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Trick of the Trade: Unveiling the Importance of Feedstock Chemistry in *Trichoderma*-Organic Amendments-Based Bio-Stimulants

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Abstract: We investigated the effect of *Trichoderma harzianum* in combination with biochar or other organic feedstocks, i.e., fish meal, Medicago, and maize straw, on the growth of *Lens culinaris*, *Zea mays*, *Oryza sativa*, and *Glycine max*. Biochar and other organic feedstocks were characterized by ^{13}C-CPMAS NMR spectroscopy. Fish and Medicago had low C/N and high N content, while biochar, maize, and AC (Activated Carbon) had high C/N. pH ranged from 9.38 for biochar to 5.67 for AC. ^{13}C-CPMAS NMR showed large chemical changes in organic mixtures leading to aromatic C-type enrichment in the presence of biochar or AC. Biochar and organic feedstocks inoculated with *T. harzianum* showed different effects, ranging from inhibition to crop stimulation. Overall, out of 88 cases, *T. harzianum* inoculum had a positive effect on root length in 46 cases (52.2%). The effect of fungal inoculum was particularly positive when combined with AC or biochar and when non-pyrogenic amendments were present. In contrast, a negative effect was observed when *T. harzianum* was inoculated with N-rich non-stabilized organic amendments. Further research is needed to identify the specific mechanisms underlying the inhibitory and bio-stimulatory effects of *Trichoderma* mixtures with organic amendment for the right combinations of raw materials that maximize crop productivity.

Keywords: beneficial microbes; biochar; rice; maize; ^{13}C CPMAS NMR; *terra preta*

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1. Introduction

Bio-stimulants for plants are products containing mineral elements, organic compounds, and/or microorganisms that stimulate natural plant processes and whose effects are partially independent of their nutrient content [1]. The importance of bio-stimulants for sustainable agriculture has increased significantly in recent years. The reduction in the number of fungicides that can be used to control fungal diseases [2] and the increase in the cost of mineral fertilizers [3] have encouraged the research and marketing of a wide range of bio-stimulant products. Bio-stimulants can be produced from a variety of organic matter of plant (e.g., crop residues, tree pruning, algae) and animal (e.g., sewage, manure, blood) origin, with the possible integration of beneficial microorganisms [4]. There are numerous beneficial microorganisms used as bio-stimulants, including bacteria of the genera *Bacillus*, *Pseudomonas*, *Streptomyces*, and fungi of the genus *Trichoderma*, which are the most commonly used [5].

The genus *Trichoderma* includes species that have biocontrol and bio-stimulatory effects thanks to a variety of mechanisms such as the production of antibiotics and substances with auxin-like activity, the ability to compete in the rhizosphere, the induction of resistance in

plants, and mycoparasitic activity [6]. Thanks to these properties, *Trichoderma* has become one of the most widely used beneficial microorganisms in the world [7]. *Trichoderma* strains can be used alone, in combination with other microorganisms, including mycorrhizal fungi to form microbial consortia [8], or mixed with organic amendments. For example, *Trichoderma* can be added after the thermophilic phase of the composting process to obtain fortified compost [9]. In this context, the combined use of *Trichoderma* with biochar has been evaluated and proposed in recent years.

According to the IBI (International Biochar Initiative) [10], biochar is a solid substance produced by the pyrolysis of biomass in a low oxygen environment. It is a carbon-rich substance with a wide range of uses in agricultural systems. Depending on the chemical composition of the feedstock and the pyrolysis conditions, i.e., temperatures, duration, and availability of oxygen, different forms of biochar can be produced [11]. The benefits of biochar to agricultural yields have been known since ancient times. The earliest evidence is provided by pre-Columbian peoples who created "*terra preta*" soils in the Amazon basin [12]. The advantages of using biochar as an organic amendment to increase agricultural yields and suppress soilborne pathogens have been demonstrated by extensive scientific research [13,14]. The beneficial effects of biochar are caused by several mechanisms, such as its liming ability [15], improved water retention in the soil [16], adsorption of potentially toxic phytochemicals [17], and stimulation of a good microbiota that helps in the suppression of pathogens in the soil [18]. Biochar is generally characterized by a high specific surface area and a highly porous structure. These properties explain the ability of biochar to adsorb phytotoxic organic compounds [19] with a variety of applications including silage production to limit the accumulation of fungal mycotoxins and pesticides [20] and remediation of water and soil contamination [21]. The porous structure has been exploited to use biochar as a carrier for beneficial microorganisms. Postma et al. [22] reported that the plant growth-promoting rhizobacteria *Pseudomonas chlororaphis*, *Peanibacillus polymyxa*, and *Streptomyces pseudovenezuelae* effectively colonized biochar derived from animal bones. Regarding fungi, some studies reported that *Trichoderma* strains can colonize, grow, and survive the porous structure of biochar and hydrochar [23].

Finding effective methods to increase the efficacy of microbial-based bio-stimulants, including the use of organic amendments, is an urgent concern. In this context, the systematic study of the interaction between beneficial microorganisms and organic amendments is a fundamental step towards the development of effective and reliable products. The aim of this work was to investigate the possibility of producing low-cost and effective plant-based bio-stimulants from a mixture of *Trichoderma* and organic amendments. To this end, we investigated the bio-stimulant effects of five organic amendments with different chemical properties (i.e., two biochar species, fish meal, Medicago, and maize straw) used alone or in all possible combinations with *Trichoderma harzianum* strain *T22* on the growth of four important crops, namely *Lens culinaris*, *Glycine max*, *Oryza sativa*, and *Zea mays*. The organic amendments were chemically characterized by ^{13}C-cross-polarisation magic angle spinning (CPMAS) nuclear magnetic resonance spectroscopy (NMR) and standard elemental analyses. The specific objectives of this study were to achieve the following: (i) evaluate the effect of biochar and other organic amendments, alone and in mixtures with *T. harzianum*, on the growth of four plants, i.e., lentil, maize, rice, and soybean; (ii) explore the relationship between the initial chemical traits of the organic starting material and the observed effect of bio-stimulation.

2. Materials and Methods

2.1. Organic Feedstock and Trichoderma harzianum Strain

Five feedstocks with different chemical properties were selected: (1) pelleted fish meal, hereafter referred to as fish; (2) *Medicago sativa* straw (referred to as Medicago); (3) *Zea mays* straw (referred to as Maize); (4) biochar prepared from sawdust and pyrolyzed at 550 °C (referred to as Biochar); (5) activated carbon (obtained from Sigma-Aldrich Co., St. Louis,

MO, USA, referred to as AC). Here, we used AC as a standard high porosity material to compare biochar and other non-pyrogenic feedstocks.

2.2. Trichoderma-Organic Amendment Mixture Preparation

The five feedstocks were used individually and in combination. Specifically, a loading ratio of 50:50 (*w*/*w*) of finely ground feedstocks was used in the two-substance mixture. The following six mixtures were prepared: AC + fish, AC + Medicago, AC + Maize, biochar + fish, biochar + Medicago, biochar + Maize. Each 500-mL flask contained 25 g of each feedstock for a total of 50. For the pure materials, biochar, AC, fish, Medicago, and Maize in amounts of 50 g were added to 500-mL flasks. The feedstocks used alone (i.e., biochar, AC, fish, Medicago, and Maize) and the six mixtures (AC + fish, AC + Medicago, AC + Maize, biochar + fish, biochar + Medicago, biochar + Maize) were then used without and with inoculum of *T. harzianum* strain *T22*. The inoculum was prepared by adding 3 mL of a conidial suspension at a concentration of 1×10^7 to the flasks. Then, 250 mL of distilled water was added to the flasks, which were then placed in an orbital shaker and incubated at 24 °C. We chose two incubation times (2 and 100 days) to evaluate the degradation of pure feedstocks and their combination with and without *T. harzianum* inoculum. A total of 11 types of feedstocks were prepared and incubated for 2 or 100 days with and without *T. harzianum* inoculum, resulting in a total of 44 combinations.

2.3. Trichoderma-Organic Amendment Chemical Characterization

Centrifugation (2395 g, 10 min) of the suspension in the flasks separated the liquid and bulk fractions of the starting materials and their mixtures at each sampling time point (2 and 100 days). The liquid fraction was immediately used for the bioassay. The bulk fraction from the centrifugation process was instead subjected to ^{13}C CPMAS NMR analysis to characterize the chemical properties of the organic matter.

After drying, organic feedstocks were chemically characterized for total carbon (C) and total nitrogen (N) by microsample flash combustion (samples of 5 mg—Elemental Analyzer NA 1500 Fison 1108 Elemental Analyzer, Thermo Fisher Scientific, Waltham, Ma, USA). The pH and electrical conductivity (EC) of the liquid fraction were measured using a pH meter and a conductometer, respectively. Untreated feedstocks and materials incubated for 100 days were also characterized using ^{13}C solid state analysis CPMAS NMR. This method allows evaluation of the chemical changes that occur in the organic carbon fractions during decomposition [24]. The ^{13}C CPMS NMR spectra were acquired using a Bruker AV -300 NMR spectrometer (Bruker Instrumental Inc., Billerica, MA, USA) equipped with a Magic Angle Spinning (MAS) and a 4 mm diameter probe. The calibrated values for the recording were as follows: 2 sec recycling time; 1H power for CP 92.16 W: 1H-90° pulse 2.85 µs, ^{13}C power for CP 150, 4 W, 1 ms contact time, 2000 scans, and 20 ms acquisition time. The data were analysed by dividing the spectra into seven regions to identify the organic carbon types [25]. Specifically, the following seven chemical shift ranges were used: 0 to 45 ppm = alkyl-C + α amino-C; 46 to 60 ppm = methoxyl- and N-alkyl-C; 61 to 90 ppm = O-alkyl-C; 91 to 110 ppm = di-O-alkyl-C; 111 to 140 ppm = H- and C-substituted aromatic C; 141 to 160 ppm = O-substituted aromatic C; 161 to 190 ppm = carbonyl-C. The relative value contribution for a given region was quantified by integrating Mestre-Nova 6.2 and then expressed as a percentage of the total area. For each chemical characterization, three replicates were conducted.

2.4. Trichoderma-Organic Amendment Bioassay

The bioassay, termed the 'root growth' experiment, was conducted to evaluate the bio-stimulatory effect of pure organic feedstocks and their mixtures on the growth of four plants: lentils (*Lens culinaris*), soybeans (*Glycine max*), maize (*Zea mays*), and rice (*Oryza sativa*). Briefly, two sterile filter paper disks (Whatman Grade 1) were placed in Petri dishes (diameter: 9 cm) and 5 mL of the liquid fraction was added. The controls consisted of sterile distilled water. Petri dishes were placed in a growth cabinet with a temperature

of 24 °C and no light. After 120 h for lentils and soybean and 168 h for maize and rice, the length of seedling roots was measured. Five replicates were used for each treatment, consisting of ten seeds in a Petri dish for each plant species. A total of 885 experimental units containing 8850 seeds were prepared (11 NPOA types and mixtures × 2 incubation times × 2 *T. harzianum* inoculum × 4 target species × 5 replicates plus the sterile water control).

2.5. Data Analyses and Visualization

Bioassay data were normalized with respect to controls, and significant differences in root length were tested with a three-way analysis ANOVA using feedstock type, incubation time, and *T. harzianum* inoculum as independent factors. Three-way analysis ANOVA was performed separately for the four target plant species, using Tukey's HSD post-hoc test to test for pairwise differences. Statistical analyses were carried out using STATISTICA 14.1.0 software (StatSoft Inc., Tulsa, OK, USA).

The effect of *T. harzianum* inoculum on root length was also analyzed using a modified version of the RII (relative interaction index), a symmetrically distributed index that ranges from +1 to −1 and is commonly used in plant ecology [26]. In this case, we named the index *Trichoderma* Interaction Index (TII), where negative values of TII indicate a negative effect of the inoculum, while positive values indicate a positive effect of the fungal inoculum. In this case, we calculated the TII as follows:

$$\text{TII} = (\text{Root T} - \text{Root NT})/(\text{Root T} - \text{Root NT})$$

where Root T and Root NT are the root length measured in the presence of *T. harzianum* and without the inoculum, respectively. *t*-tests (with two tails, $p < 0.05$) were performed to determine whether TII values were significantly different from zero.

Finally, to analyze the relationship between the effect of *T. harzianum* and the chemistry of the organic feedstocks, a comprehensive correlation analysis was performed between TII and the organic carbon, total nitrogen, C/N ratio, pH, EC, and ^{13}C CPMAS NMR fractions of the studied material.

3. Results

3.1. Organic Feedstock and Mixture Chemical Characterization

Fish and Medicago both had low C/N ratios and high N contents (Table 1). In contrast, biochar, maize, and AC had high C/N ratios due to low N content, with AC and biochar having very high C content. The pH showed a wide range, from 9.38 for biochar pyrolyzed at 550 °C to 5.67 for AC. Fish had an intermediate EC value, maize and Medicago had a high EC value, and biochar and AC had a low EC value. The pH of both pure feedstocks and mixtures slightly increased during incubation, while the EC value decreased (Table 1). EC of mixtures decreased during incubation when biochar and AC were present, especially after 100 days.

Significant variation was observed with respect to the C-types of the original organic starting materials (Table 2). In fish and Medicago, there were significant signals for alkyl-C (0−45 ppm), methoxyl- and N-alkyl-C (46−60 ppm), and carbonyl-C (161−190 ppm) fractions. In contrast, maize had a high relative abundance of the fractions di-O-alkyl-C (91−110 ppm) and O-alkyl-C (61−90 ppm), which are associated with sugars and polysaccharides. In both biochar and AC, the H- and C-substituted aromatic C fractions (111−140 ppm) were predominant. Due to the prevalence of aromatic C types, the compositions of biochar and AC were comparable and changed only minimally during decomposition (Table 2). Due to the very high relative abundance of the O-alkyl C type, undecomposed maize differed from all other materials; however, after decomposition, the O-alkyl C fraction decreased significantly (Table 2). Decomposed mixtures showed an intermediate composition between pyrogenic and non-pyrogenic raw materials, such as AC + fish, AC + Medicago, biochar—Medicago, and biochar—Maize. Overall, aromatic fractions increased in the decomposed combination compared to the original non-pyrogenic

feedstocks (Table 2). In addition, the carbonyl C, O-alkyl C, alkyl C, and di-O-alkyl C fractions increased during incubation for all mixtures compared to the initial relative amounts found in AC and biochar.

Table 1. Content of organic carbon (C), total nitrogen (N), C/N ratio, H/C ratio, pH, and electric conductivity (EC) of different organic feedstocks before and after incubation for 2 and 100 days.

	C (%)	N (%)	C/N	H/C	pH	EC (mS/cm)
Medicago	38.29	3.90	9.81	0.17	5.78	3.365
Maize	41.32	0.51	81.01	0.19	7.11	2.743
Biochar	77.71	0.41	189.53	0.03	9.38	0.150
AC	78.43	1.39	56.42	0.02	5.76	0.234
Fish	39.14	6.09	6.42	0.04	6.31	1.326
Medicago—2 d	40.10	3.93	10.20	0.18	5.82	3.450
Maize—2 d	40.38	0.49	82.40	0.19	7.05	2.650
Biochar—2 d	74.57	0.50	149.62	0.03	7.49	0.290
AC—2 d	76.56	1.47	52.08	0.02	5.67	0.264
Fish—2 d	43.12	6.06	7.12	0.05	6.25	1.295
AC + Fish—2 d	59.84	3.76	29.60	0.04	5.96	0.779
AC + EM—2 d	58.33	2.70	31.14	0.10	5.75	1.857
AC + Maize—2 d	58.47	0.98	67.24	0.11	6.36	1.457
Biochar + Fish—2 d	58.85	3.28	78.37	0.04	6.87	0.792
Biochar + Medicago—2 d	57.33	2.21	79.91	0.11	6.66	1.870
Biochar + Maize—2 d	57.47	0.49	116.01	0.11	7.27	1.470
Medicago—100 d	39.23	3.12	12.57	0.19	6.81	3.214
Maize—100 d	41.57	0.41	101.39	0.20	7.14	2.124
Biochar—100 d	73.83	0.48	153.81	0.03	7.54	0.245
AC—100 d	74.12	1.44	51.47	0.02	5.98	0.212
Fish—100 d	38.08	5.14	7.41	0.06	7.12	1.012
AC + Fish—100 d	56.10	3.29	29.44	0.04	6.55	0.612
AC + Medicago—100 d	56.68	2.28	32.02	0.10	6.40	1.713
AC + Maize—100 d	57.85	0.93	76.43	0.11	6.56	1.168
Biochar + Fish—100 d	55.96	2.81	80.61	0.05	7.33	0.628
Biochar + Medicago—100 d	56.53	1.80	83.19	0.11	7.18	1.729
Biochar + Maize—100 d	57.70	0.45	127.60	0.11	7.34	1.184

Table 2. Relative abundance of 13C CPMAS NMR spectra of undecomposed organic feedstock before and after 100 days of incubation. Different letters within indicate statistically significant differences (Duncan post hoc test, $p < 0.05$).

	Alkyl C (0−45)	Methoxyl and N-alkyl C (46−60)	O-Alkyl C (61−90)	di-O-Alkyl C (91−110)	H and C-Sub. aromatic C (111−140)	O-Sub. Aromatic C (141−160)	Carbonyl C (161−190)
Medicago	22.97	10.13	38.53	8.75	6.46	2.01	11.15
Maize	9.61	8.39	61.07	14.16	2.93	1.21	2.63
Biochar	9.30	4.17	5.56	7.07	61.71	4.41	7.79
AC	7.04	4.98	10.49	15.41	49.34	7.11	5.63
Fish	27.82	16.32	31.59	6.00	5.93	3.00	9.34
AC + Fish—2 d	17.43	10.65	21.04	10.70	27.63	5.05	7.49
AC + Medicago—2 d	15.01	7.55	24.51	12.08	27.90	4.56	8.39
AC + Maize—2 d	8.32	6.69	35.78	14.78	26.14	4.16	4.13
Biochar + Fish—2 d	18.56	10.24	18.57	6.53	33.82	3.70	8.57
Biochar + Medicago—2 d	16.13	7.15	22.04	7.91	34.09	3.21	9.47
Biochar + Maize—2 d	9.45	6.28	33.31	10.61	32.32	2.81	5.21
Medicago—100 d	8.63	10.69	47.71	14.39	10.79	4.01	3.78
Maize—100 d	7.58	10.00	54.77	15.58	7.31	2.47	2.29
Biochar—100 d	2.45	0.41	1.01	4.62	75.91	13.63	1.98
AC—100 d	8.66	3.13	3.37	10.65	58.54	6.89	8.77
Fish—100 d	17.42	9.99	29.04	9.92	10.83	6.75	16.05
AC + Fish—100 d	21.57	7.96	16.49	8.67	32.17	5.83	7.30
AC + Medicago—100 d	5.13	3.24	32.41	13.11	35.56	4.98	5.58
AC + Maize—100 d	3.88	7.58	44.19	15.02	22.49	3.44	3.38
Biochar + Fish—100 d	5.58	2.03	2.37	11.76	67.48	8.84	1.94
Biochar + Medicago—100 d	9.84	7.13	27.43	11.87	32.74	6.95	4.05
Biochar + Maize—100 d	6.08	5.68	33.45	12.78	31.87	7.77	2.37

3.2. Effect of Organic Feedstock and Their Mixture on Crop Growth

The effects of organic mixtures on plants were also significantly affected by the feedstock type and incubation time (Table S1). Undecomposed fish caused strong inhibition in all crops. Medicago straw caused slight inhibition in soybean and rice and weak stimulation in maize and lentils. Maize straw largely inhibited root growth of maize but did not affect soybean, whereas it stimulated root growth of rice and especially lentils. AC inhibited root growth of maize, did not affect soybean, but stimulated lentils and rice. Finally, biochar stimulated both grasses (maize and rice) but slightly inhibited lentils and soybean. All feedstock inhibitory effects were reduced after decomposition (Table S1). Although the inhibitory effect of fish on root length decreased after 100 days of incubation, the overall effect remained unfavorable. A slight inhibitory effect of Medicago straw on rice and soybean was still observed, while maize and lentils were stimulated. Decomposed maize straw increased the root length of all plants tested, with maize and lentil roots lengthening 183% and 300%, respectively, compared to controls.

The effects of the mixtures on root growth were also influenced by the feedstock type, incubation time, and target crop. For maize, root length ranged from inhibition (−10% for AC + maize 2 d) to very strong stimulation (+116% for Biochar + Medicago 2 d), with a significant positive effect compared with the untreated control in 9 out of a total of 12 mixtures (Table S1). The response of rice in the mixtures ranged from −51% in Biochar + Fish 2 d to +74% compared with the control in the AC + Medicago 100 d treatment. In lentils, root length was stimulated in 11 out of 12 cases compared to the control. Inhibition of root length (−12%) was observed only in the case of Biochar + Fish 2 d. In contrast, strong stimulation was observed in the other mixtures, peaking in the case of AC + Maize 100 d (+154%). In soybean, root length was stimulated by mixtures in only 4 out of 12 cases, with the lowest values for AC + Medicago 100 d (−64%) and the highest in the case of AC + Maize 2 d (+32%).

3.3. Effect of Trichoderma and Organic Feedstock on Crop Growth

The effects of Trichoderma inoculant on root length, as determined by TII, varied greatly depending on the feedstock type, mixture, and target crop (Figure 1). In maize, TII was negative in 13 cases and positive in 9 cases, ranging from almost complete inhibition (TII = −0.98 in fish 2 d) to strong stimulation (TII = +0.86 in biochar + maize 2 d). The response of rice to Trichoderma was highly variable, with a negative TII in 12 cases and a positive one in 10 cases. The worst results were obtained with biochar + fish 2 d, while substantial stimulation was recorded with fish 100 d (Figure 1). In soybean, TII was positive in 11 cases and negative in 11 cases. The lowest TII values were recorded in Medicago 2 d and fish 2 d, while the most positive response was recorded in biochar + Maize 100 d, AC + Medicago 100 d, and biochar + fish 100 d. Specifically, the TII value was positive in 16 cases and negative in only 6 cases. The highest TII values were obtained with AC 2 d and Fish 100 d (Figure 1).

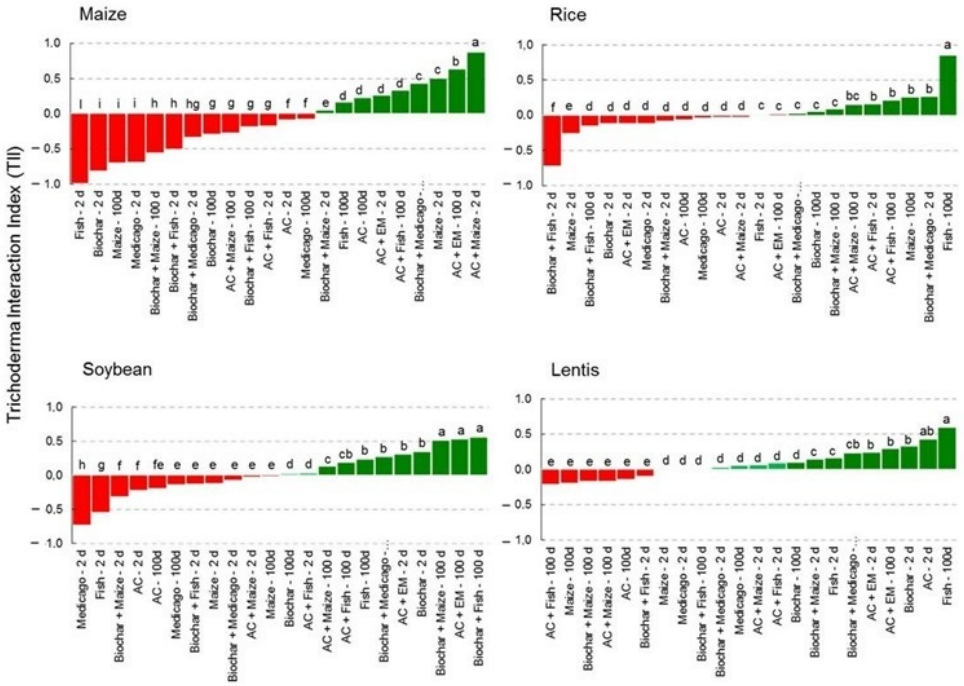

Figure 1. Responses of maize, rice, soybean, and lentils after being treated with organic feedstocks and their mixtures after 2 and 100 days of incubation in the absence and presence of *Trichoderma harzianum* T22 inoculum. Plant responses are expressed with the *Trichoderma* Interaction Index: positive values indicating stimulation and negative value inhibition compared to the same organic feedstock but without the inoculum with *T. harzianum*. Different letters indicate statistically significant differences within each target species (Duncan post hoc test, $p < 0.05$).

Regarding the relationship between TII and organic feedstock chemistry, no statistically significant correlations were found with organic carbon, total nitrogen, C/N ratio, H/C ratio, pH, and electrical conductivity (Figure 2). For the ^{13}C CPMAS NMR data, we found a positive correlation between the TII of maize and the di-O-alkyl C type, between the TII of soybean and the aromatic C type, and between the carbonyl C types and the TII of lentils. Negative correlations were found between the TII of soybean and the alkyl C and methoxyl C types, and in all other cases the correlations were weak and not statistically significant (Figure 2).

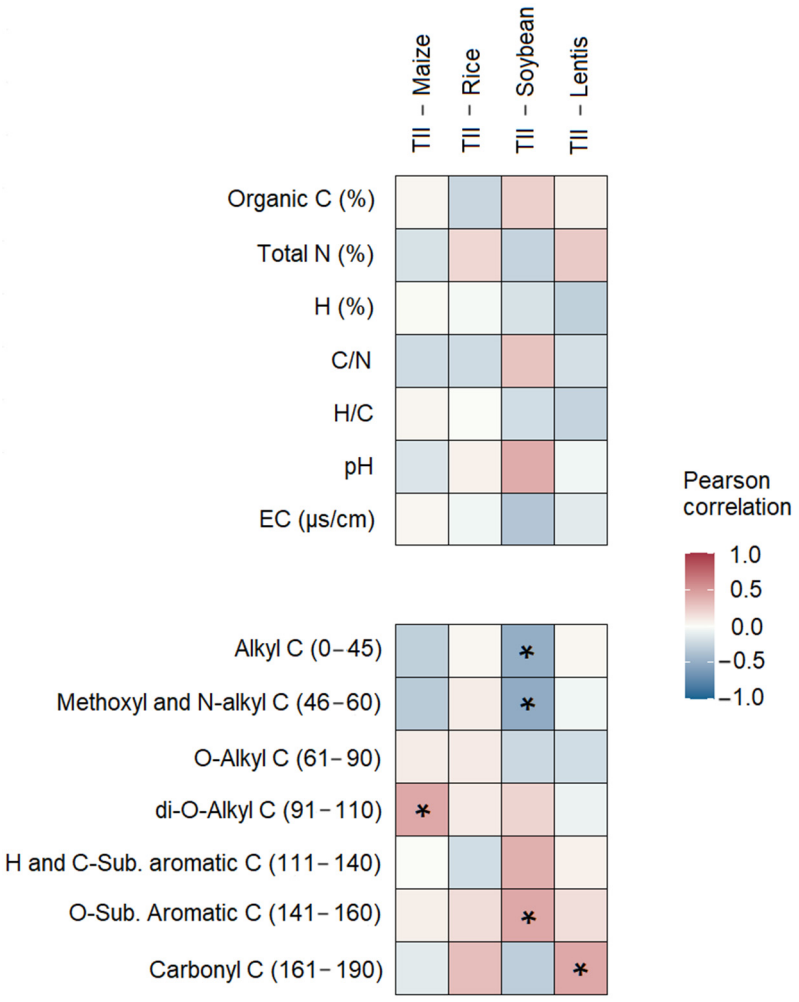

Figure 2. Heat-plot of correlation (Pearson's r) between Trichoderma Interaction Index (TII) for the four crops with elemental and 13C CPMAS NMR chemical characteristics of organic feedstocks. Asterisks indicate statistical significance for r ($p < 0.05$, after controlling for multiple comparisons according to Bonferroni's correction).

4. Discussion

Positive effects of *Trichoderma* inoculation on the growth of maize [27], rice [28], lentils [29], and soybean [30] have already been reported. However, doubts remain about the consistency and reliability of the bio-stimulatory effect related to variations in pH, soil texture, chemical composition, and native microbiota, as well as the adaptation of inoculated strains to the local microclimate. Here, we add to this list the variability caused by the application of organic amendments to the soil. Indeed, we found that the combination of organic amendments with *T. harzianum* had different effects on the root growth of the four plants studied, ranging from complete inhibition (e.g., in Medicago 2 d) to strong stimulation (e.g., in fish 100 d). Notably, the observed variability in the *Trichoderma*

interaction index was not correlated with carbon, nitrogen, C/N ratio, and pH of the organic amendments used, highlighting the complex interaction between organic matter and beneficial microbes. The synergistic and inhibitory effects on plant growth by organic amendments mixed with *T. harzianum* have not been fully elucidated.

The weakening of the plant upon contact with phytotoxic organic compounds could result in the interaction with *Trichoderma* changing from symbiotic to parasitic [31]. In this regard, undecomposed fish and Medicago straw alone have a significant inhibitory effect on root growth, a result consistent with previous findings reported for N-rich organic amendments [32]. Here, we found that inoculation with *T. harzianum* in the presence of undecomposed N-rich amendments further reduced plant growth. This indicates that the level of decomposition of organic amendments might be a key factor in determining their compatibility with *Trichoderma* inoculation. Alonso-Ramírez et al. [33] reported that *T. harzianum* can spread and invade the vascular tissue of *Arabidopsis thaliana* mutants that do not form a callose cell wall of root cells. As a result, without the activity of salicylic acid, plants were unable to prevent the spread of *T. harzianum* in the vascular system, which behaves like a pathogen and leads to plant collapse. Abiotic stress, such as the presence of phytotoxic organic compounds, could weaken the metabolic pathway of salicylic acid, resulting in plant weakening and promoting pathogenic behavior of *Trichoderma*. These results have relevant implications for agricultural use, as they indicate that the combined application of *T. harzianum* with non-stabilized N-rich amendments (e.g., fresh manure, fish, and meat meal) should be avoided.

Overall, our study revealed that out of 88 cases, the *T. harzianum* inoculum showed positive TII in 46 cases (52.2%), indicating a positive effect compared to the non-inoculated control. The effect of fungal inoculum was particularly effective when combined with AC or biochar and when non-pyrogenic amendments were present. For example, soybeans and lentils showed increased TII levels (>+0.5) when combined with decomposed Medicago straw and AC for 100 days. The positive contribution of biochar or AC is evidenced by the positive correlation between TII and aromatic C content of ^{13}C CPMAS NMR spectra. Several mechanisms could explain the synergistic effect of biochar and AC with *T. harzianum* inoculum. The addition of biochar or AC to a non-pyrogenic amendment could attenuate the phytotoxic effect of N-rich materials [32], thanks to its known ability to adsorb phytotoxic organic molecules [19,20]. Second, *T. harzianum* could exploit the porosity of biochar and the availability of organic carbon through the non-pyrogenic fraction to increase its population. In this regard, Hagemann et al. [34] demonstrated the formation of a nutrient-rich microbial complex and coating during biochar co-composting, covering the pore surfaces of biochar particles. The formation of such a nutrient-rich and highly biodiverse coating is associated with slow nitrate release, which helps explain the valuable growth-promoting effect of co-composted biochar. Previous studies have focused on examining the chemical composition of the coating [35–37], with less attention paid to the microbial composition. The present study also did not investigate the population density of *T. harzianum* in organic matrices during the decomposition process, an aspect that will certainly be explored in future studies. Indeed, to fully understand the factors causing differences among the treatments and to optimize the agricultural use of *Trichoderma*, further research is essential. Future studies should explore the population density of *T. harzianum* in different organic matrices during the decomposition process to identify the most suitable combinations of organic substrates. Additionally, investigating the microbial composition of the nutrient-rich coating formed during biochar co-composting could shed light on the mechanisms behind the growth-promoting effect of co-composted biochar. It is tempting to hypothesize that combinations of biochar and non-pyrogenic organic amendments could provide both a safe environment and food sources for the beneficial microbes, both of which would be critical in the early stages of soil or rhizosphere colonization. Determining the preferred food profile could also be very helpful in this situation to determine the appropriate combination of organic substrates [38]. Overall, our study highlights the complexity of the interaction between *Trichoderma*, organic amendments, and plants. The

varying responses observed in different combinations underscore the need for careful consideration when applying *Trichoderma* in agricultural practices. By gaining a deeper understanding of the factors influencing these interactions, we can develop more effective and reliable strategies for utilizing *Trichoderma* as a bio-stimulant to enhance crop growth and agricultural productivity.

5. Conclusions

Our results are another step towards the development of new biofertilizers based on biochar or AC in combination with non-pyrogenic organic amendments and the further addition of beneficial microbes. Our analysis combining ^{13}C CPMAS NMR is a first step towards identifying the right chemical mixture to promote *T. harzianum* establishment and beneficial activity. Overall, our work shows that an efficient method for converting organic feedstock into plant biofertilizer involves the right combination of biochar and organic amendments at a high loading rate. Our study also has the merit of showing the high variability in the effect of *T. harzianum*, ranging from complete inhibition to strong stimulation. The study of the mechanisms of the complex interaction between beneficial microbes, organic amendments, and plants will be essential to make the use of these agents predictable and reliable in the agricultural field. The study of the population dynamics of *Trichoderma* interacting with the native microbiome will be a fundamental aspect for the development of functionalized organic amendments with significant bio-stimulation of plants.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9090957/s1, Table S1: Root length of Maize, Rice, Soybean, and Lentis after treated with organic feedstocks and their mixtures after 2 and 100 days of incubation. Root length values are expressed as percentage compared to control with distiller water (100%), different letters indicate statistically significant differences within each target species in column (Duncan post hoc test, $p < 0.05$).

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