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Rhizoctonia solani and Bacterial Inoculants Stimulate Root Exudation of Antifungal Compounds in Lettuce in a Soil-Type Specific Manner

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Abstract: Previous studies conducted on a unique field site comprising three contrasting soils (diluvial sand DS, alluvial loam AL, loess loam LL) under identical cropping history, demonstrated soil type-dependent differences in biocontrol efficiency against *Rhizoctonia solani*-induced bottom rot disease in lettuce by two bacterial inoculants (*Pseudomonas jessenii* RU47 and *Serratia plymuthica* 3Re-4-18). Disease severity declined in the order DS > AL > LL. These differences were confirmed under controlled conditions, using the same soils in minirhizotron experiments. Gas chromatography-mass spectrometry (GC-MS) profiling of rhizosphere soil solutions revealed benzoic and lauric acids as antifungal compounds; previously identified in root exudates of lettuce. Pathogen inoculation and pre-inoculation with bacterial inoculants significantly increased the release of antifungal root exudates in a soil type-specific manner; with the highest absolute levels detected on the least-affected LL soil. Soil type-dependent differences were also recorded for the biocontrol effects of the two bacterial inoculants; showing the highest efficiency after double-inoculation on the AL soil. However, this was associated with a reduction of shoot growth and root hair development and a limited micronutrient status of the host plants. Obviously, disease severity and the expression of biocontrol effects are influenced by soil properties with potential impact on reproducibility of practical applications.

Keywords: lettuce; soil microbiome; root exudates; plant health

1. Introduction

Pathogen-related yield losses are among the most prominent limitations in crop production [1]. The use of resistant cultivars, application of chemical pesticides, as well as crop rotation [2] represent approaches aimed to reduce the incidence and severity of pathogen attacks [1,3]. However, particularly for soil-borne pathogenic fungi with a wide host spectrum, such as *Rhizoctonia*, *Fusarium*, or *Pythium*, the development of efficient control strategies remains a highly challenging task. Long persistence of the pathogen in soils as achieved by the formation of sclerotia [4,5] and saprophytic growth stages, as well as restricted availability of resistant crop cultivars or fungicides are major limiting

factors [6]. However, distinct soil-microbial populations can induce suppression of pathogens [7], and the general suppressiveness of soils against plant pathogens [8] is a characteristic of soil quality and health [9]. This implies that biological control of pathogens via microbial antagonists is a realistic strategy, provided that it is possible to identify specific control strains, which can be transferred to pathogen-affected soils to achieve a specific soil suppressiveness [8] by harnessing the beneficial soil microbiomes against pathogens [10]. However, the exploitation of microbial inoculants as biocontrol agents in crop production systems is frequently hampered by inconsistent results at the field scale [3], likely linked to plant performance. In many cases there is still a substantial lack of knowledge about the factors determining the successful establishment of biocontrol systems.

The rhizosphere of the host plants is the major site for plant-microbial interactions, with rhizodeposition, i.e., the release of organic carbon through plant roots, as the major driving force [11,12]. During these interactions, both beneficial microorganisms and unfavorable pathogens are attracted by the plant roots [12–14] with rhizodepositions acting as signals and as carbon and nitrogen sources, but also as components of plant defense against pathogens. Constant microbiome–root interactions mediate nutrient turnover in the rhizosphere, which enables the plants to acquire nutrients and to receive benefits during growth phases [15,16], while soil-borne pathogens adapt to the rhizosphere, compete with beneficial soil microbes for nutrient availability, and can harm the host plant. Rhizodeposition is highly variable and influenced by many abiotic and biotic factors. It comprises passive losses of organic compounds, as well as highly regulated secretory processes with adaptive functions [17,18]. Due to the central role of rhizodeposits in shaping rhizosphere-microbial communities, a more detailed understanding of the factors determining rhizodeposition with impact on plant pathogens and microbial antagonists in the rhizosphere, will provide important information on the conditions required for successful establishment of biocontrol systems. As an example, biological control of *Rhizoctonia solani* (Kühn) teleomorph *Thanatephorus cucumeris* (A.B. Frank Donk) [6] by bacterial strains of *Pseudomonas jessenii* and *Serratia plymuthica* has been proposed as a promising approach against bottom rot disease in lettuce (*Lactuca sativa*) [2,19–23].

To reveal differences in biocontrol efficiency linked to the soil type and not influenced by local climate or cropping history, Schreiter et al. [23] used a unique experimental field plot system, with three different soil types stored at the same field site for 10 years under the same agricultural management. Clear soil type-dependent differences in the bacterial community structure of the bulk soils and the corresponding lettuce rhizospheres were detected. This was associated with distinct quantitative patterns of low-molecular weight compounds in the rhizosphere soil solutions, mainly representing rhizodeposits, detected by microsampling and gas chromatography-mass spectrometry (GC-MS) profiling in a parallel minirhizotron study conducted with the same soils [16]. Particularly, benzoic acid and lauric acid were of special interest besides other organic acids, various amino acids, amines, sugars, and sugar alcohols. It is documented that these two compounds exhibit antifungal activity against *Rhizoctonia solani*, *Pythium ultimum*, *Sclerotinia sclerotiorum*, and others [24,25], and have been previously identified in root exudates of lettuce grown in soil-free systems in hydroponic culture [26]. Inoculation of the three soils with *Rhizoctonia solani* AG1-IB (*R. solani*) revealed a strong soil type-dependent effect on disease severity under field conditions, while the rhizosphere competence and the biocontrol activity of the pre-inoculated bacterial biocontrol strains *Pseudomonas jessenii* RU47 and *Serratia plymuthica* 3Re-4-18 were much less affected by soil type differences [3].

Based on this background information, the present study addressed the question of whether similar plant-pathogen interactions and biocontrol effects are reproducible using the same field soils in a minirhizotron study to enable the characterization of antifungal root exudates in the rhizosphere soil solution and their potential relationships with the presence of the pathogen and/or the bacterial inoculants. Lettuce plants (*Lactuca sativa* L. cv. Tizian) were grown in minirhizotrons, equipped with removable root observation windows for non-destructive micro-sampling of rhizosphere soil solution with sorption filters applied to the surface of different root zones, followed by re-extraction and GC-MS analysis [16].

2. Results

2.1. Plant Biomass Production is Affected by Rhizoctonia solani AG1-IB

The severity of *R. solani*-induced bottom rot disease was clearly influenced by the soil type and increased in the order loess loam (LL) < alluvial loam (AL) < diluvial sand (DS) (Table 1), as indicated by declining shoot biomass production. While shoot biomass of lettuce plants grown on the LL soil was not significantly affected by *R. solani* inoculation, shoot biomass significantly declined by 38% on the AL soil. The lowest biomass of non-inoculated control plants was recorded on DS soil, where the plants died within the first week after pathogen inoculation (Table 1).

Table 1. Shoot dry mass (g plant^{-1}) of *Lactuca sativa* L. cv. Tizian grown on three different soils without (control) and with inoculation of *Rhizoctonia solani* AG1-IB (+*Rhizoctonia*). (++ Plants died within the first week after *Rhizoctonia solani* inoculation).

Treatment	Diluvial Sand	Alluvial Loam	Loess Loam
Control	0.22 ± 0.2	1.13 ± 0.1 a	1.33 ± 0.5 a
+*Rhizoctonia*	++	0.70 ± 0.3 b	1.45 ± 0.2 a

Means ± standard errors of four independent replicates. Different characters indicate significant differences for a given soil type (*t*-test, $p = 0.05$).

2.2. Biocontrol Activity of Pseudomonas jessenii RU47 and Serratia plymuthica 3Re-4-18

2.2.1. Effects on Shoot Growth

On the DS soil, no biocontrol activity of the bacterial inoculants against *R. solani* was detectable due to rapid seedling decay during the first week after pathogen inoculation (Table 1). The decline of shoot biomass on the AL soil induced by *R. solani* inoculation (Table 1) was mitigated by pre-inoculation with *P. jessenii*, reaching dry matter production not significantly different from the control treatment without *R. solani* inoculation (Figure 1b). Visual scoring of plant damage (Figure 2) revealed the most prominent biocontrol effect by double-inoculation with *P. jessenii* and *S. plymuthica* (+*P. jess./S. ply.*), indicated by survival of all plants in response to *R. solani* inoculation. Despite high biocontrol activity (Figure 2), the lowest shoot biomass production was recorded in the double-inoculated variants in both treatments with and without *R. solani* inoculation (Figure 1a,b). Single inoculation revealed that this effect could be mainly attributed to the presence of *S. plymuthica* (Figure 1b). By contrast, on the LL soil, no significant treatment effects were recorded and shoot biomass production ranged between min. 1.3 g pot^{-1} and max. 1.7 g pot^{-1} (Supplementary Figure S1).

(a)

Figure 1. *Cont.*

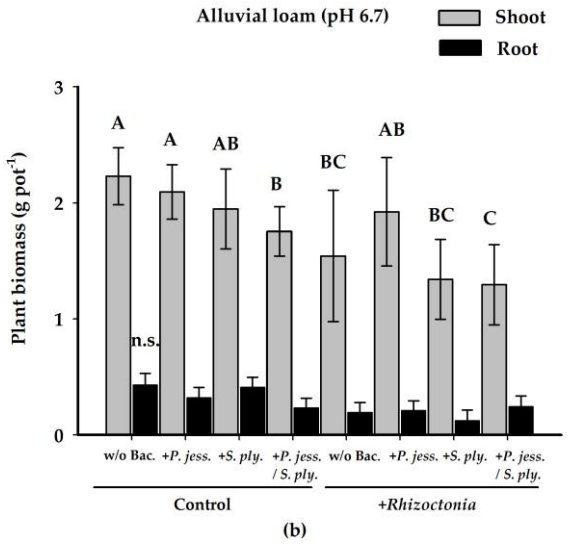

Figure 1. Habitus (**a**) and shoot and root dry mass (g pot^{-1}) (**b**) of *Lactuca sativa* L. cv. Tizian. The plants were grown on alluvial loam without bacterial inoculation (w/o Bac.), pre-inoculated with *Pseudomonas jessenii* RU47 (+*P. jess.*), *Serratia plymuthica* 3Re-4-18 (+*S. ply.*), a combination of both (+*P. jess*/*S. ply.*), with and without (Control) subsequent inoculation with *Rhizoctonia solani* AG1-IB (+*Rhizoctonia*). Means ± standard errors of four independent replicates. Different characters indicate significant differences (one-way ANOVA, Holm-Sidak test, $p = 0.05$).

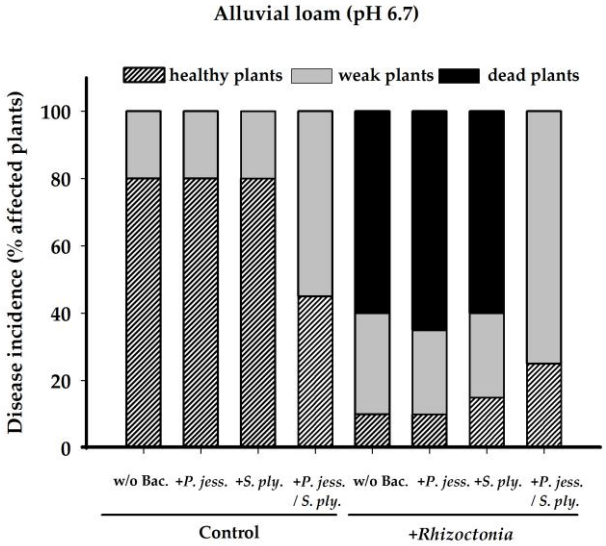

Figure 2. Visual scoring of disease incidence of *Lactuca sativa* L. cv. Tizian, expressed as percentage of healthy, weak (growth depression and leaf necrosis due to fungal infection), and dead plants. The plants were grown on alluvial loam without bacterial inoculation (w/o Bac.), pre-inoculated with *Pseudomonas jessenii* RU47 (+*P. jess.*), *Serratia plymuthica* 3Re-4-18 (+*S. ply.*), a combination of both (+*P. jess*/*S. ply.*), with and without (Control) subsequent inoculation with *Rhizoctonia solani* AG1-IB (+*Rhizoctonia*).

2.2.2. Effects on Root Growth and Morphology

No significant treatment effects were recorded for root biomass of lettuce plants, neither on the AL (Figure 1), nor on the LL soil. A trend for lower root biomass production was detectable for the AL soil in the treatments with *R. solani* inoculation (Figure 1). However, in all treatments with *S. plymuthica* inoculation on the AL soil, root hair length (Figure 3b) was significantly reduced by 30–50% in the presence of *R. solani.* Similarly, root hair density (Figure 3c) declined after pre-inoculation

with *S. plymuthica*, both in the control treatment and after inoculation with *R. solani*. No comparable effects were detectable on the LL soil, where root hair length ranged between 0.71 mm and 0.87 mm in all treatments (Supplementary Figure S2).

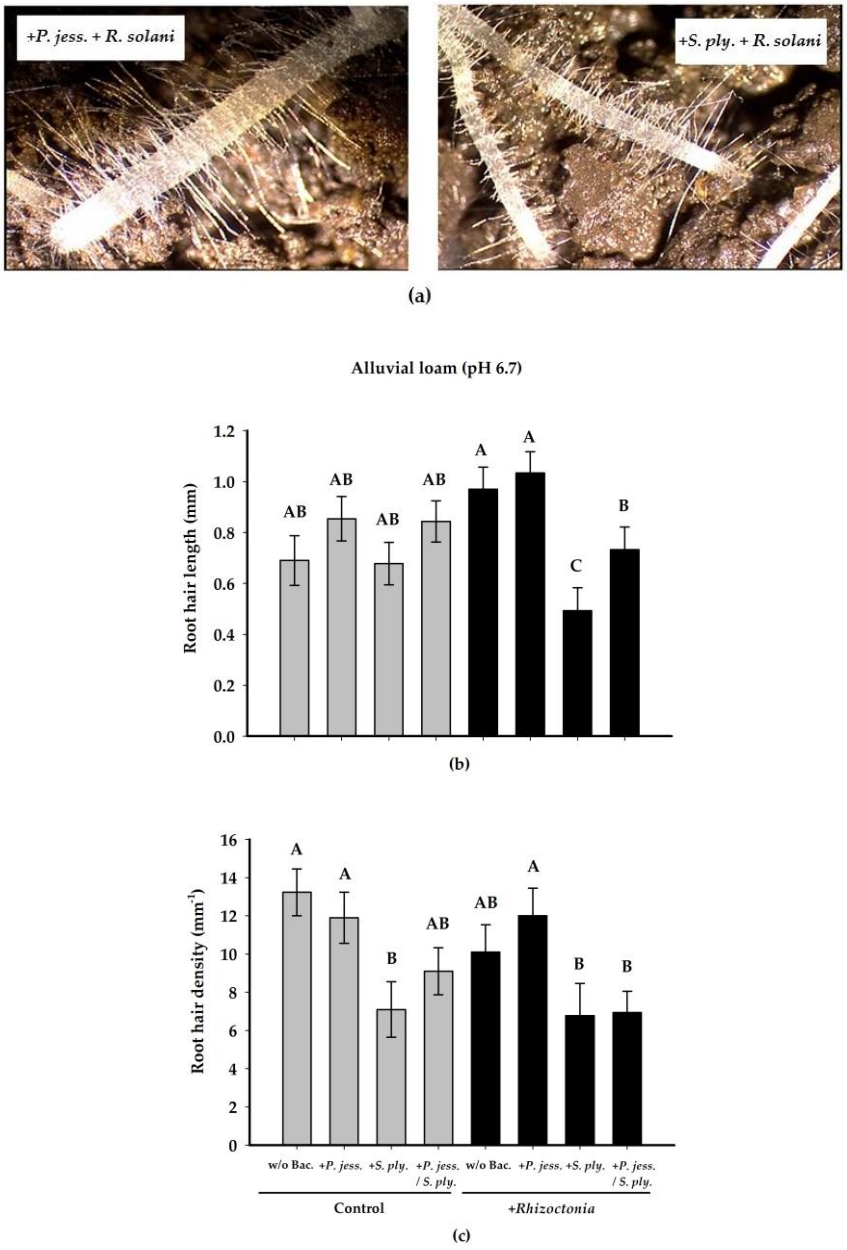

Figure 3. Root hair development (**a**), root hair length (mm) (**b**) and root hair density (mm^{-1}) (**c**) of *Lactuca sativa* L. cv. Tizian. The plants were grown on alluvial loam without bacterial inoculation (w/o Bac.), pre-inoculated with *Pseudomonas jessenii* RU47 (+*P. jess.*), *Serratia plymuthica* 3Re-4-18 (+*S. ply.*), a combination of both (+*P. jess*/*S. ply.*), with and without (Control) subsequent inoculation with *Rhizoctonia solani* AG1-IB (+*Rhizoctonia*). Means ± standard errors of four independent replicates. Different characters indicate significant differences (one-way ANOVA, Holm-Sidak test, $p = 0.05$).

2.2.3. Plant-Nutritional Status

For the macronutrients N, P, K, and Mg supplied to the soils as fertilizers in sufficient amounts prior to plant cultivation, no significant treatment effects or characteristic deficiency symptoms were recorded for the lettuce plants grown on the AL and the LL soil (not shown). However, on AL

soil, micronutrient shoot concentrations of Zn, Mn, and Fe (Figure 4a–c) of the treatments with *R. solani* inoculation showed a trend of decline in the order w/o Bac. > *P. jessenii* > *S. plymuthica* > *P. jessenii* +*S. plymuthica*, finally reaching critical values close to the deficiency thresholds [27] for the double-inoculation. In the control treatment without *R. solani* inoculation, this effect was restricted to lettuce plants with double-inoculation with *P. jessenii* and *S. plymuthica* (+*P. jess./S. ply.*).

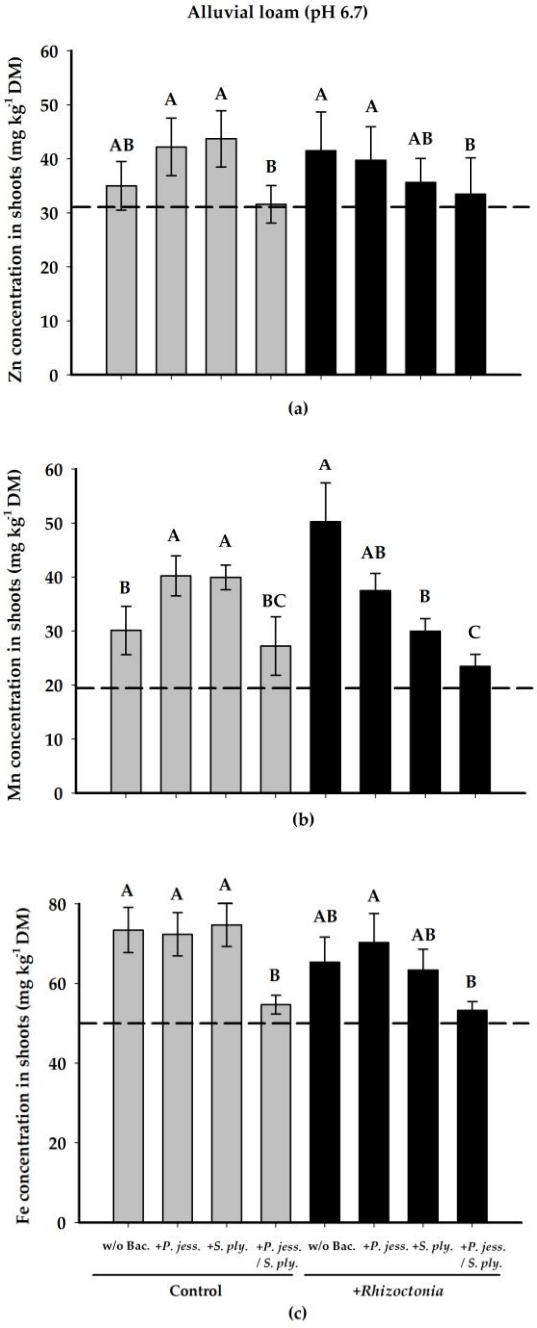

Figure 4. Micronutrient concentrations of Zn (**a**) Mn (**b**) and Fe (**c**) in shoots (mg kg^{-1} DM) of *Lactuca sativa* L. cv. Tizian. The plants were grown on alluvial loam without bacterial inoculation (w/o Bac.), pre-inoculated with *Pseudomonas jessenii* RU47 (+*P. jess.*), *Serratia plymuthica* 3Re-4-18 (+*S. ply.*), a combination of both (+*P. jess/S. ply.*), with and without (Control) subsequent inoculation with *Rhizoctonia solani* AG1-IB (+*Rhizoctonia*). Means ± standard errors of four independent replicates. The dotted lines indicate the threshold levels for micronutrient deficiency. Different characters indicate significant differences (one-way ANOVA, Holm-Sidak test, $p = 0.05$).

For lettuce plants grown on LL soil, no significant treatment effects in shoot micronutrient concentrations were recorded. Shoot concentrations of Mn ranged between 29.4 mg kg^{-1} DM and 53.4 mg kg^{-1} DM, and Zn between 20.4 mg kg^{-1} DM and 29.5 mg kg^{-1} DM (Supplementary Figure S3). No nutrient analysis was conducted for the plants on DS soil due to the rapid decay already during the first week after *R. solani* inoculation.

2.2.4. Antifungal Compounds in the Rhizosphere Soil Solution

The antifungal compounds benzoic acid and lauric acid [24,25], previously identified in root exudates of lettuce [26], were detected by GC-MS analysis (Table 2) after collection by micro-sampling with sorption filters [16] from lettuce plants in 1–2 cm subapical lateral root zones and in more basal parts of the root zones (8–9 cm behind the root tip). On the AL soil, *R. solani* inoculation significantly increased the concentrations of benzoic acid (Figure 5a) collected from subapical root zones by 234% and in the basal root zones by 296%. Lauric acid concentrations (Figure 5b) tended to be increased only in the basal parts of the root zones, as compared to the control treatment without *R. solani* inoculation (Table 2). In contrast, on the LL soil, only trends for increased root exudation of benzoic acid (11% subapical, 29% basal) and lauric acids (+22% subapical) were induced by *R. solani* inoculation but no significant effects in comparison to the control treatment without *R. solani* inoculation were observed. In general, lauric acid concentrations in samples collected on the LL soil were higher than on AL soil.

Table 2. Benzoic and lauric acid concentrations in rhizosphere soil solutions collected by micro-sampling with sorption filters in 1–2 cm subapical regions of young roots and from older basal root zones (8–9 cm) of *Lactuca sativa* L. cv. Tizian. The plants were grown on alluvial loam treated without (control), with *Rhizoctonia solani* (+*R. solani*), with *Pseudomonas jessenii* (+*P. jess.*) and *Serratia plymuthica* (+*S. ply.*), with *Rhizoctonia solani* and one bacterial inoculant (+*R. solani* +*P. jess*; +*R. solani* +*S. ply.*), with a combination of both bacterial inoculants (+*P. jess*/*S. ply.*) and with *Rhizoctonia solani* and both bacterial inoculants (+*R. solani* +*P. jess./S. ply.*). Relative values based on peak areas (gas chromatography-mass spectrometry (GC-MS) analysis) after subtraction of background levels in bulk soil samples.

Treatment	Benzoic Acid				Lauric Acid			
	Subapical (Young)		Basal (Old)		Subapical (Young)		Basal (Old)	
	AL	LL	AL	LL	AL	LL	AL	LL
Control	3.92 a	5.00 a	2.77 a	6.30 a	1.76 a	4.29 a	0.14 a	14.00 a
+*R. solani*	13.07 b	5.53 a	10.97 b	8.12 a	0.85 a	5.25 a	1.36 ab	9.56 a
+*P. jess.*	12.84 b	10.54 b	12.98 b	13.58 ab	1.00 a	10.55 b	1.52 b	21.80 a
+*S. ply.*	13.08 b	10.49 b	12.75 b	9.58 ab	0.51 a	10.66 b	1.01 ab	9.40 a
+*R. solani* +*P. jess.*	10.75 b	9.62 b	13.65 b	15.53 b	0.92 a	9.29 b	1.34 ab	25.03 a
+*R. solani* +*S. ply.*	11.71 b	8.63 b	12.68 b	15.79 b	1.00 a	7.82 ab	0.94 ab	29.87 a
+*P. jess./S. ply.*	12.84 b	N.d.	12.63 b	N.d.	0.85 a	N.d.	0.93 ab	N.d.
+*R. solani* +*P. jess./S. ply.*	12.14 b	N.d.	11.31 b	N.d.	0.43 a	N.d.	1.09 ab	N.d.

Means of three independent replicates. In each column different characters indicate significant differences (one-way ANOVA, Holm-Sidak test, $p = 0.05$). N.d. = not determined. DS: Diluvial sand, AL: alluvial loam, LL: loess loam.

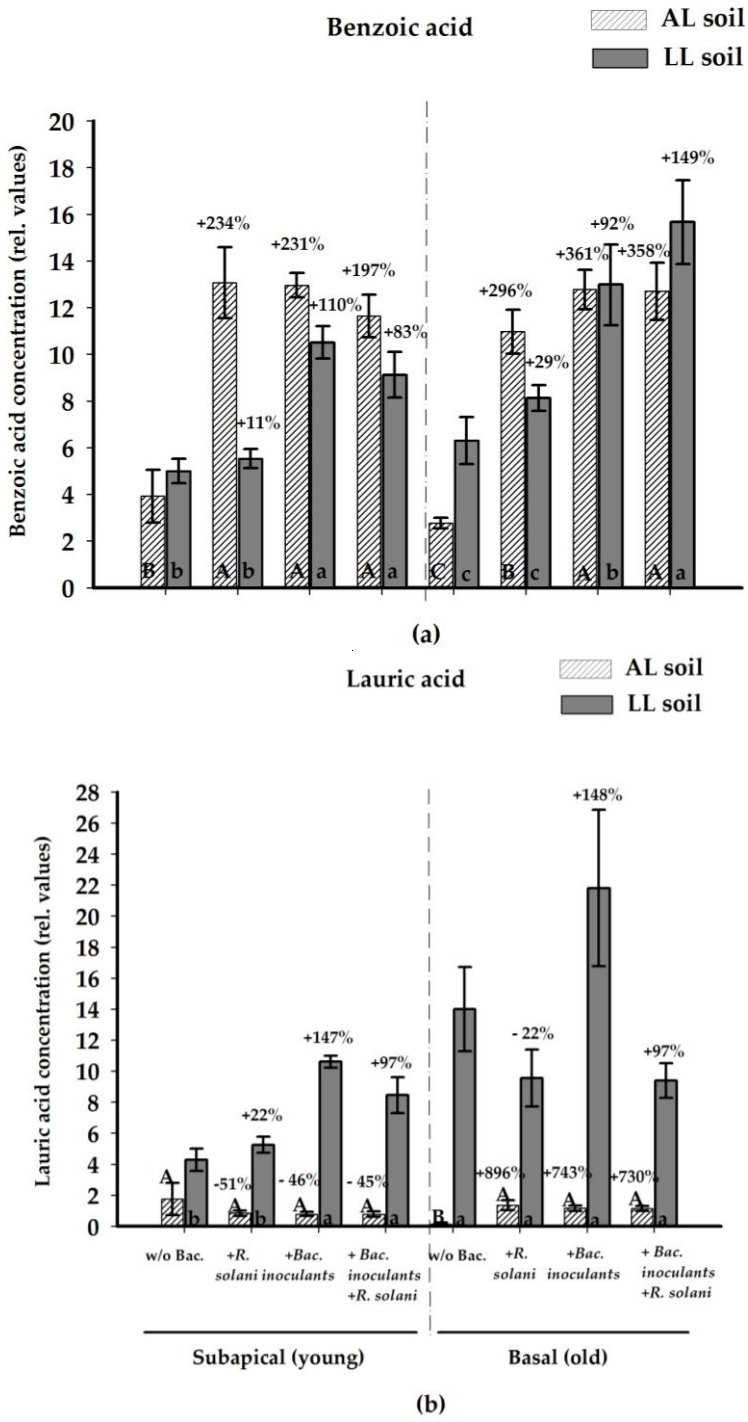

Figure 5. Concentrations and relative changes (% compared to the untreated controls) of benzoic acid (**a**) and lauric acid (**b**) in rhizosphere soil solutions collected from 1–2 cm subapical regions of young roots and from older basal root zones (8–9 cm basal roots) of *Lactuca sativa* L. cv. Tizian. The plants were grown on alluvial loam (AL) and loess loam (LL) treated without bacterial inoculation (w/o Bac.), with *Rhizoctonia solani* AG1-IB (+*R. solani*), with bacterial inoculants (+*Bac. inoculants* = *Pseudomonas jessenii* RU47 and/or *Serratia plymuthica* 3Re-4-18) and with a combination of bacterial inoculants and *Rhizoctonia solani* AG1-IB (+*Bac. inoculants* +*R. solani*). Relative values based on peak area (GC-MS analysis) after subtraction of background levels in bulk soil samples. Means ± standard errors of 3–9 independent replicates. For each soil type, different characters indicate significant differences (one-way ANOVA, Holm-Sidak test, $p = 0.05$).

Compared to the non-inoculated control treatment, pre-inoculation with *P. jessenii*, *S. plymuthica* and a combination of both (+*P. jess*/*S. ply.*) increased root exudation of benzoic acid (Figure 5a) in the subapical and basal root zones on the AL soil between 197% and 361% and on the LL soil between 83% and 149%. Since no significant differences between the bacterial inoculants were recorded (Table 2), Figure 5 summarizes the effects of all bacterial inoculants. An increased exudation of lauric acid was observed in the basal root zones on AL soil (730% and 743%) and additionally on LL soil in the subapical root zones (97% and 147%). The highest lauric acid concentrations and the highest levels of antifungal root exudates in general were recorded on LL soil (Figure 5).

3. Discussion

3.1. Plant Pathogen Interactions

Similar to previously reported field observations [3], soil type-dependent differences in severity of bottom rot disease in lettuce, induced by inoculation with *R. solani* AG1-IB (predominant in field-grown lettuce in Germany [28]), were confirmed in the present minirhizotron study. The experiment was conducted under controlled conditions on three different soils (DS: diluvial sand, pH 6.1; AL: alluvial loam, pH 6.7; LL: loess loam, pH 7.1) with the same cropping history during the last 10 years. Shoot biomass production of *R. solani*-inoculated lettuce plants declined in the order LL > AL > DS soil (Table 1), reflecting the negative impact of the pathogen on lettuce growth as previously reported on the same soils under field conditions [23]. However, in minirhizotron culture, disease symptoms on lettuce grown on DS soil appeared much faster, and in contrast to the field experiments all plants died already during the first week after pathogen inoculation. The higher conduciveness for bottom rot disease of the DS soil observed in all experiments may be caused by the bigger pore sizes and better oxygen availability in sandy soils, which enables more rapid hyphal growth of *R. solani* towards the host plant [3,29]. The more severe disease symptoms in the minirhizotron study may be attributed to higher rooting densities in the limited soil volume of the minirhizotrons and due to shorter spatial distances between the pathogen and the host plant in comparison to field conditions with larger soil volume. Moreover, the constant temperature conditions in the growth chamber (23–25 °C) in the optimum range for hyphal growth of *R. solani* AG1-IB [28] could further promote the infection process, particularly on the DS soil with the weakest plant development even in the non-inoculated control (Table 1). Accordingly, Schreiter et al. [3] reported a certain background infection potential for bottom rot disease in all investigated soils, even without artificial inoculation with *R. solani* AG1-IB, with the lowest disease severity on LL soil, in comparison to disease severity for plants grown on DS and AL soil.

On the AL soil, *R. solani* inoculation resulted in a 60% reduction in shoot biomass (Table 1) and 40% of the plants finally survived (Figure 2). In contrast, no apparent bottom rot symptoms were detected on the LL soil (Table 1) and supported the observed results of higher soil suppressiveness in the field experiment [3], reflected also by the lowest infection potential on the non-inoculated control soils [3]. Accordingly, on the AL soil, pathogen inoculation significantly increased the concentrations of benzoic and lauric acids (Table 2, Figure 5) in the rhizosphere soil solutions (234% to ca. 900%), previously identified as root exudates of lettuce with documented antifungal activity against *Rhizoctonia*, *Fusarium*, and other pathogenic fungi [24,25]. In contrast, only a non-significant trend for increased root exudation of the antifungal compounds by a maximum of 30% was detected in the rhizosphere on the LL soil with antifungal suppressive potential (Table 2; Figure 5). These findings suggest that the release of the antifungal root exudates is part of the pathogen defense response in lettuce, as previously reported also for *Fusarium*-resistant peanut cultivars [30]. *Rhizoctoniasolani*-affected plants on the AL soil obviously responded with increased exudation of antibiotic compounds, while only a weak response was triggered in the *R. solani* inoculated plants grown on the LL soil with low *R. solani* infection potential.

Besides an increased release of antifungal root exudates in response to *R. solani* infection on AL soil in basal parts of the root system close to the inoculation sites, benzoic acid was also detected in the youngest root zones (1–2 cm behind the root tip), up to 30 cm below the *R. solani* infection sites (Table 2, Figure 5). *Rhizoctonia solani* infection sites are usually located at the root-shoot junction and at the lower leaves with direct soil contact [28,29]. Thus, release of antifungal root exudates, even five weeks after inoculation with the pathogen, may indicate a systemic response of lettuce to *R. solani* infection. Accordingly, previous studies have also reported the systemic induction of biosynthetic pathways for aromatic compounds in response to *R. solani* infection and their potential role in pathogen defense in different crops, such as potato and rice [29,31].

3.2. Disease Suppression by Bacterial Inoculants

Two bacterial strains, *Pseudomonas jessenii* RU47 and *Serratia plymuthica* 3Re-4-18 [32], characterized for good biocontrol effects against bottom rot disease in previous experiments [6,21], were selected as biocontrol strains used for pre-inoculation of the lettuce plants. On the DS soil with the weakest plant development even in the controls without pathogen inoculation (Table 1), and with the most severe bottom rot symptoms, no protective effects of the bacterial inoculants against *R. solani* were recorded. In contrast, on the AL soil, particularly pre-inoculation with *P. jessenii* RU47 tended to increase biomass production of *R. solani*-affected plants (Figure 1). However, the most pronounced biocontrol effect was evaluated by a double-inoculation with both bacterial strains where, in contrast to the other treatments, all plants survived (Figure 2). The increased expression of antagonistic effects after double inoculation with different biocontrol strains has been similarly reported in previous studies [33] and may be attributed to additive effects of different antibiotics, cellulolytic enzymes, and induction of plant defense responses, but also to a broader activity spectrum under different environmental conditions [34]. By contrast, no additional beneficial effects of pre-inoculation with the bacterial inoculants were observed on the least conductive LL soil (Supplementary Figure S1) where the lettuce plants did not show any visible symptoms of bottom rot disease.

Interestingly, similar to *R. solani* infection, inoculation with the bacterial inoculants also increased the exudation of benzoic and lauric acids on the AL soil. Moreover, the same response was recorded on the more suppressive LL soil where *R. solani* inoculation had only marginal effects on the release of the antifungal root exudates (Table 2, Figure 5). In accordance with the absence of disease symptoms, the highest total levels of the antifungal compounds (sum of benzoic and lauric acids) were detected in root exudates of lettuce plants grown on the LL soil (Table 2, Figure 5), which may reflect a particularly intense expression of pathogen defense mechanisms. Even in the variant without pathogen inoculation, high background levels of lauric and benzoic acids were detectable on the LL soil (Table 2, Figure 5). This raises the question of whether generally high levels of antifungal root exudates contributed to the particularly low conductivity for *Rhizoctonia* bottom rot disease on this soil. Interestingly, Schreiter et al. [3] observed a soil type-dependent rhizosphere effect of the lettuce plants on the abundance of bacteria with the capacity to degrade aromatic hydrocarbons (particularly *Sphingomonas*), declining in the order DS > AL > LL soil, which follows the soil type-dependent expression in severity of *Rhizoctonia*-induced disease symptoms (Table 1). This may reflect a declining capacity for degradation of the antifungal root exudates as secondary metabolites by members of the indigenous microflora, resulting in the highest accumulation on LL soil, which may be also responsible for the low background infection potential for bottom rot disease on this soil [3]. Apart from benzoic and lauric acids, Neumann et al. [16] also reported particularly high levels of sugars, amino acids, and organic acids in the rhizosphere soil solutions collected from lettuce plants grown on the LL soil, associated with the highest shoot biomass production (Table 1). This may indicate a generally higher capacity for root exudation due to stronger plant development on the LL soil, associated with a higher photosynthetic capacity as a major driving force for rhizodeposition [17].

The exudation of benzoic acid was more strongly induced by bacterial inoculants or by pathogen–inoculant combinations than by sole inoculation with *R. solani* (Figure 5). These findings

suggest that pre-inoculation with the bacterial inoculants can exert long-lasting priming effects on induction of plant defense reactions in the form of antifungal root exudates still detectable five to six weeks after the last inoculation, as similarly reported for the induction of pathogen defense responses by various rhizosphere bacteria [35]. This is also in line with the high rhizosphere competence of the selected bacterial inoculants reported by Schreiter et al. [23], since the observed release stimulation of the antifungal root exudates, even in the youngest roots at the end of the culture period indicates that the inoculants were still active.

Root colonization with bacterial inoculants is often preferentially located in the more basal parts of the root system [25,34] close to the initial inoculation sites. However, similar to the effects of *R. solani* inoculation, increased root exudation of benzoic and lauric acids induced by the bacterial inoculants was detectable on both soils, not only in older parts of the root system, but even in the young root tips (Table 2, Figure 5). This suggests a systemic effect for bacterial inoculants also. A systemic induction of plant defense responses in a combination of aromatic compounds has been previously proposed for the genera *Pseudomonas* and *Serratia* [36,37]. This spatial exudation pattern may contribute to a protective effect not only against *Rhizoctonia* bottom rot, but also against other more typical root pathogens [24,25].

3.3. Negative Side Effects of the Bacterial Inoculants

Although the most pronounced biocontrol effect was observed in treatments with double inoculation of *P. jessenii* and *S. plymuthica* on the AL soil, indicated by the absence of any dead plants after *R. solani* infection (Figure 2), this effect was associated with lower biomass production (Figure 1). Additionally, reduced root hair length and density (Figure 3) and a decline of the plant micronutrient status (Figure 4) close to the deficiency thresholds [27] was detectable.

The decline of plant micronutrient status was associated with the expression of typical symptoms of micronutrient limitation (Supplementary Figure S4). These effects could be attributed to the presence of *S. plymuthica*, since they were not only detectable after double-inoculations, but to a lesser extent also for single inoculation with *S. plymuthica*. The inhibitory effects were particularly expressed in *R. solani*-affected plants. Similarly, Schreiter et al. [23] reported a reduction of shoot growth under field conditions, associated with high rhizosphere abundance of *S. plymuthica*. Inhibition of plant growth and induction of micronutrient limitation may be related to the fact that both inoculant strains are effective producers of siderophores [38,39], known as efficient chelators for iron but also other divalent metal cations [40]. A comparison of the nutritional status of lettuce plants cultivated on the three investigated soils without pathogen inoculation [16] revealed the lowest micronutrient levels close to the deficiency threshold on the AL soil. Under these conditions, micronutrient availability to lettuce plants additionally stressed by *R. solani* infection may be negatively affected by competitive interactions with efficient micronutrient-chelating siderophores released by the bacterial inoculants. Moreover, bacterial siderophores are also discussed as inducers of systemic plant defense responses [38,41] and this may apply also for root exudation of benzoic acid, similarly observed in the present study.

Additionally, in *S. plymuthica*- and double-inoculated lettuce plants grown on the AL soil, both the elongation and density of the root hairs were significantly reduced. Growth and formation of root hairs are usually increased as an adaptive response to improve iron acquisition in so-called strategy I plants (dicotyledonous plants, such as lettuce). The stimulation of root hair development is triggered by Fe-deficiency-induced ethylene production [18,42]. However, a wide-spread feature of many plant growth-promoting bacteria is the suppression of excessive ethylene production with inhibitory effects on plant growth by enzymatic degradation of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) via ACC desaminase [43] or by other yet unidentified mechanisms as discussed also for *S. plymuthica* [44]. This would explain the inhibitory effect on Fe deficiency-induced root hair development in *S. plymuthica*-inoculated lettuce plants (Figure 4c), which would further counteract nutrient acquisition on the AL soil with low micronutrient solubility, where root hair development is of particular importance [42]. Interestingly, these effects were not detectable on the LL soil, indicating

that the inhibitory effects of the microbial inoculants represent a soil-specific feature. As a possible explanation, plants grown on the LL soil were less affected by *R. solani* and may therefore exhibit a higher level of tolerance against nutrient limitation.

4. Materials and Methods

4.1. Lettuce Cultivation

Lettuce (*Lactuca sativa* L. cv. Tizian) cultivation, harvest, and root exudate sampling were performed as described by Neumann et al. [16]. To achieve homogenous plant development, lettuce seedlings were pre-cultivated in seed trays in a peat culture substrate–sand mixture (7:3 *w*/*w*; TKS1 Anzuchtsubstrat, Floragard, Germany) to the two-leaf stage (BBCH 12). Thereafter, minirhizotrons (36 × 11.5 × 2.5 cm) equipped with removable root observation windows were used for plant cultivation. Three contrasting soil types originating from a unique long-term field plot with a 10-year identical crop management history, located at the Leibniz Institute of Vegetable and Ornamental Crops, Großbeeren (Germany), were used for plant cultivation. Soil characteristics were: (i) Arenic-Luvisol pH 6.1 (diluvial sand = DS) with silty sand and 5.5% clay; (ii) Gleyic-Fluvisol pH 6.7 (alluvial loam = AL) heavy sandy loam with 27.5% clay; and (iii) Luvic-Phaeozem pH 7.1 (Loess loam = LL), medium content of clayey silt with 17.2% clay. Macronutrient fertilization with 100 mg N kg^{-1} soil, 100 mg P kg^{-1} soil, 150 mg K kg^{-1} soil and 50 mg Mg kg^{-1} soil (supplied as Ca $(NO_3)_2$; Ca $(H_2PO_4)_2$; K_2SO_4; and $MgSO_4$) was applied to cover the plant demand during the culture period. The soil moisture level was adjusted to 18–20% *w*/*w* and controlled gravimetrically throughout the culture period. The transplanting of two pre-cultivated lettuce seedlings was undertaken at BBCH 12 and the minirhizotrons were fixed at an angle of 45° to stimulate root growth along the root observation window for exudate sampling. The lettuce plants were cultivated in a growth chamber with a 16 h light period at 200 µmol m^{-2} s^{-1}, 60% rel. humidity, and a day/night temperature regime of 25 °C/23 °C. Final plant harvest was conducted eight weeks after sowing (BBCH 19). For further analysis, roots were washed out from soil with sieves (0.5 to 1 mm) and their fresh weight was recorded. Root hair length (mm) and root hair density (mm^{-1}) were recorded with a video macroscope (Stemi2000-C, Zeiss, Oberkochen, Germany), and analysed with the Axion Vision 30.0 software system (Zeiss, Oberkochen, Germany). For plant analysis of mineral nutrients, the fresh shoot biomass was recorded and dried at 60 °C.

4.2. Inoculation with Bacterial Inoculants

Application of bacterial inoculants was performed according to Schreiter et al. [3]. Seed treatment was conducted with a 29 °C overnight culture of *Pseudomonas jessenii* RU47 and *Serratia plymuthica* 3Re-4-18 on King's B agar (Merck KGaA, Darmstadt, Germany) supplemented with rifampicin (75 mg mL^{-1}). Bacterial cells were suspended in sterile 0.3% NaCl solution (*w*/*v*) and adjusted to a cell density of 10^8 colony forming units (CFU) mL^{-1} by spectrophotometric determination. Lettuce seeds were coated with 250 µL of the bacterial cell suspensions per 100 seeds by dripping on the seed surface under vigorous shaking. At the end of the pre-culture period of the plants on peat culture substrate (BBCH 12), a second inoculation was performed by root drenching according to Schreiter et al. [3] with 20 mL of bacterial suspension in 0.3% NaCl solution per plant.

4.3. Pathogen Inoculation

The *Rhizoctonia solani* AG1-IB isolate 7/3 from the strain collection of the Leibniz Institute of Vegetable and Ornamental Crops, Großbeeren (Germany) was used as model pathogen. The inoculum was multiplied over three weeks at 25 °C on Petri dishes using potato extract glucose agar as the growth medium (Roth, Karlsruhe, Germany), previously inoculated with two agar blocks each (2 cm diameter) of a stock culture. The inoculation of the pathogen into the soil of the minirhizotrons was performed directly after transplanting of the lettuce seedlings (BBCH 12) from the pre-culture medium.

After opening of the root observation windows, seven *Rhizoctonia*-infected agar blocks (2 cm diameter) were inserted into the soil at a distance of 5 cm to the roots, avoiding direct root contact.

4.4. Nutrient Analysis of Plant Biomass

For analysis of mineral nutrients in the shoot biomass, 500 mg of dried plant material was ashed in a muffle furnace for 5 h at 500 °C. Thereafter, the samples were extracted twice with 2 mL of 3.4 M HNO_3 to precipitate SiO_2. The plant ash was dissolved in 2 mL of 4 M HCL and boiled for 2 min after 1:10 dilution with hot deionized water. Subsequently, 0.1 mL Cs/La buffer was added to 4.9 mL ash solution and Fe, Zn, and Mn concentrations were determined using atomic absorption spectrometry (AAS, Unicam 939, Offenbach/Main, Germany). Spectrophotometric determination of orthophosphate (Hitachi U-3300 Spectrophotometer, Hitachi Ltd. Corporation, Chiyoda, Tokio, Japan) was conducted after addition of molybdate-vanadate color reagent according to the method of Gericke and Kurmies [45]). Determination of Mg was performed by atomic absorption spectrometry, while K and Ca were measured by flame emission photometry (ELEX 6361, Eppendorf, Hamburg, Germany).

4.5. Analysis of Rhizosphere Soil Solution

Microsampling of rhizosphere soil solutions was conducted with sorption filters placed onto the surface of lateral roots growing along the root observation window in subapical root zones (1–2 cm behind the root tip) and older parts of the root system (8–9 cm behind the root tip) according to the method described by Haase et al. [46]. The exudate sampling was performed five weeks after transplanting during vegetative growth, when carbohydrate partitioning to the roots and root exudation are considered most active [47,48]. After a collection period of 4 h, for each minirhizotron, ten sorption filters of the respective root zones were pooled and stored at −20 °C for further analysis.

The sorption filters were re-extracted with 80% methanol. After centrifugation, extracts were dried at 30 °C in a Speed Vac Concentrator (Savant, Farmington, CT, USA) at 30 °C and stored at −80 °C for further analysis. For GC-MS analysis, the residues were re-dissolved in 200 µL methanol, transferred into GC-MS glass vials and evaporated to dryness at 30 °C. Derivatization was performed online directly prior to injection using a MPS Autosampler (Gerstel, Mühlheim a.d.R., Germany) by adding 25 µL methoxyhydroxymethylamine (20 mg mL^{-1} in pyridine) and incubated for 2 h at 37 °C, 350 rpm. Thereafter, 50 µL MSTFA (*N*-Trimethylsilyl-*N*-methyl trifluoroacetamide) as a silylating reagent that forms volatile derivatives for GC-MS analysis including standard alkanes from Sigma C7–C30 (0.1% v/v) were added and incubated for 30 min at 37 °C, 350 rpm. One µL aliquots were analyzed by an Agilent7890 gas chromatograph (Agilent, Santa Clara, CA, USA) in the splitless mode, coupled to a TOF mass spectrometer GCT Premier (Waters Corporation, Eschborn, Germany). Separation was performed on a Rxi®5 Sil MS Integra column (Restek, Bellefonte, PA, USA) with 0.25 mm inner diameter and 0.25 µm film thickness, including a 5 m guard column according to Lippmann et al. [49]. Injection temperature was adjusted to 240 °C. The temperature program for GC separation was: 3 min 80 °C isothermal followed by a ramp of 5 °C min^{-1} to 300 °C for 5 min. Mass spectrometry (MS) data was recorded with Mass Lynx 4.1 (Waters Corporation, Milford, Massachusetts, USA) at a rate of 10 spectra s^{-1} in a range of 50–700 m/z. The metabolites were identified automatically with the internal software ChromaLynx (Waters Corporation, Milford, Massachusetty, USA) using the NIST 5 library and interesting components were verified manually by comparison with reference spectra. Relative quantification was based on comparative analysis of peak area.

5. Conclusions

The results of the present study demonstrate that even under controlled conditions, the soil type effects on the expression of bottom rot disease severity previously described in field experiments [3,23] were still detectable and even more strongly expressed. This underlines the importance of the culture conditions for determining the expression of disease severity and for antagonistic interactions with biocontrol agents. Our study showed for the first time that the release of benzoic and lauric acids as

root exudates with antifungal activity was triggered by the presence of the pathogen *Rhizoctonia solani* and even more strongly by the bacterial inoculants *Pseudomonas jessenii* RU47 and *Serratia plymuthica* 3Re4-18 in a soil type-specific manner. The highest level of antifungal root exudates was detectable in the rhizosphere soil solutions of the LL soil with the lowest conductivity for bottom rot disease. These findings strongly suggest a role of the antifungal root exudates in the defense reaction of lettuce against bottom rot disease as a biocontrol mechanism of *P. jessenii* RU47 and *S. plymuthica* 3Re4-18. This further underlines the central role of the host plant status which in turn is determined by physical and chemical soil properties, the interactions with the respective soil microbiomes, and of course climatic conditions. Obviously, high rhizosphere competence and a high antagonistic potential of bacterial inoculants are important, but not the only features determining a successful establishment of biocontrol effects in lettuce cultivation.

Supplementary Materials: The following materials are available online at www.mdpi.com/2073-4395/7/2/44/s1. Figure S1. Shoot dry mass of *Lactuca sativa* L. cv. Tizian. The plants were grown on loess loam without bacterial inoculation (w/o Bac.), pre-inoculated with *Pseudomonas jessenii* RU47 (+*P. jess.*), *Serratia plymuthica* 3Re-4-18 (+*S. ply.*), a combination of both (+*P. jess*/*S. ply.*), with and without (Control) subsequent inoculation with *Rhizoctonia solani* AG1-IB (+*Rhizoctonia*). Means ± SE of four independent replicates. Figure S2. Root hair length (mm) of *Lactuca sativa* L. cv. Tizian. The plants were grown on loess loam without bacterial inoculation (w/o Bac.), pre-inoculated with *Pseudomonas jessenii* RU47 (+*P. jess.*), *Serratia plymuthica* 3Re-4-18 (+*S. ply.*), a combination of both (+*P. jess*/*S. ply.*), with and without (Control) subsequent inoculation with *Rhizoctonia solani* AG1-IB (+*Rhizoctonia*). Means ± SE of four independent replicates. Figure S3. Micronutrient concentration of Zn (a) and Mn (b) in shoots (mg kg^{-1} DM) of *Lactuca sativa* L. cv. Tizian. The plants were grown on loess loam without bacterial inoculation (w/o Bac.), pre-inoculated with *Pseudomonas jessenii* RU47 (+*P. jess.*), *Serratia plymuthica* 3Re-4-18 (+*S. ply.*) a combination of both (+*P. jess*/*S. ply.*) with and without (Control) subsequent inoculation with *Rhizoctonia solani* AG1-IB (+*Rhizoctonia*). Means ± SE of four independent replicates. Figure S4. Symptoms of micronutrient deficiencies (Zn, Mn, Fe) in *Lactuca sativa* L. cv. Tizian grown in a hydroponic culture system with controlled supply of mineral nutrients: 2 mM Ca $(NO_3)_2$, 0.25 mM KH_2PO_4, 0.7 mM K_2SO_4, 0.1 mM KCl, 0.5 mM $MgSO_4$, 80 µM/10 µM Fe-EDTA, 10 µM H_3BO_3, 0.5 µM/0.1 µM $ZnSO_4$, 0.5 µM/0.1 µM $MnSO_4$, 0.2 µM $CuSO_4$ and 0.01 µM $(NH_4)_6Mo_7O_{24}$.

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References

1. Oerke, E.-C. Crop losses to pests. *J. Agric. Sci.* **2006**, *144*, 31–43. [CrossRef]
2. Grosch, R.; Schneider, J.H.M.; Kofoet, A.; Feller, C. Impact of continuous cropping of lettuce on the disease dynamics of bottom rot and genotypic diversity of *Rhizoctonia solani* AG 1-IB. *J. Phytopathol.* **2011**, *159*, 35–44. [CrossRef]
3. Schreiter, S.; Sandmann, M.; Smalla, K.; Grosch, R. Soil type dependent rhizosphere competence and biocontrol of two bacterial inoculant strains and their effects on the rhizosphere microbial community of field-grown lettuce. *PLoS ONE* **2014**, *9*, 1–11. [CrossRef] [PubMed]
4. Hallmann, J. *Einführung in die Thematik- Bedeutung bodenbürtiger Schaderreger*; Julius KühnInstitut: Münster, Germany, 2010; Volume 15, pp. 2–9.
5. Mitsuro, H.; Tadao, U. The Role of the overwintered Plant Debris and Sclerotia as Inoculum in the field occurred with Sugarbeet Root Rot. *Ann. Phytopathol. Soc. Jpn.* **1982**, *48*, 628–633.
6. Grosch, R.; Faltin, F.; Lottmann, J.; Kofoet, A.; Berg, G. Effectiveness of 3 antagonistic bacterial isolates to control *Rhizoctonia solani* Kühn on lettuce and potato. *Can. J. Microbiol.* **2005**, *51*, 345–353. [CrossRef] [PubMed]
7. Bonilla, N.; Gutiérrez-Barranquero, J.A.; De Vicente, A.; Cazorla, F.M. Enhancing soil quality and plant health through suppressive organic amendments. *Diversity* **2012**, *4*, 475–491. [CrossRef]

8. Weller, D.M.; Raaijmakers, J.M.; Gardener, B.B.M.; Thomashow, L.S. Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annu. Rev. Phytopathol.* **2002**, *40*, 309–348. [CrossRef] [PubMed]
9. Garbeva, P.; Postma, J.; Van Veen, J.A.; Van Elsas, J.D. Effect of above-ground plant species on soil microbial community structure and its impact on suppression of *Rhizoctonia solani* AG3. *Environ. Microbiol.* **2006**, *8*, 233–246. [CrossRef] [PubMed]
10. Bakker, M.G.; Manter, D.K.; Sheflin, A.M.; Weir, T.L.; Vivanco, J.M. Harnessing the rhizosphere microbiome through plant breeding and agricultural management. *Plant Soil* **2012**, *360*, 1–13. [CrossRef]
11. Bais, H.P.; Weir, T.L.; Perry, L.G.; Gilroy, S.; Vivanco, J.M. The role of root exudates in rhizosphere interactions with plants and other organisms. *Annu. Rev. Plant Biol.* **2006**, *57*, 233–266. [CrossRef] [PubMed]
12. Raaijmakers, J.M.; Paulitz, T.C.; Steinberg, C.; Alabouvette, C.; Moënne-Loccoz, Y. The rhizosphere: A playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant Soil* **2009**, *321*, 341–361. [CrossRef]
13. Badri, D.V.; Vivanco, J.M. Regulation and function of root exudates. *Plant Cell Environ.* **2009**, *32*, 666–681. [CrossRef] [PubMed]
14. Chaparro, J.M.; Badri, D.V.; Bakker, M.G.; Sugiyama, A.; Manter, D.K.; Vivanco, J.M. Root Exudation of Phytochemicals in Arabidopsis Follows Specific Patterns That are Developmentally Programmed and Correlate with Soil Microbial Functions. *PLoS ONE* **2013**, *8*, 1–10. [CrossRef]
15. Marschner, P.; Neumann, G.; Kania, A.; Weiskopf, L.; Lieberei, R. Spatial and temporal dynamics of the microbial community structure in the rhizosphere of cluster roots of white lupin (*Lupinus albus* L.). *Plant Soil* **2002**, *246*, 167–174. [CrossRef]
16. Neumann, G.; Bott, S.; Ohler, M.A.; Mock, H.-P.; Lippmann, R.; Grosch, R.; Smalla, K. Root exudation and root development of lettuce (*Lactuca sativa* L. cv. Tizian) as affected by different soils. *Front. Microbiol.* **2014**, *5*, 1–6. [CrossRef] [PubMed]
17. Neumann, G.; Römheld, V. The release of root exudates as affected by the plant physiological status. In *The Rhizosphere: Biochemistry and Organic Substances at the Soil-Plant Interface*; Pinton, R., Varanini, Z., Nannipieri, P., Eds.; CRC Press: Boca Raton, FL, USA, 2007; pp. 23–72.
18. Neumann, G. Root exudates and nutrient cycling. In *Soil Biology Volume 10*; Marschner, P., Rengel, Z., Eds.; Springer: Berlin, Germany, 2007; pp. 123–157.
19. Aziz, N.H.; El-Fouly, M.Z.; El-Essawy, A.A.; Khalaf, M.A. Influence of bean seedling root exudates on the rhizosphere colonization by *Trichoderma lignorum* for the control of *Rhizoctonia solani*. *Bot. Bull. Acad. Sin.* **1997**, *38*, 33–38.
20. Alabouvette, C.; Olivain, C.; Steinberg, C. Biological control of plant diseases: The European situation. *Eur. J. Plant Pathol.* **2006**, *114*, 329–341. [CrossRef]
21. Scherwinski, K.; Grosch, R.; Berg, G. Effect of bacterial antagonists on lettuce: Active biocontrol of *Rhizoctonia solani* and negligible, short-term effects on nontarget microorganisms. *FEMS Microbiol. Ecol.* **2008**, *64*, 106–116. [CrossRef] [PubMed]
22. Grosch, R.; Dealtry, S.; Schreiter, S.; Berg, G.; Mendonça-Hagler, L.; Smalla, K. Biocontrol of *Rhizoctonia solani*: Complex interaction of biocontrol strains, pathogen and indigenous microbial community in the rhizosphere of lettuce shown by molecular methods. *Plant Soil* **2012**, *361*, 343–357. [CrossRef]
23. Schreiter, S.; Ding, G.C.; Heuer, H.; Neumann, G.; Sandmann, M.; Grosch, R.; Kropf, S.; Smalla, K. Effect of the soil type on the microbiome in the rhizosphere of field-grown lettuce. *Front. Microbiol.* **2014**, *5*, 1–13. [CrossRef] [PubMed]
24. Yoon, M.; Seo, K.H.; Lee, S.H.; Choi, G.J.; Jang, K.S.; Choi, Y.H.; Cha, B.; Kim, J.-C. Antifungal Activity of Benzoic Acid from *Bacillus subtilis* GDYA-1 against Fungal Phytopathogens. *Res. Plant Dis.* **2012**, *18*, 109–116. [CrossRef]
25. Walters, D.R.; Walker, R.L.; Walker, K.C. Lauric Acid Exhibits Antifungal Activity against Plant Pathogenic Fungi. *J. Phytopathol.* **2003**, *151*, 228–230. [CrossRef]
26. Lee, J.G.; Lee, B.Y.; Lee, H.J. Accumulation of phytotoxic organic acids in reused nutrient solution during hydroponic cultivation of lettuce (*Lactuca sativa* L.). *Sci. Hortic.* **2006**, *110*, 119–128. [CrossRef]
27. Bergmann, W. *Ernährungsstörungen bei Kulturpflanzen*; Gustav Fischer Verlag: Jena, Germany, 1988.
28. Grosch, R.; Schneider, J.H.M.; Kofoet, A. Characterisation of *Rhizoctonia solani* anastomosis groups causing bottom rot in field-grown lettuce in Germany. *Eur. J. Plant Pathol.* **2004**, *110*, 53–62. [CrossRef]

29. Lehtonen, M.J.; Somervuo, P.; Valkonen, J.P.T. Infection with *Rhizoctonia solani* induces defense genes and systemic resistance in potato sprouts grown without light. *Phytopathology* **2008**, *98*, 1190–1198. [CrossRef] [PubMed]
30. Li, X.G.; Zhang, T.L.; Wang, X.X.; Hua, K.; Zhao, L.; Han, Z.M. The composition of root exudates from two different resistant peanut cultivars and their effects on the growth of soil-borne pathogen. *Int. J. Biol. Sci.* **2013**, *9*, 164–173. [CrossRef] [PubMed]
31. Jayaraj, J.; Bhuvaneswari, R.; Rabindran, R.; Muthukrishnan, S.; Velazhahan, R. Oxalic acid-induced resistance to *Rhizoctonia solani* in rice is associated with induction of phenolics, peroxidase and pathogenesis-related proteins. *J. Plant Interact.* **2010**, *5*, 147–157. [CrossRef]
32. Lugtenberg, B.; Dekkers, L. What makes *Pseudomonas* bacteria rhizosphere competent. *Environ. Microbiol.* **1999**, *1*, 9–13. [CrossRef] [PubMed]
33. Berg, G. Plant-microbe interactions promoting plant growth and health: Perspectives for controlled use of microorganisms in agriculture. *Appl. Microbiol. Biotechnol.* **2009**, *84*, 11–18. [CrossRef] [PubMed]
34. Zachow, C.; Fathehi, J.; Cardinale, M.; Tilcher, R.; Berg, G. Strain-specific colonization pattern of *Rhizoctonia* antagonists in the root system of sugar beet. *FEMS Microb. Ecol* **2010**, *74*, 124–135. [CrossRef] [PubMed]
35. Van Loon, L.C.; Bakker, P.A.H.M. PGPR: Biocontrol and Biofertilization. In *Springer, Dordrecht, The Netherlands*; Siddiqui, Z.A., Ed.; Springer: Dordrecht, The Netherlands, 2005; pp. 39–66.
36. Adesina, M.F.; Grosch, R.; Lembke, A.; Vatchev, T.D.; Smalla, K. In vitro antagonists of *Rhizoctonia solani* tested on lettuce: Rhizosphere competence, biocontrol efficiency and rhizosphere microbial community response: Research article. *FEMS Microbiol. Ecol.* **2009**, *69*, 62–74. [CrossRef] [PubMed]
37. De Vleesschauwer, D.; Chernin, L.; Höfte, M.M. Differential effectiveness of *Serratia plymuthica* IC1270-induced systemic resistance against hemibiotrophic and necrotrophic leaf pathogens in rice. *BMC Plant Biol.* **2009**, *16*, 1–16. [CrossRef] [PubMed]
38. Adesina, M.F.; Lembke, A.; Costa, R.; Speksnijder, A.; Smalla, K. Screening of bacterial isolates from various European soils for in vitro antagonistic activity towards *Rhizoctonia solani* and *Fusarium oxysporum*: Site-dependent composition and diversity revealed. *Soil Biol. Biochem.* **2007**, *39*, 2818–2828. [CrossRef]
39. Adam, E.; Müller, H.; Erlacher, A.; Berg, G. Complete genome sequences of the *Serratia plymuthica* strains 3Rp8 and 3Re4-18, two rhizosphere bacteria with antagonistic activity towards fungal phytopathogens and plant growth promoting abilities. *Stand. Genom. Sci.* **2016**, *11*, 2–8. [CrossRef] [PubMed]
40. Brandel, J.; Humbert, N.; Elhabiri, M.; Schalk, I.J.; Mislin, G.L.A.; Abrecht-Gary, A.-M. Pyochelin, a siderophore of *Pseudomonas aeruginosa*: Physicochemical characterization of the iron (III), copper (II) and zinc (II) complexes. *Dalton Trans.* **2012**, *41*, 2820–2834. [CrossRef] [PubMed]
41. Audenaert, K.; Patterey, T.; Cornelis, P.; Höfte, M. Induction of Systemic Resistance to *Botrytis cinerea* in Tomato by *Pseudomonas aeruginosa* 7NSK2: Role of Salicylic Acid, Pyochelin, and Pyocyanin. *Mol. Plant-Microbe Interact.* **2002**, *15*, 1147–1156. [CrossRef] [PubMed]
42. Neumann, G.; Römheld, V. Root-induced changes in the availability of nutrients in the rhizosphere. In *Plant Roots, The Hidden Half*; Waisel, Y., Eshel, A., Kafkafi, U., Eds.; CRC Press: Boca Raton, FL, USA, 2002; pp. 617–649.
43. Glick, B.R.; Cheng, Z.; Czarny, J.; Duan, J. Promotion of plant growth by ACC deaminase-producing soil bacteria. In *New Perspect. Approaches Plant Growth-Promoting Rhizobacteria Resarch*; Bakker, P.A.H.M., Raaijmakers, J.M., Bloemberg, G., Höfte, M., Lemanceau, P., Cooke, B.M., Eds.; Springer: Dordrecht, The Netherlands, 2007; pp. 329–339.
44. Dandurishvili, N.; Toklikishvili, N.; Ovadis, M.; Eliashvili, P.; Giorgobiani, N.; Keshelava, R.; Tediashvili, M.; Vainstein, A.; Khmel, I.; Szegedi, E.; et al. Broad-range antagonistic rhizobacteria *Pseudomonas fluorescens* and *Serratia plymuthica* suppress Agrobacterium crown gall tumours on tomato plants. *J. Appl. Microbiol.* **2010**, *110*, 341–352. [CrossRef] [PubMed]
45. Gericke, S.; Kurmis, B. Die kolorimetrische Phosphorsäurebestimmung mit Ammonium-Vanadat-Molybdat und ihre Anwendung in der Pflanzenanalyse. *Zeitschrift der Pflanzenernährung/Bodenkd* **1958**, *59*, 235–247.
46. Haase, S.; Neumann, G.; Kania, A.; Kuzyakov, Y.; Römheld, V.; Kandeler, E. Elevation of atmospheric CO_2 and *N*-nutritional status modify nodulation, nodule-carbon supply, and root exudation of *Phaseolus vulgaris* L. *Soil Biol. Biochem.* **2007**, *39*, 2208–2221. [CrossRef]
47. Marschner, H. *Mineral Nutrition of Higher Plants*, 2nd ed.; Academic Press: London, UK, 1995.

48. Weisskopf, L.; Abou-Mansour, E.; Fromin, N.; Tomasi, N.; Santelia, D.; Edelkott, I.; Neumann, G.; Aragno, M.; Tabacchi, R.; Martinoia, E. White lupin has developed a complex strategy to limit microbial degradation of secreted citrate required for phosphate acquisition. *Plant Cell Environ.* **2006**, *29*, 919–927. [CrossRef] [PubMed]
49. Lippmann, R.; Kaspar, S.; Rutten, T.; Melzer, M.; Kumlehn, J.; Matros, A.; Mock, H.P. Protein and metabolite analysis reveals permanent induction of stress defense and cell regeneration processes in a tobacco cell suspension culture. *Int. J. Mol. Sci.* **2009**, *10*, 3012–3032. [CrossRef] [PubMed]