtilis*, NO_3^--N, the applied amount of *S. cerevisiae* and 15–25 cm NH_4^+-N explained 22.8% (p = 0.002), 5.3% (p = 0.128), 6.9% (p = 0.064) and 4.6% (p = 0.156) of the variation in the physiological indices, respectively. *B. subtilis* is the most important factor.

Table 7 showed the relationship between soil N, EC (the mean value of the three layers) and physiological indices. NO_3^--N had a positive correlation with root activity in 2018 (r = 0.606) and 2019 (0.394) as well as GS (r = 0.314, 0.467), while NH_4^+-N had a negative correlation with GS and a positive correlation with soluble sugar (r = 0.299, 0.137). Soil EC had a positive correlation with root activity (r = 0.449) in 2019 and a positive correlation with NO_3^--N in 2018 (0.237) and 2019 (0.435).

Table 7. Correlation analysis results of soil N, EC and physiological indices at S71 in 2019.

Year	Soil Indices	EC	Chl a + b	Root Activity	Soluble Sugar	MDA	Protein	POD	CAT	SOD	GS
2018	NO_3^--N	0.237	0.184	0.606 **	0.092	−0.211	0.571 **	−0.394	−0.535 *	−0.603 **	0.314
	NH_4^+-N	−0.1	0.617 **	0.381	0.299	−0.253	−0.382	0.06	0.299	0.339	−0.201
	EC	–	−0.024	−0.011	0.218	−0.389	0.149	−0.177	−0.115	−0.184	0.208
2019	NO_3^--N	0.435 *	0.224	0.394	0.445 *	0.164	−0.048	0.449 *	0.134	0.324	0.467 *
	NH_4^+-N	0.051	0.07	−0.362	0.137	0.215	0.035	−0.393	−0.256	−0.331	−0.336
	EC	–	0.027	0.449 *	−0.097	0.212	−0.334	0.08	0.09	0.035	0.097

Note: * and ** represent that there was a significant correlation at $p < 0.05$ and $p < 0.01$, respectively.

4. Discussion

4.1. The Salt Content of Reclaimed Water and Microorganism Changed the Soil EC

As increasing the amount of reclaimed water will increase soil salinity [30], salts in irrigation water should be controlled to allow sustainable irrigation [31]. In this study, the EC of soil restored to fresh water irrigation was lower than that of reclaimed water irrigation, which is an important basis for the combination of saline and fresh water irrigation [32]; that is, to control the soil salinity within a certain range, because reclaimed water irrigation with different salt content significantly affected soil silt and clay content and pH value [33]. We found that the EC of experimental soil is similar in 2018 and 2019 (Table 1), but the EC of reclaimed water in 2019 is 312 μS/cm higher than that in 2018 (Table 2). Although the irrigation amount in 2019 is lower than that in 2018 (Table S1), at S61, the EC of 5–15 cm soil layer in 2019 is 459 μS/cm higher than that in 2018, but the EC of 0–5 cm and 15–25 cm layers was close, which indicated that the soil salinity increased significantly under the condition of high EC reclaimed water irrigation (in 2019), which directly led to the effect of different years on soil EC exceeding that of microorganism treatments (Table 4). However, with the use of fresh water irrigation at S61–S71, the soil salt gradually moved to the bottom layer (15–25 cm) (Table 4). Although there were differences in the effect of different proportion of microorganisms on reducing soil EC between 2018 and 2019, J2 treatment could significantly reduce soil EC, which further confirmed the positive effect of mixed inoculation of the two microorganisms [34]. Nevertheless, water with an EC above 750 μS/cm might cause yield loss to crops [35,36]. Therefore, when using reclaimed water for irrigation in agricultural production, apart for controlling the irrigation level of reclaimed water [30] and irrigation cycle [37], it is also necessary to pay attention to the salt content of reclaimed water.

4.2. Effect of Microorganisms on Soil NO_3^--N and NH_4^+-N

The documented benefits of the inoculation of plants with beneficial microbes include reduced pathogen infection; improved fertiliser use efficiency; and improved resistance to abiotic stress factors such as drought, mineral deficiency, salinity and phosphate solubilisation [38,39]. Yeast in the soil can promote crop growth [40], mainly because of *p* solubilisation improved *p* uptake [41], and induced indole acetic acid production [42]. *B. subtilis* facilitates the maintenance of ion balance and water movement in plant tissues. N is essential for plant growth, particularly in the early and middle stages, and as long as the root system receives NO_3^--N, aboveground crop growth will not be limited [43]. Currently, our understanding of the effect of mixed application of *B. subtilis* and *S. cerevisiae* on soil nitrogen remains poor.

In the present study, *B. subtilis* and *S. cerevisiae* could improve soil NH_4^+-N concentrations; however, their effects on soil NO_3^--N varied in the two years of study. The addition of *B. subtilis* and *S. cerevisiae* accelerated oxygen (O_2) consumption in the soil and created an increasingly anaerobic environment, which was conducive for denitrification. The soil was alkaline, which was also conducive for N denitrification. As Table S3 shows, air temperature and humidity in 2019 were lower than in 2018; temperature influences N mineralisation and *B. subtilis* and *S. cerevisiae* reproduction [44,45], which can alter soil nitrogen supply. While yeast stimulated the nitrification of added ammonium, the hydrolysis

of urea, and the subsequent nitrification of the released ammonium [46], the reclaimed water improved soil salinity. High salinity concentrations could inhibit nutrient absorption by roots and affect N transformation [47,48]. In addition, the presence of NO_3^--N could promote NH_4^+-N absorption [49]. Although NH_4^+-N in soil is converted easily into NO_3^-–N under irrigation, how *B. subtilis* and *S. cerevisiae* improve NH_4^+-N requires further investigation, particularly regarding the roles of soil nitrifying and denitrifying bacteria.

Most notably, the nitrate nitrogen content in the 15–25 cm soil layer was higher than 0–15 cm, which increased the risk of nitrate nitrogen loss [50] and caused non-point source pollution [51]. Moreover, the higher soil salt content in the 15–25 cm layer inhibited the absorption of nitrogen by roots. Therefore, it is necessary to reduce the nitrate nitrogen migration to the lower layer through cutting down the amount of irrigation or fertilisation, which is in favour of making full use of the nitrogen provided by reclaimed water [52].

4.3. The Effect of Soil Salinity and Nitrate Nitrogen on Physiological Indexes

Wanas (2002) [53] reported that yeast enhanced the formation of chlorophyll and delayed its degradation and senescence in bean plants. In 2018 and 2019, chlorophyll (Chl a + b) and carotenoid concentrations were enhanced by application of *B. subtilis* and *S. cerevisiae*; one of the reasons for this is that *B. subtilis* and *S. cerevisiae* could increase soil nutrient supply, and mineral nutrition increases chlorophyll concentrations [54]. Upregulation of nitrate and ammonium uptake genes together with the N assimilation genes increased N content and relative nitrogen use efficiency, and then increased the chlorophyll content [55]. Furthermore, a combination of bryophytes and soil yeast could also increase chlorophyll concentrations significantly [56], and the decrease in soil EC (Table 4) is beneficial to the increase of chlorophyll content [57].

Yeast facilitates root colonisation of rice seedlings [16], and the change in soil nutrients after inoculation could promote root growth. In 2018, the application of *B. subtilis* and *S. cerevisiae* increased root activity, which was the opposite in 2019 excluding in the J2 treatment. A potential reason for this is that long-term salt stress decreased root activity [58,59]. The EC of reclaimed water in 2019 increased by 312 μS/cm compared to that in 2018 (Table 2), which meant the soil EC of all treatments in 2019 increased by 25.97% compared to that in 2018, and 5–15 cm soil EC at S61 in 2019 increased markedly when compared to that in 2018 (Table S2). The root activity is closely related to the utilisation of nitrogen in rice [60], the decrease in root activity reduced the absorption of nitrogen by plants, as nitrate uptake depends on internal factors related to N demand of the plant, rather than on nitrate availability in the soil volume [61], which explains the reason why soil NO_3^--N of the treatments in 2019 is higher than that in 2018 (Figure 1). Moreover, Khelil et al. [62] also found that greater N rates from RW did not improve total N plant uptake. Besides, the soluble sugar content of leaves in 2019 was higher than that in 2018, which was also the effect of soil salinity [63].

Rice planting in saline soil significantly increased MDA [24]. Our results show that inoculating microorganisms can reduce MDA in leaves. Inoculation of *Aspergillus aculeatus* reduced O_2 accumulation and MDA concentrations in rice roots, and increased antioxidant enzyme activity (SOD, POD and CAT) [64]. In another study, inoculation with yeast (*Macrophomina phaseolina*) decreased MDA concentrations in sunflower plants [19]. GS activity is an indicator of NH_4^+-N tolerance capacity of root systems [65]; we also observed that 15–25 cm NH_4^+-N was positively correlated with GS in 2018, and GS activity was correlated negatively with 0–5 cm NH_4^+-N in 2019.

CAT and SOD can control the potential damage caused by excess reactive oxygen species under environmental stress [66] and maintain normal crop growth. Compared to CK, the mean value of SOD, CAT and protein concentrations of J1–J5 treatments decreased in 2018 and increased in 2019 (Table 6), which is mainly because soil salinity in 2019 is higher than that in 2018, and salt stress could increase protein level in the leaves [67] and cause physiological disorder of rice [68,69]. Despite the fact that yeast provides hormones, vitamins, enzymes, amino acids and minerals for plants [14], and *B. subtilis* could secrete

plant hormones or metabolites with plant hormone activity [70], based on our results, the positive effect of microorganisms was weakened by higher soil salinity, as the plant height in 2018 was higher than that in 2019 (Table 5). The decrease in air temperature and the increase in nitrogen application could increase soluble protein in leaves [71,72], which are also the reasons why the soluble protein of leaves in 2019 is lower than that in 2018, as the temperature in 2019 is lower than that in 2018 (Table S3), and the NH_4^+-N in 0–25 cm soil layer is lower than that in 2018 (Figure 1b,d). In addition, sowing time also had significant effect on rice growth [73], the date of sowing and transplanting in 2019 is earlier than that in 2018 (Table 3), which could decrease rice yield (Table 5). Specifically, the effect of J1 treatment on rice was relatively stable, redundancy analyses results also confirmed that *B. subtilis* had greater influence than *S. cerevisiae* (Figure 4) and J1 treatment was beneficial to increase the yield (Table 5), mainly because *B. subtilis* has strong adaptability to extreme conditions and enhances the host's resistance to plant pathogens [74]. Further, the amount of Na^+ and Cl^- in leaves increased with the increase of salt content in reclaimed water [50], and the photosynthesis of leaves was inhibited [75], which is not conducive to dry matter accumulation. Furthermore, considering the potential influence of soil temperature and moisture content, inoculated bacteria may not have a positive role, similar to the differences in NO_3^--N in 2018 and 2019. Based on the characteristics of *B. subtilis* and *S. cerevisiae*, changes in soil *p* and soil enzyme activity should also be investigated to better understand the changes in plant physiology.

5. Conclusions

In the present study, we investigated NO_3^--N and NH_4^+-N concentrations in the soil 10 days after the inoculation of *B. subtilis* and *S. cerevisiae* under reclaimed water–fresh water combined irrigation, and experiments were carried out in 2018 and 2019 using reclaimed water with different EC. The inoculation of *B. subtilis* and *S. cerevisiae* increased the amount of soil NH_4^+-N and leaf chlorophyll. Both single and mixed application of *B. subtilis* and *S. cerevisiae* increased soluble sugar concentrations in leaves, but mixed application could reduce MDA concentrations and GS activity in leaves. Reclaimed water with high EC (2019) increased soil EC compared to reclaimed water with low EC (2018), which led to more severe stress on rice and weakened the ability of microorganisms to increase the physiological activity of rice, resulting in a decrease in plant height and yield. NO_3^--N influenced the physiological indices the most, especially rice root activity and leaf GS, while *B. subtilis* contributed more to the changes in physiological indices than *S. cerevisiae*. The combination of *B. subtilis* and *S. cerevisiae* has unstable influence on the growth of rice, which is easily affected by soil EC and air temperature under pot experiment, and the application of 5 g *B. subtilis* was recommended after irrigation with reclaimed water.

Supplementary Materials: The following are available online at https://www.mdpi.com/2073-4441/13/6/773/s1, Table S1: Irrigation amount at S1–S71; Table S2: Soil electrical conductivity (EC) at S61; Table S3: Air temperature and humidity at S1–S61.

Author Contributions: Conceptualisation, X.Q.; methodology, H.L.; software, H.L.; formal analysis, H.L.; investigation, Y.H.; resources, Z.Z.; data curation, Y.H.; writing—original draft preparation, H.L.; writing—review and editing, S.u.R.; supervision, X.Q.; project administration, D.Q. and P.L.; funding acquisition, X.Q. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Program of the National Natural Science Foundation of China (51679241, 51879268), the Key Research and Development and Promotion Projects of Henan Province (212400410790), and the Natural Science Foundation of Henan Province (212300410139).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: We greatly thank all anonymous reviewers for their constructive comments, which improved the manuscript.

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

References

1. Song, P.; Li, Y.; Zhou, B.; Zhou, C.; Zhang, Z.; Li, J. Controlling mechanism of chlorination on emitter bio-clogging for drip irrigation using reclaimed water. *Agric. Water Manag.* **2017**, *184*, 36–45. [CrossRef]
2. Kumar, U.; Shahid, M.; Tripathi, R.; Mohanty, S.; Kumar, A.; Bhattacharyya, P.; Lal, B.; Gautam, P.; Raja, R.; Panda, B.B.; et al. Variation of functional diversity of soil microbial community in sub-humid tropical rice-rice cropping system under long-term organic and inorganic fertilization. *Ecol. Indic.* **2017**, *73*, 536–543. [CrossRef]
3. Yang, W.; Lin, K.; Wu, C.; Chang, Y.; Chang, Y. Effects of waterlogging with different water resources on plant growth and tolerance capacity of four herbaceous flowers in a bioretention basin. *Water* **2020**, *12*, 1619. [CrossRef]
4. Bastida, F.; Torres, I.F.; Abadía, J.; Romero-Trigueros, C.; Ruiz-Navarro, A.; Alarcón, J.J.; García, C.; Nicolás, E. Comparing the impacts of drip irrigation by freshwater and reclaimed wastewater on the soil microbial community of two citrus species. *Agric. Water Manag.* **2018**, *203*, 53–62. [CrossRef]
5. Lyu, S.; Chen, W.; Wen, X.; Chang, A.C. Integration of hydrus-1d and modflow for evaluating the dynamics of salts and nitrogen in groundwater under long-term reclaimed water irrigation. *Irrig. Sci.* **2018**, *37*, 35–47. [CrossRef]
6. Erel, R.; Eppel, A.; Yermiyahu, U.; Ben-Gal, A.; Levy, G.; Zipori, I.; Schaumann, G.E.; Mayer, O.; Dag, A. Long-term irrigation with reclaimed wastewater: Implications on nutrient management, soil chemistry and olive (*Olea europaea* L.) performance. *Agric. Water Manag.* **2019**, *213*, 324–335. [CrossRef]
7. Deng, S.; Yan, X.; Zhu, Q.; Liao, C. The utilization of reclaimed water: Possible risks arising from waterborne contaminants. *Environ. Pollut.* **2019**, *254*, 113020. [CrossRef]
8. Tunc, T.; Sahin, U. The changes in the physical and hydraulic properties of a loamy soil under irrigation with simpler-reclaimed wastewaters. *Agric. Water Manag.* **2015**, *158*, 213–224. [CrossRef]
9. Perez-Montano, F.; Alias-Villegas, C.; Bellogin, R.A.; del Cerro, P.; Espuny, M.R.; Jimenez-Guerrero, I.; Lopez-Baena, F.J.; Ollero, F.J.; Cubo, T. Plant growth promotion in cereal and leguminous agricultural important plants: From microorganism capacities to crop production. *Microbiol. Res.* **2014**, *169*, 325–336. [CrossRef]
10. Radhakrishnan, R.; Hashem, A.; Abd Allah, E.F. Bacillus: A biological tool for crop improvement through bio-molecular changes in adverse environments. *Front. Physiol.* **2017**, *8*, 667. [CrossRef]
11. Stein, T. *Bacillus subtilis* antibiotics: Structures, syntheses and specific functions. *Mol. Microbiol.* **2005**, *56*, 845–857. [CrossRef]
12. Zhang, H.; Kim, M.S.; Krishnamachari, V.; Payton, P.; Sun, Y.; Grimson, M.; Farag, M.A.; Ryu, C.M.; Allen, R.; Melo, I.S.; et al. Rhizobacterial volatile emissions regulate auxin homeostasis and cell expansion in arabidopsis. *Planta* **2007**, *226*, 839–851. [CrossRef]
13. Reque, P.M.; Pinilla, C.M.B.; Gauterio, G.V.; Kalil, S.J.; Brandelli, A. Xylooligosaccharides production from wheat middlings bioprocessed with *Bacillus subtilis*. *Food Res. Int.* **2019**, *126*, 108673. [CrossRef]
14. Barnett, J.A.; Payne, R.W.; Yarrow, D. *Yeasts Characteristics Andidentification*; Cambridge University Press: London, UK, 1990.
15. Sarabia, M.; Jakobsen, I.; Grønlund, M.; Carreon-Abud, Y.; Larsen, J. Rhizosphere yeasts improve P uptake of a maize arbuscular mycorrhizal association. *Appl. Soil Ecol.* **2018**, *125*, 18–25. [CrossRef]
16. Amprayn, K.-O.; Rose, M.T.; Kecskés, M.; Pereg, L.; Nguyen, H.T.; Kennedy, I.R. Plant growth promoting characteristics of soil yeast (*Candida tropicalis* HY) and its effectiveness for promoting rice growth. *Appl. Soil Ecol.* **2012**, *61*, 295–299. [CrossRef]
17. Mohamed, S.E. Photochemical studies on common bean (*Phaseolus vulgaris* L.) plants as affected by foliar fertilizer and active dry yeast under sandy soil conditions. *Egypt. J. Appl. Sci.* **2005**, *20*, 539–559.
18. Gollner, M.J.; Püschel, D.; Rydlová, J.; Vosátka, M. Effect of inoculation with soil yeasts on mycorrhizal symbiosis of maize. *Pedobiologia* **2006**, *50*, 341–345. [CrossRef]
19. Nafady, N.A.; Hashem, M.; Hassan, E.A.; Ahmed, H.A.M.; Alamri, S.A. The combined effect of arbuscular mycorrhizae and plant-growth-promoting yeast improves sunflower defense against macrophomina phaseolina diseases. *Biol. Control* **2019**, *138*, 104049. [CrossRef]
20. Alam, M.; Khaliq, A.; Sattar, A.; Shukla, R.; Anwar, M.; Dharni, S. Synergistic effect of arbuscular mycorrhizal fungi and *Bacillus subtilis* on the biomass and essential oil yield of rose-scented geranium (*Pelargonium graveolens*). *Arch. Agron. Soil Sci.* **2011**, *57*, 889–898. [CrossRef]
21. Bhowmik, S.N.; Singh, C.S. Mass multiplication of am inoculum: Effect of plant growth-promoting rhizobacteria and yeast in rapid culturing of *Glomus mosseae*. *Curr. Sci.* **2004**, *86*, 705–709.
22. Zalacáin, D.; Martínez-Pérez, S.; Bienes, R.; García-Díaz, A.; Sastre-Merlín, A. Salt accumulation in soils and plants under reclaimed water irrigation in urban parks of Madrid (Spain). *Agric. Water Manag.* **2019**, *213*, 468–476. [CrossRef]
23. Frukh, A.; Siddiqi, T.O.; Khan, M.I.R.; Ahmad, A. Modulation in growth, biochemical attributes and proteome profile of rice cultivars under salt stress. *Plant Physiol. Biochem.* **2020**, *146*, 55–70. [CrossRef]
24. Gerona, M.E.B.; Deocampo, M.P.; Egdane, J.A.; Ismail, A.M.; Dionisio-Sese, M.L. Physiological responses of contrasting rice genotypes to salt stress at reproductive stage. *Rice Sci.* **2019**, *26*, 207–219. [CrossRef]
25. Ur Rahman, S.; Xuebin, Q.; Zhao, Z.; Du, Z.; Imtiaz, M.; Mehmood, F.; Hongfei, L.; Hussain, B.; Ashraf, M.N. Alleviatory effects of silicon on the morphology, physiology, and antioxidative mechanisms of wheat (*Triticum aestivum* L.) roots under cadmium stress in acidic nutrient solutions. *Sci. Rep.* **2021**, *11*, 1958. [CrossRef]

26. Feng, J.; Dong, P.; Li, R.; Li, C.; Xie, X.; Shi, Q. Effects of wood fiber properties on mold resistance of wood polypropylene composites. *Int. Biodeterior. Biodegrad.* **2019**, *140*, 152–159. [CrossRef]
27. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254. [CrossRef]
28. Bon, I.C.; Salvatierra, L.M.; Lario, L.D.; Morató, J.; Pérez, L.M. Prospects in cadmium-contaminated water management using free-living cyanobacteria (*Oscillatoria* sp.). *Water* **2021**, *13*, 542. [CrossRef]
29. Bai, F.; Qi, X.; Li, P.; Qiao, D.; Wang, J.; Du, Z.; She, Y.; Guo, W.; Lu, H. Depression of groundwater table and reduced nitrogen application jointly regulate the bacterial composition of nirS-type and nirK-type genes in agricultural soil. *Water* **2020**, *12*, 3459. [CrossRef]
30. Han, Y.; Qiao, D.; Qi, X.; Li, P.; Guo, W.; Cui, B.; Lu, H.; Zhao, Y.; Bai, F.; Pang, Y. Effects of reclaimed water irrigation levels on soil salinity and composition of soil bacteria community. *Trans. Chin. Soc. Agric. Eng.* **2020**, *36*, 106–117. [CrossRef]
31. Cornel, P.; Müller, K. Salt content of reclaimed water from sanitation facilities in informal settlements and management options for sustainable agricultural irrigation. *Water Pract. Technol.* **2015**, *10*, 99–109. [CrossRef]
32. Soothar, R.K.; Zhang, W.Y.; Zhang, Y.Q.; Tankari, M.; Mirjat, U.; Wang, Y.S. Evaluating the performance of saltmed model under alternate irrigation using saline and fresh water strategies to winter wheat in the north china plain. *Environ. Sci. Pollut. Res.* **2019**, *26*, 34499–34509. [CrossRef] [PubMed]
33. Hu, C.; Wang, H.; Wu, Y.; Lu, J.; Li, Y. Migration characteristics and its differences of reclaimed water salinity in different subtropical soils. *Trans. Chin. Soc. Agric. Eng.* **2018**, *34*, 99–107. [CrossRef]
34. Etesami, H.; Alikhani, H.A. Co-inoculation with endophytic and rhizosphere bacteria allows reduced application rates of N-fertilizer for rice plant. *Rhizosphere* **2016**, *2*, 5–12. [CrossRef]
35. Richards, L.A. *Diagnosis and Improvement of Saline and Alkali Soils. U.S.D.A. Agriculture Handbook No. 60*; USDA: Washington, DC, USA, 1954.
36. Ayers, R.S.; Westcot, D.W.; Agl. Water quality for agriculture. *FAO Irrig. Drain. Pap.* **1976**, *29*, 628–630. [CrossRef]
37. Lu, S.B.; Zhang, X.L.; Liang, P. Influence of drip irrigation by reclaimed water on the dynamic change of the nitrogen element in soil and tomato yield and quality. *J. Clean. Prod.* **2016**, *139*, 561–566. [CrossRef]
38. Falih, A.M.; Wainwright, M. Nitrification, s-oxidation and p-solubilization by the soil yeast *Williopsis californica* and by *Saccharomyces cerevisiae*. *Mycol. Res.* **1995**, *99*, 200–204. [CrossRef]
39. Mirabal Alonso, L.; Kleiner, D.; Ortega, E. Spores of the mycorrhizal fungus *Glomus mosseae* host yeasts that solubilize phosphate and accumulate polyphosphates. *Mycorrhiza* **2008**, *18*, 197–204. [CrossRef]
40. Sarabia, M.; Cornejo, P.; Azcón, R.; Carreón-Abud, Y.; Larsen, J. Mineral phosphorus fertilization modulates interactions between maize, rhizosphere yeasts and arbuscular mycorrhizal fungi. *Rhizosphere* **2017**, *4*, 89–93. [CrossRef]
41. Nakayan, P.; Hameed, A.; Singh, S.; Young, L.-S.; Hung, M.-H.; Young, C.-C. Phosphate-solubilizing soil yeast *Meyerozyma guilliermondii* cc1 improves maize (*Zea mays* L.) productivity and minimizes requisite chemical fertilization. *Plant Soil* **2013**, *373*, 301–315. [CrossRef]
42. Nassar, A.H.; El-Tarabily, K.A.; Sivasithamparam, K. Promotion of plant growth by an auxin-producing isolate of the yeast williopsis saturnus endophytic in maize (*Zea mays* L.) roots. *Biol. Fertil. Soils* **2005**, *42*, 97–108. [CrossRef]
43. Walch-Liu, P.; Neumann, G.; Engels, C. Response of shoot and root growth to supply of different nitrogen forms is not related to carbohydrate and nitrogen status of tobacco plants. *J. Plant Nutr. Soil Sci.* **2001**, *164*, 97–103. [CrossRef]
44. Zak, D.; Holmes, W.; MacDonald, N.; Pregitzer, K. Soil temperature, matric potential, and the kinetics of microbial respiration and nitrogen mineralization. *Soil Sci. Soc. Am. J.* **1999**, 63. [CrossRef]
45. Salehi, E.; Nasab, S.B.; Mohammadi, A.S. The effect of combination of saline water and fresh water on physical and chemical properties of soil with silt loam texture. *Researcher* **2015**, *7*, 9–16. [CrossRef]
46. Wainwright, M.; Falih, A.M.K. Involvement of yeasts in urea hydrolysis and nitrification in soil amended with a natural source of sucrose. *Mycol. Res.* **1996**, *100*, 307–310. [CrossRef]
47. Daliakopoulos, I.N.; Apostolakis, A.; Wagner, K.; Deligianni, A.; Koutskoudis, D.; Stamatakis, A.; Tsanisa, I.K. Effectiveness of *Trichoderma harzianum* in soil and yield conservation of tomato crops under saline irrigation. *Catena* **2019**, *175*, 144–153. [CrossRef]
48. Arsova, B.; Foster, K.J.; Shelden, M.C.; Bramley, H.; Watt, M. Dynamics in plant roots and shoots minimize stress, save energy and maintain water and nutrient uptake. *New Phytol.* **2020**, *225*, 1111–1119. [CrossRef] [PubMed]
49. Kronzucker, H.J.; Glass, A.D.M.; Siddiqi, M.Y.; Kirk, G.J.D. Comparative kinetic analysis of ammonium and nitrate acquisition by tropical lowland rice: Implications for rice cultivation and yield potential. *New Phytol.* **2000**, 471–476. [CrossRef]
50. Romero-Trigueros, C.; Nortes, P.; Alarcón, J.J.; Nicolás, E. Determination of ^{15}N stable isotope natural abundances for assessing the use of saline reclaimed water in grapefruit. *Environ. Eng. Manag. J.* **2014**, *13*, 2525–2530. [CrossRef]
51. Anane, M.; Selmi, Y.; Limam, A.; Jedidi, N.; Jellali, S. Does irrigation with reclaimed water significantly pollute shallow aquifer with nitrate and salinity? An assay in a perurban area in north Tunisia. *Environ. Monit. Assess.* **2014**, *186*, 4367–4390. [CrossRef] [PubMed]
52. Guo, W.; Qi, X.; Xiao, Y.; Li, P.; Andersen, M.; Zhang, Y.; Zhao, Z. Effects of reclaimed water irrigation on microbial diversity and composition of soil with reducing nitrogen fertilization. *Water* **2018**, *10*, 365. [CrossRef]
53. Wanas, A.L. Response of faba bean (*Vicia faba*, L.) plants to seed soaking application with natural yeast and carrot extracts. *Ann. Agric. Sci.* **2002**, *40*, 83–102.

54. Feng, G.; Zhang, F.S.; Li, X.L.; Tian, C.Y.; Tang, C.; Rengel, Z. Improved tolerance of maize plants to salt stress by arbuscular mycorrhiza is related to higher accumulation of soluble sugars in roots. *Mycorrhiza* **2002**, *12*, 185–190. [CrossRef]
55. Saha, C.; Mukherjee, G.; Banka, P.; Seal, A. A consortium of non-rhizobial endophytic microbes from typha angustifolia functions as probiotic in rice and improves nitrogen metabolism. *Plant Biol.* **2016**, 18. [CrossRef]
56. Boby, V.U.; Balakrishna, A.N.; Bagyaraj, D.J. Interaction between *Glomus mosseae* and soil yeasts on growth and nutrition of cowpea. *Microbiol. Res.* **2008**, *163*, 693–700. [CrossRef] [PubMed]
57. Khaliq, A.; Zia Ul Haq, M.; Aslam, F.; Matloob, A.; Hussain, S. Salinity tolerance in wheat cultivars is related to enhanced activities of enzymatic antioxidants and reduced lipid peroxidation. *Clean Soil Air Water* **2015**, *43*, 1248–1258. [CrossRef]
58. Gu, J.; Hu, B.; Jia, Y.; Sha, H.; Li, J.; Ma, C.; Zhao, H. Effects of salt stress on root related traits and yield of rice. *Crops* **2019**, 176–182. [CrossRef]
59. Negrao, S.; Courtois, B.; Ahmadi, N.; Abreu, I.; Saibo, N.; Oliveira, M. Recent updates on salinity stress in rice: From physiological to molecular responses. *Crit. Rev. Plant Sci.* **2011**, *30*, 329–377. [CrossRef]
60. Cai, K.; Luo, S.; Duan, S. The response of the rice root system to nitrogen conditions underroot confinement. *Acta Ecol. Sin.* **2003**, *23*, 1109–1116.
61. Cerezo, M.; Camanes, G.; Flors, V.; Primo-Millo, E.; Garcia-Agustin, P. Regulation of nitrate transport in citrus rootstocks depending on nitrogen availability. *Plant Signal. Behav.* **2007**, *2*, 337–342. [CrossRef] [PubMed]
62. Khelil, M.; Rejeb, S.; Hanchi, B.; Destain, J. Effects of irrigation water quality and nitrogen rate on the recovery of ^{15}N fertilizer by sorghum in field study. *Commun. Soil Sci. Plant Anal.* **2013**, 44. [CrossRef]
63. Zhu, M.; Jin, F.; Shao, X.; Gao, X.; Geng, Y.; Wang, S. Effects of soda saline-alkali stress on physiological characteristics of rice in different concentrations. *Adv. Mater. Res.* **2014**, *1010*, 1225–1229. [CrossRef]
64. Xie, Y.; Li, X.; Huang, X.; Han, S.; Amombo, E.; Wassie, M.; Chen, L.; Fu, J. Characterization of the cd-resistant fungus *Aspergillus aculeatus* and its potential for increasing the antioxidant activity and photosynthetic efficiency of rice. *Ecotoxicol. Environ. Saf.* **2019**, *171*, 373–381. [CrossRef]
65. Lea, P.L. *Nitrogen Metabolism of Plant*; Oxford University Press: New York, NY, USA, 1992.
66. Sofo, A.; Dichio, B.; Xiloyannis, C.; Masia, A. Effects of different irradiance levels on some antioxidant enzymes and on malondialdehyde content during rewatering in olive tree. *Plant Sci.* **2004**, *166*, 293–302. [CrossRef]
67. Berteli, F.; Corrales, E.; Guerrero, C.; Ariza, M.; Pliego, F.; Valpuesta, V. Salt stress increases ferredoxin-dependent glutamate synthase activity and protein level in the leaves of tomato. *Physiol. Plant.* **2008**, *93*, 259–264. [CrossRef]
68. Ghoulam, C.; Foursy, A.; Fares, K. Effects of salt stress on growth, inorganic ions and proline accumulation in relation to osmotic adjustment in five sugar beet cultivars. *Environ. Exp. Bot.* **2002**, *47*, 39–50. [CrossRef]
69. Kumar, V.; Khare, T. Differential growth and yield responses of salt-tolerant and susceptible rice cultivars to individual (Na^+ and Cl^-) and additive stress effects of nacl. *Acta Physiol. Plant.* **2016**, *38*, 170. [CrossRef]
70. Kilian, M.; Steiner, U.; Krebs, B.; Junge, H.; Schmiedeknecht, G.; Hain, R. FZB24® *Bacillus subtilis*—Mode of action of a microbial agent enhancing plant vitality. *Pflanzenschutz-Nachrichten Bayer* **2000**, *1*, 72–93.
71. Liu, Q.; Wu, X.; Li, T.; Ma, J.; Zhou, X. Effects of elevated air temperature on physiological characteristics of flag leaves and grain yield in rice. *Chil. J. Agric. Res.* **2013**, *73*, 85–90. [CrossRef]
72. Du, J.; Shen, T.; Xiong, Q.; Zhu, C.; Peng, X.; He, X.; Fu, J.; Ouyang, L.; Bian, J.; Hu, L.; et al. Combined proteomics, metabolomics and physiological analyses of rice growth and grain yield with heavy nitrogen application before and after drought. *BMC Plant Biol.* **2020**, *20*, 556. [CrossRef] [PubMed]
73. Clerget, B.; Bueno, C. The effect of aerobic soil conditions, soil volume and sowing date on the development of four tropical rice varieties grown in the greenhouse. *Funct. Plant Biol.* **2013**, *40*, 79–88. [CrossRef]
74. Garcia-Gutierrez, M.S.; Ortega-Alvaro, A.; Busquets-Garcia, A.; Perez-Ortiz, J.M.; Caltana, L.; Ricatti, M.J.; Brusco, A.; Maldonado, R.; Manzanares, J. Synaptic plasticity alterations associated with memory impairment induced by deletion of CB2 cannabinoid receptors. *Neuropharmacology* **2013**, *73*, 388–396. [CrossRef]
75. James, R.A.; Munns, R.; von Caemmerer, S.; Trejo, C.; Miller, C.; Condon, T. Photosynthetic capacity is related to the cellular and subcellular partitioning of Na^+, K^+ and Cl^- in salt-affected barley and durum wheat. *Plant Cell Environ.* **2006**, *29*. [CrossRef] [PubMed]