Science of the Total Environment xxx (xxxx) 179595

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com

Effect of *Streptomyces* spp. metabolites and the combination of biochar and compost on *Fusarium graminearum* inhibition, triticale growth, and soil properties

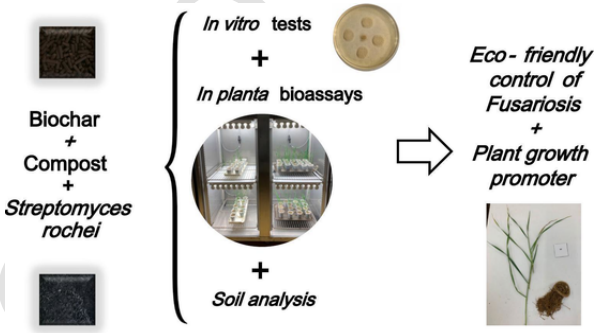
Laura Buzón-Durán [a,*], Beatriz Molinuevo-Salces [b], Mari Cruz García-González [b], Mercedes Sánchez-Báscones [b], Claudia Vitoria [c,d], Carmo Horta [c,d]

[a] Department of Agricultural and Forestry Engineering, ETSIIAA, Universidad de Valladolid, Avenida de Madrid 44, 34004 Palencia, Spain
[b] Department of Agroforestry Sciences, ETSIIAA, Universidad de Valladolid, Avenida de Madrid 44, 34004 Palencia, Spain
[c] Instituto Politécnico de Castelo Branco, Escola Superior Agrária, Quinta da Senhora de Mércules, 6001-909 Castelo Branco, Portugal
[d] CERNAS-IPCB Research Centre for Natural Resources, Environment and Society, Instituto Politécnico de Castelo Branco, 6001-909 Castelo Branco, Portugal

HIGHLIGHTS

- Biochar combined with *S. rochei* inhibits 100 % of fungal sporangia germination.
- *S. rochei* filtrates with biochar/compost inhibit pathogens.
- Secondary metabolites from *S. rochei* exhibit antimicrobial properties.
- Biochar, compost, and *S. rochei* improve soil quality and enzymatic activity.
- *S. rochei* filtrates with biochar/compost promote triticale growth.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Jose Julio Ortega-Calvo

Keywords:
Fusarium graminearum
Streptomyces spp.
Biochar
Compost
Antifungal
Secondary metabolites

ABSTRACT

Fusarium graminearum is the most harmful pathogen associated with Fusarium Head Blight (FHB) disease in triticale. Among the strategies that can be envisaged for its control, the reuse of organic residues for the production of secondary metabolites from *Streptomyces* spp. is particularly promising. The study presented herein focuses on the assessment of the antagonistic capacity of the culture filtrates of *Streptomyces rochei* alone, with compost, with biochar or with both of them, and their culture filtrates against *F. graminearum*. Firstly, the secondary metabolites were characterized by gas chromatography–mass spectrometry, with 5-Hydroxymethylfurfural, 2–3 Butanediol, Oxime-, methoxy-phenyl and acid butanoic being the most abundant chemical species. Subsequently, the capacity of *S. rochei* to inhibit the growth of the pathogen was tested in dual culture plate assays, finding 83 % inhibition. Sporangial tests showed that the mixture of *S. rochei* and biochar can inhibit 100 % of sporangia germination. Micropot trials conducted on triticale using the crop filtrates not only inhibited pathogen growth with all treatments but also improved crop growth. Hence, the cul-

* Corresponding author.
E-mail address: laura.buzon@uva.es (L. Buzón-Durán).

https://doi.org/10.1016/j.scitotenv.2025.179595
Received 18 February 2025; Received in revised form 14 April 2025; Accepted 1 May 2025
0048-9697/

ture filtrates of biochar, compost, compost and biochar, and the selected *Streptomyces* spp. culture filtrates may be put forward as promising protection treatments for the sustainable control of fusariosis.

1. Introduction

Triticale, a hybrid of wheat and rye, is susceptible to Fusarium Head Blight (FHB), with *Fusarium graminearum* being the most commonly isolated pathogen associated with FHB symptoms (Dinolfo et al., 2020). Besides triticale, the presence of *F. graminearum* has been reported in wheat (Xu et al., 2022), barley (Imboden et al., 2018), corn and rye (Logrieco et al., 1990), among others. When environmental conditions, such as temperature and humidity, are optimal for its occurrence, FHB is the prevalent disease in crops, which leads mainly to a reduction in yield and quality of grains. The susceptibility of triticale to FHB and the potential erosion of its genetic base underscore the importance of broadening its genetic variation to enhance resistance to pathogens, including *Fusarium* spp. (Góral et al., 2020). Furthermore, the subsequent higher accumulation of *Fusarium* spp. toxins in its grain, particularly deoxynivalenol (DON), posing risks to human and animal health. The genetic resistance of triticale cultivars to FHB and mycotoxin accumulation has been a subject of investigation, highlighting the complex interactions between the pathogen and the host plant (Góral et al., 2016).

The transition from the extensive use of chemical fungicides towards biocontrol strategies for managing fungal diseases in plants is of paramount importance. Chemical fungicides have historically been a cornerstone of disease management in agriculture, but their overreliance has led to detrimental effects on the environment and human health (Ons et al., 2020). Excessive use of synthetic chemicals has raised concerns about environmental toxicity and the development of resistance in fungal pathogens, which has prompted the search for alternative, eco-friendly disease management strategies (Singh and Chhatpar, 2011). Biocontrol strategies, which encompass the use of biological control agents (BCAs) such as fungi, bacteria, and other microorganisms, offer a less toxic and safer approach to reducing the severity of various crop diseases. These BCAs employ diverse mechanisms to protect plants against pathogenic invasion, including mycoparasitism, antibiosis, competition and induced resistance. Actinobacteria, in particular, have been shown to play a significant role in preventing important diseases in major crops and exhibit significant antagonistic activity against a variety of soil and airborne plant pathogens (Renuka and Ugandhar, 2024). The use of biocontrol agents presents several advantages, including their environmentally safe, sustainable and highly specific nature (dos Santos Gomes et al., 2021). They also offer the potential for broad-spectrum antagonistic activities against various phytopathogens. Furthermore, the combination of BCAs with chemical fungicides has been proposed as an integrated approach to enhance disease control while reducing the reliance on synthetic chemicals (McLaughlin et al., 2023).

In summary, the transition towards biocontrol strategies for managing fungal diseases in plants is driven by the need for more sustainable and environmentally friendly disease management practices. The potential of biocontrol agents to offer effective disease control while minimizing the negative impacts associated with chemical fungicides makes them a promising alternative for the future of agriculture. *Streptomyces* spp. are recognized as promising biocontrol agents due to their ability to produce a wide array of secondary metabolites with antimicrobial properties, making them effective in controlling plant pathogens. More than 7600 biological compounds, over 7500 biologically active secondary metabolites and approximately 75 % of all antibiotics are produced by *Streptomyces* spp. Because of this, *Streptomyces* is now the main source of antibiotics used in drug research (Alam et al., 2022). The secondary metabolites produced by *Streptomyces* spp. include antibiotics, antifungals and antibacterials, which play a crucial role in inhibiting the growth of plant pathogens and preventing plant-fungal infections (Alam et al., 2022; Khan et al., 2023). These bioactive compounds are essential for the biocontrol activity of *Streptomyces* spp., as they exhibit antagonistic activities against diverse plant diseases and have the potential to suppress the development of plant pathogens (Le et al., 2022). The production of these secondary metabolites is dependent on the nature of the signals received and sent by *Streptomyces* spp. and is influenced by various environmental factors such as nutrient availability and stress conditions. The diverse range of bioactive compounds produced by *Streptomyces* spp. enables them to effectively inhibit the growth of plant pathogens and contribute to the overall health and protection of plants (Khan et al., 2023). Therefore, the extensive secondary metabolism of *Streptomyces* spp. is of great importance in their potential application as biocontrol agents for managing fungal diseases in plants (Harir et al., 2018).

In addition, biochar and compost have been shown to significantly enhance soil quality and promote plant growth, particularly under challenging conditions such as saline irrigation. The combined application of biochar and compost has been found to improve soil fertility, water holding capacity and nutrient availability, leading to enhanced plant growth and productivity (Agegnehu et al., 2017). Moreover, the addition of biochar and compost has been demonstrated to increase soil organic carbon, cation exchange capacity and soil microbial biomass, which are essential for maintaining healthy soil and promoting plant growth (Liu et al., 2021). Furthermore, the slow decomposition of biochar in the soil results in long-term improvements in soil physiochemical properties, while the organic materials in compost encourage the growth of beneficial microorganisms, leading to increased nutrient availability and improved soil structure (Bello et al., 2023). Additionally, the application of biochar-compost mixtures has been identified as a new option for soil improvement, contributing to enhanced compost quality, nutritional value, safety and stability (Qian et al., 2023).

Because of the wide repertoire of activities of the chemical species present in *Streptomyces* spp. secondary metabolites, the aim of this study was: (1) to identify by gas chromatography–mass spectrometry (GC–MS) the secondary metabolites with antifungal and plant growth promoting activity generated by four treatments, namely *Streptomyces*, *Streptomyces*-biochar, *Streptomyces*-compost, and *Streptomyces*-compost-biochar; (2) to evaluate in vitro the antifungal activity of the secondary metabolites obtained in the four treatments against *F. graminearum*, through dual plate assays, sporangial germination mycelial growth-inhibition tests; (3) to test ex situ the antifungal activity of those secondary metabolites using triticale as plant test inoculated with *F. graminearum* and (4) to evaluate some soil fertility properties according to the four treatments tested to observe improvements in those soil properties compared to the original soil.

2. Materials and methods

2.1. Fungal isolate, reagents, biochar, compost, soil and seeds

The bacteria of the genus *Streptomyces*, *Streptomyces rochei* (DSM 41729), was purchased from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen; Braunschweig, Germany). The *Fusarium graminearum* Schwabe 1839 (CECT 20487) isolate came from CECT (Spanish Type Culture Collection, Valencia, Spain).

Phosphate buffer (for microbiology, APHA, pH 7.2), ethyl acetate (CAS 141-78-6), p-Nitrophenol solution (CAS 100-02-7) and citric acid (CAS 77-92-9) were supplied by Sigma-Aldrich Química S.A. (Madrid, Spain). Starch Casein Agar (SCA), Potato Dextrose Agar (PDA), Potato Dextrose Broth (PDB), Triptone Soye broth (TSB) and Yeast Malst Agar (ISP2), were purchased from Becton, Dickinson and Company (Franklin

Lakes, NJ, USA). TRIS (CAS 77-86-1), sodium bicarbonate (CAS 144-55-8), phosphate standard (CAS 7664-38-2), calcium standard (CAS 7697-37-2), magnesium standard (CAS 7697-37-2). Potassium antimonyl tartrate (CAS 28300-74-5), ammonium molybdate (CAS 13106-76-8), sodium bicarbonate (CAS 144-55-8), Tween® 20 (CAS 9005-64-5), potassium dichromate (CAS 7778-50-9), and sulfuric acid (CAS 7664-93-9) were acquired by Merck KGaA (Darmstadt, Germany). *p*-Nitrophenyl-β-glucoside (CAS 2492-87-7) was provided by Tokyo Chemical Industry (París, Francia). Ammonium acetate (CAS 631-61-8), boric acid (CAS 10043-35-3), ascorbic acid (CAS 50-81-7), maleic acid (CAS 110-16-7), ferrous sulfate hexahydrate (CAS 7783-85-9) and sodium hidroxyde (CAS 1310-73-2), were purchased by Chem Lab Supplies (Johannesburg, South Africa). Lanthanum (III) chloride heptahydrate (CAS 10025-84-0), calcium chloride (CAS 10043-52-4), lithium chloride (CAS 7447-41-8) and hydrogen chloride solution (CAS 7647-01-0) were acquired by Honeywell/Fluka (Seelze, Germany).

The biochar was obtained from the pyrolysis at 700 °C of untreated wood of fine sieve residues from woodchip production. The compost was initially performed from a mixture of digestate of pig slurry with oat straw and horse manure. This mixture was composted for seven months until a mature compost was obtained. Then, the final compost was pelletized. Table S1, showed the data sheet for biochar whereas Table S2 included the data sheet for compost and compost pellets. Compost, biochar and triticale seeds (×*Triticosecale* Wittmack var. Misionero) were supplied by the Escola Superior Agraria, Instituto Politecnico de Castelo Branco (Castelo Branco, Portugal).

2.2. Preparation of secondary metabolites of Streptomyces spp. and preparation of F. graminearum sporangial suspension

Lyophilized *S. rochei* (DSMZ 41729) was inoculated in TSB at 28 °C for 24 h and was seeded on SCA medium plates at 28 °C for 10 days. The plates were stored at 4 °C. Secondary metabolites were obtained following the method described by Sadigh-Eteghad et al. (2011) with some modifications. In the liquid culture medium (i.e. PDB), it was added 1 % of biochar, 1 % of compost or 1 % of compost and 1 % of biochar, resulting in four treatments. These treatments were treatment 1 (T1-*Streptomyces rochei*), treatment 2 (T2-*Streptomyces rochei*-biochar), treatment 3 (T3-*Streptomyces rochei*-compost) and treatment 4 (T4-*Streptomyces rochei*-compost-biochar). After fermentation, each final solution of the four cultures was treated with 50 mL of phosphate buffer and then sonicated for 5 min (pH 6.4). To quantify the concentration of bioactive compounds in the prepared solutions, the procedure described in Pazhanimurugan et al. (2016) was followed. First, the culture filtrates were centrifuged to separate the supernatant, which was then extracted with 100 mL of ethyl acetate. After the extraction, the solvent was evaporated under reduced pressure, allowing the metabolites to be concentrated. Finally, the residue was lyophilized to obtain the metabolites in a dry form. The concentration was determined by measuring the mass of the lyophilized extract and correlating it with the initial volume of the culture. This process resulted in culture filtrates with an approximate concentration of 2.0 $mg{\cdot}mL^{-1}$ (1.88 $mg{\cdot}mL^{-1}$ for secondary metabolites from T1-*S. rochei*, 1.90 $mg{\cdot}mL^{-1}$ for T2-*S. rochei* - biochar, 1.86 $mg{\cdot}mL^{-1}$ for T3-*S. rochei* - compost, and 1.9 $mg{\cdot}mL^{-1}$ for T4-*S. rochei* - compost - biochar).

For the preparation of *F. graminearum* conidial suspension, lyophilized *F. graminearum* (CECT 20487) was inoculated in PDB at 25 °C for 24 h and was seeded on PDA medium plates at 25 °C for 7 days. Subsequently, 5 plugs (10 mm in diameter) were inoculated in 150 mL of PDB. Seven-day-old PDB cultures (150 mL cultures kept at 26 °C, 135 rpm in an orbital stirrer incubator) were harvested for *F. graminearum* conidia. The suspension was filtered twice to eliminate hyphal fragments before measuring the spore concentration using a hemocytometer (Weber Scientific International Ltd., Teddington, Middlesex, UK). It was then amended with 0.2 % Tween 20® and adjusted to a final concentration of 1×10^6 spores (conidia)$\cdot mL^{-1}$.

2.3. Gas chromatography–mass spectrometry analysis of Streptomyces spp. secondary metabolites

To identify the secondary metabolites produced by *S. rochei* DSM 41729 in the four treatments tested; part of the culture filtrates was set aside. The culture filtrates were centrifuged, and the bioactive components were extracted from the supernatant using 100 mL of ethyl acetate. The concentrated mixture was then freeze-dried. Subsequently, 5 mL of HPLC-grade methanol was mixed with 25 mg of the freeze-dried aqueous extract to create a 5 $mg{\cdot}mL^{-1}$ solution. This was filtered and utilized for GC–MS analysis at the Research Support Services (STI) at Universidad de Alicante (Alicante, Spain), a quadrupole mass spectrometer model 5975C and a gas chromatograph model 7890A (both from Agilent Technologies, Santa Clara, CA, USA) were used to conduct the GC–MS study.

Chromatographic conditions: 1 μL injection volume; spitless mode with an injector temperature of 280 °C; initial oven temperature of 60 °C, 2 min; ramping up to a final temperature of 300 °C, 15 min at 10 $°C{\cdot}min^{-1}$. The Agilent Technologies HP-5MS UI chromatographic column, measuring 30 m in length, 0.250 mm in diameter, and 0.25 μm in thickness, was employed to separate the chemicals. Conditions for the mass spectrometer: 230 °C for the mass spectrometer's electron impact source and 150 °C for the quadrupole; 70 eV for ionization energy. The components were identified by computer matching with the National Institute of Standards database and by comparing their mass spectra and retention times with those of the authentic compounds and by computer matching with the database of the National Institute of Standards and Technology (NIST11).

2.4. Antifungal activity assessment

2.4.1. In vitro tests

Dual plate assays: With minor adjustments, the methodology of (Ghanem et al., 2022) was applied to evaluate the antagonistic effect of *F. graminearum* against *S. rochei*. PDA plates were used as the culture medium, and to achieve a concentration of 1×10^8 $CFU{\cdot}mL^{-1}$, 4 drops of 30 μL from TSB tubes that had been previously seeded with the four treatments of *S. rochei* at 26 °C for 18 h were added. Then, a 5 mm plug of *F. graminearum* was added to the center of the plate, while another plug was inoculated on a control plate (without any treatment). At 26 °C, the plates were incubated for seven days. The experiment was run in triplicate. Eq. (1), in accordance with Zambrano et al., 2021), was used to calculate the growth inhibitory effects on the pathogenic fungi as a percentage of pathogen growth inhibition (PGI):

$$PGI = (R1 - R2)/R1 \times 100 \quad (1)$$

where R1 = growth of the pathogenic fungus in the control plate and R2 = growth of the pathogenic fungus interacting with the antagonist.

Sporangia germination experiment: 50 μL of each of the 4 treatments suspension was added to a 96-well cell culture plate containing 20 μL of *F. graminearum* sporangia suspension, mixed, and then placed in a 20 °C incubator for 24 h. For the control, the *S. rochei* suspension was replaced with sterile distilled water. There were three experimental runs. Each experimental run had three replicates. The total and empty number sporangia in the field of view were recorded, and the mean values were recorded according to Eq. (2) (De Vrieze et al., 2018):

$$\text{Percentage Inhibition I } (\%) = \left[(R1 - R2)/R1\right] \times 100 \quad (2)$$

where R1 means control sporangia germination rate and R2 means treated sporangia germination rate.

Antifungal Activity Assessment for the four tested treatments (*T1–T4*): PDA plates with four holes (5 mm, the holes were distributed in four directions with the same distance from the center) were inoculated according to the following treatments: (T1) *S. rochei* strain treatment, where the *S. rochei* suspension (1×10^8 UCF·mL^{-1}, 0.1 mL) was inoculated in the holes of PDA plates; (T2) *S. rochei* strain combined with biochar; (T3) *S. rochei* strain combined with compost and (T4) *S. rochei* strain combined with biochar and compost. The treatment of biochar and compost was obtained by mixing compost and biochar in equal proportions. For T2, T3, and T4, *S. rochei* strain suspension containing 1 % biochar or compost (OD_{570} = 1, 0.1 mL) was inoculated in the holes of the respective PDA plates. Another assay was run as a control, using an ISP2 liquid medium. The biochar or compost without *S. rochei* was used as a control. In all the plates, 5 mm diameter mycelial plugs of *F. graminearum* (seven days old) were placed at the center of the plates. All the experiment was repeated three times. After culturing in the dark for 7 d at 25 °C, the colony diameters were determined. The inhibition rate was computed according to Eq. (3):

$$\text{Inhibition rate } (\%) = \left[1 - (\text{diameter of treated}/\text{diameter of control})\right] \times 100 \quad (3)$$

2.4.2. Ex situ micropot trials

A micropot experiment with two plants of triticale (×*Triticosecale* Wittmack var. Misionero) in each pot as crop test was performed in a phytoclimatic chamber (aralab, Rio de Mouro, Portugal). Test conditions: 23 °C day and 12 °C night with 16 h of light and 70–75 % relative humidity; light intensity of 90 μmol m^{-2} s^{-1} (PAR 5000 lx), during 35 days. Pathogen (i.e. *F. graminearum*) inoculation was carried out after seed germination (5 days). A concentration of 1×10^4 conidia/mL was added to each beaker. The soil used was derived from granitic rocks (Dystric Regosol; IUSS, 2015). Each pot contained 150 g of soil sieved at 2 mm. The four studied treatments with and without the presence of *S. rochei* and *F. graminearum*, resulted in a total of 12 assays with a total of 126 pots, which are summarized in Table S3.

For each assay, 4 replications were carried out, with 2 triticale plants for replication. To maintain the same fertilization conditions, each pot was irrigated with Hoagland solution with ½ of the concentration in N for a total quantity of 0.1 g Npot^{-1}. The pH of the solution was previously adjusted to pH = 5.5 with 0.1 M KOH. To apply 0.1 g N pot^{-1}, a total of 238 mL of Hoagland solution with ½ of N was needed to each pot. Therefore, the pots were irrigated, every day with water up to 70 % field capacity and 3 times a week with Hoagland solution and water up to the mentioned field capacity. Once a week, each pot was irrigated with 5 mL of the culture filtrates with the different treatments. After 35 days of the experiment, the plants were cut, the stems were separate from the roots and the corresponding analyses were carried out. The plants and roots were weighted for quantification of the biomass production. The soil was analyzed following the Standard Operating Procedures of the Global Soil Laboratory Network (Food and Agriculture Organization of the United Nations, 2021) for: pH, conductivity, organic matter, exchangeable cation composition and phosphorus. Soil enzymes were also analyzed: β-glucosidase (Eivazi and Tabatabai, 1988), acid phosphatase and alkaline phosphatase (Tabatabai and Bremner, 1969).

2.5. Statistical analysis

Data were subjected to analysis of variance (two-way ANOVA) in IBM SPSS Statistics v.25 software. Tukey's HSD test at 0.05 probability level was used for the post hoc comparison of means of the treatments with significant differences ($p < 0.05$; $p < 0.01$ or $p < 0.001$). Principal Component Analyses (PCA) and PERMANOVA were carried out using the R program.

3. Results

3.1. GC–MS characterization of secondary metabolites for treatments 1 to 4

The main secondary metabolites identified for *S. rochei* DSM 41729 (T1) were 5-Hydroxymethylfurfural (15.1 %); 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (8.6 %); Hydrazinecarbothioimidate, *N*, *N*-dimethyl-S-ethyl (4.0 %) and Oxime, methoxy-phenyl (3.8 %). For *S. rochei* and biochar (T2) included 2,3-Butanediol (52,2 %); Silane, dimethyl (but-2-enyloxy) silyloxy (5.5 %); 1,2-Benzisothiazol-3-amine (2.1 %) and Tris(*tert*-butyldimethylsilyloxy)arsane (2.0 %). *S. rochei* and compost (T3) included Oxime-, methoxy-phenyl (33.6 %); Arsenous acid, tris(trimethylsilyl) ester (31.6 %); 13H-Dibenzo[*a*,*i*]carbazole (3.3 %) and Hexanoic acid (2.5 %). *S. rochei*, biochar and compost (T4) included: Butanoic acid (40.7 %); Acetic acid (24.2 %) and Tris(*tert*-butyldimethylsilyloxy)arsane (6,2 %). Tables S4–S7 showed the chemical species identified by GC–MS in T1 (*S. rochei*), T2 (*S. rochei* - biochar), T3 (*S. rochei* – compost) and T4 (*S. rochei* – biochar - compost).

3.2. Antifungal activity assessment

3.2.1. In vitro tests. Inhibitory effects of the application of S. rochei on F. graminearum

Regarding the antibiosis assay, dual plate assays showed a strong antagonistic effect of *S. rochei* DSM 41729 against *F. graminearum*. Fungal growth was reduced by 83 %.

The sporangia suspension of F. graminearum (1×10^4 conidia·mL^{-1}) was treated for 24 h by the four treatments displaying significant effects on sporangia germination. All treatments significantly inhibited sporangial germination of F. gramineraum and the inhibition rates were >85 %. The best treatment was T2 (*S. rochei* - biochar) with 100 % inhibition, followed by T4 (*S. rochei* - biochar - compost), with 93 % inhibition. T3 (*S. rochei* - compost) reached inhibition percentages of 85 %. In the control in the absence of *S. rochei* (T1), the germination of F. graminearum occurs whereas in the presence of the actinobacteria, no germination was found.

Regarding the antifungal activity of the tested treatments (T1–T4), the effects of *S. rochei* combined with biochar, compost or compost and biochar enhanced the inhibition rate on the growth of F. graminearum (Fig. 1). In the absence of *S. rochei*, the relative inhibition rates ranged between 0 % (control-T1) to 80 % (T2). In the presence of *S. rochei*, T2 showed the strongest inhibition. In addition, the presence of *S. rochei* resulted in higher inhibition rates, from 82 % to 98 %, if compared with those assays where no bacteria were added. The mixture of *S. rochei* together with biochar (T2) showed the best results (93 %).

3.2.2. Micropot trials

The two-way ANOVA (Fig. 2) revealed significant effects of soil treatment (Table S3) and fungal condition (absence or presence of *F. graminearum*) on fresh leaf biomass ($F_{15,48} = 11.49$, $p < 0.001$ for soil treatment; $F_{15,48} = 10.01$, $p < 0.001$ for soil treatment and absence/presence of pathogen interaction) and root biomass ($F_{15,48} = 8.75$, $p < 0.001$ for soil treatment and absence/presence of pathogen interaction). More specifically, in absence of pathogen, T2 (*S. rochei* - biochar) resulted in the highest leaf biomass (1,2 ± 0.04 g), showing significant differences with all other treatments. Regarding root biomass in absence of *F. graminearum*, T2 (*S. rochei* + biochar) also reached the highest yield (0.71 ± 0.12 g), showing significant differences with other treatments. Compost based amendments showed intermediate leaf and root biomass yields (leaf: 0.01 ± 0.004 g; root: 0.01 ± 0.002 g).

In presence of pathogen, T2 (*S. rochei* + biochar), preserved 75.8 % of leaf biomass (0.91 ± 0.18 g) and improves 160 % of root biomass (0.91 ± 0.21 g) compared to the results obtained in absence of pathogen. Compost treatments maintained stable biomass production

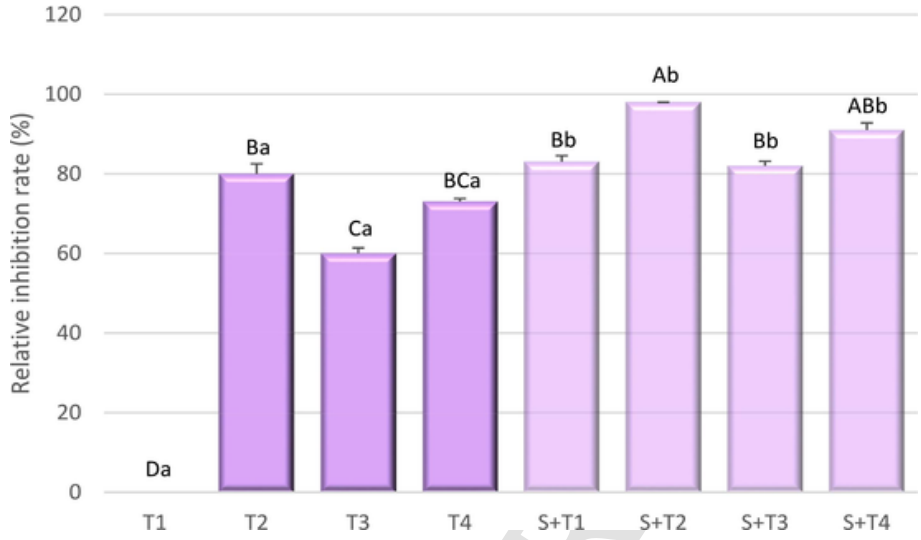


Fig. 1. Relative inhibition rate (%) of F. graminearum in presence of different treatments (T1 = negative control; T2 = biochar; T3 = compost; T4 = biochar + compost), in absence or presence of *S. rochei*. S stands for *S. rochei*. Results labelled with the same uppercase letters are not significantly different within the same condition (absence/presence of *S. rochei*). Results labelled with the same lowercase letters are not significantly different between absence/presence *S. rochei* conditions for the same treatment. Statistical significance was determined at $p < 0.05$ by Tukey's test following a two-way ANOVA. All values are presented as the average of four repetitions. Error bars represent the standard deviation of the mean of the four replicates.

across fungal conditions, with leaf biomass and root biomass varying by <35 %. However, soils in presence of pathogen, suffered near total biomass loss (leaf: 87.5 %; root: 77.1 %). The treatment with pathogen interaction was most pronounced in biochar amendments, where *S. rochei* supplementation mitigated stress, preserving root biomass at 72.8 % of control levels despite fungal presence.

Regarding leaf length, the two-way ANOVA revealed significant effects of soil treatment and fungal condition (absence or presence of *F. graminearum*) on leaf length ($F_{15,48} = 10.76$, $p < 0.001$ for soil treatment; $F_{15,48} = 13.95$, $p < 0.001$ for soil treatment and absence/presence of pathogen interaction) (Fig. 3). These results have similar patterns observed in biomass responses. In absence of pathogen, T2 (*S. rochei* + Biochar) resulted in significantly longer leaves (37.7 ± 1.7 cm) compared to other treatments. Compost-based amendments showed intermediate leaf lengths (32.5 ± 2.7 cm), while untreated controls exhibited shorter leaves (27.2 ± 2.8 cm) (Fig. 3a). In the presence of pathogen, untreated soils showed severe reductions in leaf length (7.8 ± 5.8 cm), highlighting the pathogen's detrimental impact on plant growth. However, T2 (*S. rochei* + biochar) in presence of *F. graminearum*, mitigated this stress, preserving leaf length at 31.5 ± 3.6 cm, representing a retention of 83.5 % compared to pathogen-free conditions. Compost treatments maintained stable leaf length across fungal conditions, with <15 % variation between pathogen-free and infected soils.

With respect to the results of soil analysis obtained in micropot trials after the experiment, Tables 1 and 2 showed the results obtained for the different soil parameters studied: organic matter, pH, conductivity, P (Olsen) and exchangeable cation composition of Ca^{2+}, Mg^{2+}, K^{+}, Na^{2+}, Ca^{2+}/Mg^{2+}. The percentage of organic matter increased in all the treatments if compared to the control, ranging from 3.1 % (C–; negative control) to 3.7 %, which corresponds to T4 (*S. rochei* + biochar + compost).

The pH remained acidic in all cases, with values between 3.90 and 4.26. Regarding conductivity, the lowest values were obtained with the biochar treatment (B) both in the absence (119.25 μSm^{-1}) and in the presence (880 μSm^{-1}) of *F. graminearum*, while the highest values were obtained with T3 (*S. rochei* + compost) in the absence (1460.5 μSm^{-1}) and in the presence (6365.75 μSm^{-1}) of the pathogen. In relation to phosphorus, the highest values were recorded in the control in the presence of *F. graminearum*, (35.5 $mg{\cdot}kg^{-1}$) while the lowest values occurred at T4 in the presence of the pathogen (20.2 $mg{\cdot}kg^{-1}$). Regarding the exchangeable cation composition (Table 2), there were hardly any significant differences between the different treatments.

According to the soil enzymes results (Table 3), a higher enzyme activity was observed in the presence of the fungus. β-glucosidase values ranged from 3.49 μg p-nitrophenol released h^{-1} $g^{-1}{\cdot}$soil for the biochar and compost treatment (B + C) in the presence of *F. graminearum*, (lower than the C– 3.91 μg p-nitrophenol released h^{-1} $g^{-1}{\cdot}$soil) to 26.29 μg p-nitrophenol released h^{-1} $g^{-1}{\cdot}$soil, corresponding to T1 in the presence *F. graminearum*. On the other hand, for acid phosphatase, there were no significant differences in the absence of the pathogen, however, in the presence of the fungus, the lowest value was obtained for the control (3.17 μg p-nitrophenol released $h^{-1}{\cdot}g^{-1}$ soil), with significant differences with respect to the highest value of 412.76 μg p-nitrophenol released $h^{-1}{\cdot}g^{-1}$ soil, which corresponds to T2. For alkaline phosphatase, the values were lower than for acid phosphatase. In the absence of pathogen, there was lower activity in all treatments compared to the control. On the other hand, in the presence of *F. graminearum*, 3 out of the 7 treatments (T1, T2 and T4) presented lower values than the control while the others presented higher values than the control. The treatment of biochar and compost (B + C) in the presence of *F. graminearum* doubled the value of the control.

In order to understand the influence of *F. graminearum*, *S. rochei*, and their combination on soil biochemical processes, a Principal Component Analysis (PCA) of the enzymatic activity was performed. Fig. 4 illustrates the PCA performed to evaluate the enzymatic activity profiles in soil samples under four treatment categories: Soil (untreated control), Fus (soil inoculated with *Fusarium graminearum*), Str (soil treated with *S. rochei*), and Str_Fus (soil treated with *S. rochei* and inoculated with *F. graminearum*). The PCA effectively summarized the variance in enzymatic activity data, with component 1 accounting for 54 % of the variance and component 2 explaining an additional 17 %.

Each point in the PCA plot represents the enzymatic activity profile of an individual replicate, while the ellipses denote confidence intervals for each treatment group. The analysis revealed distinct clustering patterns among the treatments, indicating significant differences in enzymatic activity profiles. Notably, the combined treatment (Str_Fus) ex-

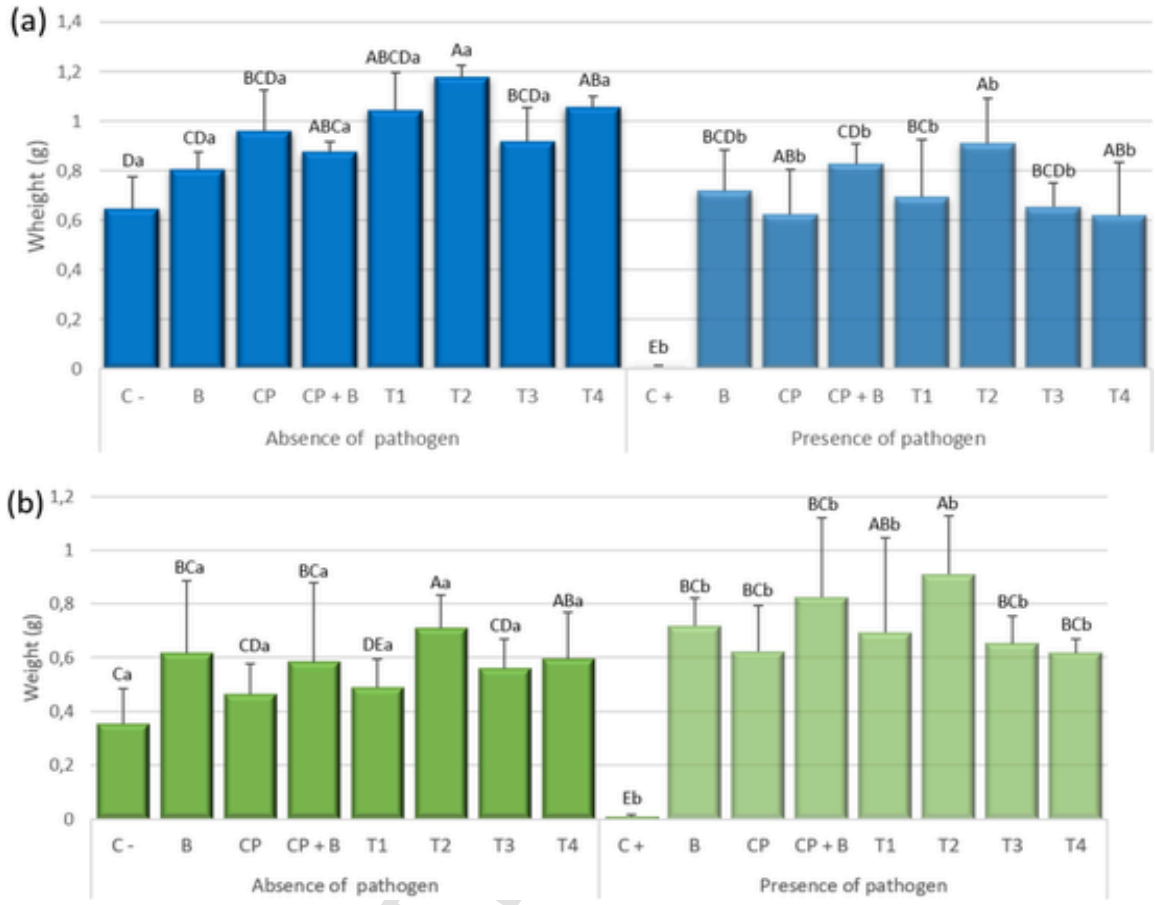


Fig. 2. Weight (g) of fresh leaf (a) and root (b) biomass in presence of different treatments (C– = negative control; B = biochar; CP = compost; CP + B = biochar + compost; T1 = *S. rochei*; T2 = *S. rochei* + biochar; T3 = *S. rochei* + compost; T4 = *S. rochei* + biochar + compost; C+ = positive control), in absence or presence of pathogen (*F. graminearum*). Results labelled with the same uppercase letters are not significantly different within the same fungal condition (absence/presence of pathogen). Results labelled with the same lowercase letters are not significantly different between fungal conditions (absence/presence of pathogen) for the same treatment. Statistical significance was determined at $p < 0.05$ by Tukey's test following a two-way ANOVA. All values are presented as the average of four repetitions. Error bars represent the standard deviation of the four replicates.

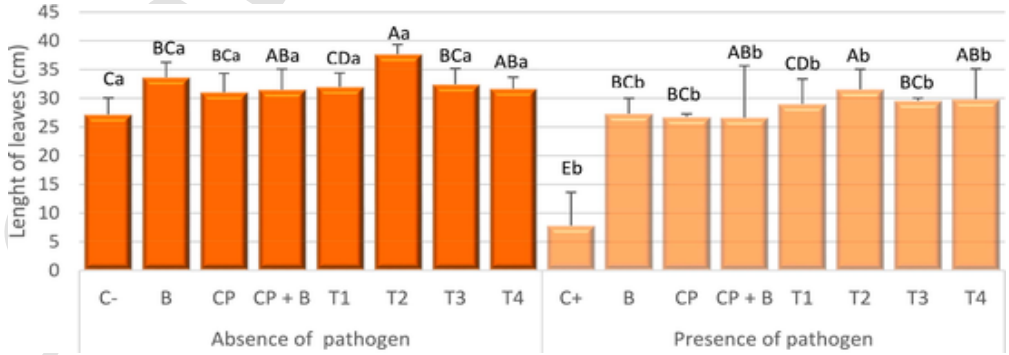


Fig. 3. Length of leaves (cm) in presence of the different treatments (C– = negative control; B = biochar; CP = compost; CP + B = biochar + compost; T1 = *S. rochei*; T2 = *S. rochei* + biochar; T3 = *S. rochei* + compost; T4 = *S. rochei* + biochar + compost; C+ = positive control) in absence or presence of pathogen (*F. graminearum*). Results labelled with the same uppercase letters are not significantly different within the same fungal condition (absence/presence of pathogen). Results labelled with the same lowercase letters are not significantly different between fungal conditions (absence/presence of pathogen) for the same treatment. Statistical significance was determined at $p < 0.05$ by Tukey's test following a two-way ANOVA. All values are presented as the average of four repetitions. Error bars represent the standard deviation of the four replicates.

Table 1
Soil parameters analysis for the treatments against *F. graminearum*.

	Treatments	Organic matter (%)	pH	Conductivity ($\mu S \cdot m^{-1}$)	$P_{(Olsen)}$ ($mg \cdot kg^{-1}$)
Absence of *F. graminearum*	C−	3.1 ± 0.2^{e}	4.15 ± 0.07^{ab}	1108 ± 181^{f}	20.7 ± 1.8^{c}
	B	3.3 ± 0.3^{cd}	4.19 ± 0.06 ^{ab}	119.25 ± 640^{h}	27.7 ± 5.4^{abc}
	CP	3.5 ± 0.3 ^{bc}	4.14 ± 0.11 ^{ab}	1398.25 ± 449^{cd}	26.3 ± 1.1 ^{abc}
	CP + B	3.5 ± 0.3 ^{bc}	4.11 ± 0.10 ^{ab}	1059 ± 266^{fg}	25.5 ± 4.8 ^{abc}
	T1	3.5 ± 0.2 ^{bc}	4.01 ± 0.08 ^{ab}	1313.5 ± 369^{d}	34.7 ± 6.1^{ab}
	T2	3.5 ± 0.3 ^{bc}	3.90 ± 0.06^{b}	1101.25 ± 300^{f}	26.5 ± 2.5 ^{abc}
	T3	3.6 ± 0.1 ^{ab}	4.16 ± 0.06 ^{ab}	1460.5 ± 286^{c}	22.5 ± 3.0^{c}
	T4	3.7 ± 0.3^{a}	4.13 ± 0.05 ^{ab}	1289.5 ± 245de	26.9 ± 1.7 ^{abc}
Presence of *F. graminearum*	C+	3.2 ± 0.2^{de}	4.12 ± 0.35 ^{ab}	1530.75 ± 129^{b}	35.5 ± 4.7^{a}
	B	3.6 ± 0.1 ^{ab}	4.16 ± 0.02 ^{ab}	880 ± 59^{g}	25.3 ± 3.8^{bc}
	CP	3.5 ± 0.3 ^{bc}	4.15 ± 0.05 ^{ab}	6274.75 ± 166^{a}	26.4 ± 4.3 ^{abc}
	CP + B	3.5 ± 0.2 ^{bc}	4.09 ± 0.02 ^{ab}	1048.75 ± 408^{fg}	22.1 ± 3.1^{c}
	T1	3.6 ± 0.3^{ab}	3.90 ± 0.02^{b}	1405.75 ± 140^{b}	28.3 ± 2.4 ^{abc}
	T2	3.2 ± 0.3^{de}	4.13 ± 0.01 ^{ab}	1223.75 ± 382^{e}	28.1 ± 5.4 ^{abc}
	T3	3.4 ± 0.2^{cd}	4.11 ± 0.01 ^{ab}	6365.75 ± 400^{a}	27.2 ± 2.9 ^{abc}
	T4	3.1 ± 0.1^{e}	4.26 ± 0.02^{a}	966.75 ± 389^{g}	20.2 ± 3.4^{c}

C− = negative control; B = biochar; CP = compost; CP + B = biochar + compost; T1 = *S. rochei*; T2 = *S. rochei* + biochar; T3 = *S. rochei* + compost; T4 = *S. rochei* + biochar + compost; C+ = positive control. Results labelled with the same uppercase letters are not significantly different at $p < 0.01$ by Tukey's test. All values are presented as the average of four repetitions.

Table 2
Soil parameter analysis for the treatments against *F. graminearum*.

		Exchangeable cation composition ($cmol_{(+)} \cdot kg^{-1}$)				
	Treatments	Ca^{2+}	Mg^{2+}	K^{+}	Ca^{2+}/Mg^{2+}	Na^{2+}
Absence of *F. graminearum*	C−	1.3 ± 0.6 ^{a}	0.6 ± 0.1 ^{a}	0.9 ± 0.1 ^{ab}	2.2 ± 0.0^{ab}	0.1 ± 0.0^{a}
	B	1.7 ± 0.8^{a}	0.8 ± 0.3 ^{a}	1.1 ± 0.3 ^{ab}	2.1 ± 0.1 ^{ab}	0.1 ± 0.0 ^{a}
	CP	1.5 ± 0.6 ^{a}	0.6 ± 0.2 ^{a}	1.2 ± 0.3 ^{ab}	2.3 ± 0.3^{a}	0.1 ± 0.0 ^{a}
	CP + B	1.4 ± 0.6 ^{a}	0.6 ± 0.1 ^{a}	0.9 ± 0.2 ^{ab}	2.2 ± 0.1 ^{ab}	0.1 ± 0.0 ^{a}
	T1	1.5 ± 0.7 ^{a}	0.7 ± 0.1 ^{a}	1.2 ± 0.2 ^{ab}	2.3 ± 0.2 ^{ab}	0.1 ± 0.0 ^{a}
	T2	1.6 ± 0.7 ^{a}	0.7 ± 0.1 ^{a}	1.3 ± 0.1^{a}	2.2 ± 0.1 ^{ab}	0.1 ± 0.0 ^{a}
	T3	1.4 ± 0.6 ^{a}	0.6 ± 0.0 ^{a}	1.1 ± 0.2 ^{ab}	2.3 ± 0.1 ^{ab}	0.1 ± 0.0 ^{a}
	T4	1.8 ± 0.8^{a}	0.8 ± 0.2 ^{a}	1.0 ± 0.1 ^{ab}	2.2 ± 0.1 ^{ab}	0.1 ± 0.0 ^{a}
Presence of *F. graminearum*	C+	1.4 ± 0.7 ^{a}	0.7 ± 0.0 ^{a}	1.2 ± 0.1 ^{ab}	2.0 ± 0.1^{b}	0.2 ± 0.0 ^{b}
	B	1.4 ± 0.6 ^{a}	0.6 ± 0.0 ^{a}	0.9 ± 0.1 ^{ab}	2.2 ± 0.1 ^{ab}	0.1 ± 0.0 ^{a}
	CP	1.2 ± 0.5 ^{a}	0.5 ± 0.0 ^{a}	0.9 ± 0.2 ^{ab}	2.3 ± 0.1^{a}	0.1 ± 0.0 ^{a}
	CP + B	1.3 ± 0.6 ^{a}	0.6 ± 0.0 ^{a}	0.8 ± 0.1^{b}	2.3 ± 0.1 ^{ab}	0.1 ± 0.0 ^{a}
	T1	1.5 ± 0.7 ^{a}	0.7 ± 0.0 ^{a}	1.1 ± 0.2 ^{ab}	2.1 ± 0.0 ^{ab}	0.1 ± 0.0 ^{a}
	T2	1.8 ± 0.8 ^{a}	0.8 ± 0.1 ^{a}	1.1 ± 0.1 ^{ab}	2.2 ± 0.1 ^{ab}	0.1 ± 0.0 ^{a}
	T3	1.6 ± 0.7 ^{a}	0.7 ± 0.1 ^{a}	1.1 ± 0.2 ^{ab}	2.2 ± 0.1 ^{ab}	0.1 ± 0.0 ^{a}
	T4	1.4 ± 0.6 ^{a}	0.6 ± 0.1 ^{a}	0.8 ± 0.2^{b}	2.4 ± 0.1^{a}	0.1 ± 0.0 ^{a}

C− = negative control; B = biochar; CP = compost; CP + B = biochar + compost; T1 = *S. rochei*; T2 = *S. rochei* + biochar; T3 = *S. rochei* + compost; T4 = *S. rochei* + biochar + compost; C+ = positive control. Results labelled with the same uppercase letters are not significantly different at $p < 0.01$ by Tukey's test. All values are presented as the average of four repetitions.

Table 3
Soil enzymes activity for the treatments against *F. graminearum*.

Treatments	Absence of *F. graminearum*			Presence of *F. graminearum*		
	β-glucosidase	Acid fosfatase	Alcaline fosfatase	β-glucosidase	Acid fosfatase	Alcaline fosfatase
C−/C+	3.91 ± 0.00^{c}	192.35 ± 0.00^{b}	81.03 ± 0.01 ^{ab}	4.70 ± 0.00^{c}	3.7 ± 0.00^{c}	58.04 ± 0.01 ^{b}
B	3.82 ± 0.07^{ab}	221.75 ± 0.01 ^{b}	73.89 ± 0.03 ^{b}	4.41 ± 0.12^{c}	221.27 ± 0.01^{b}	77.19 ± 0.01 ^{b}
CP	3.95 ± 0.04^{c}	209.21 ± 0.00^{b}	76.06 ± 0.03 ^{b}	4.78 ± 0.06^{c}	237.74 ± 0.00^{b}	60.68 ± 0.02 ^{b}
CP + B	4.30 ± 0.05^{c}	216.18 ± 0.01^{b}	74.53 ± 0.02 ^{b}	3.49 ± 0.03^{c}	227.88 ± 0.04^{b}	121.57 ± 0.01^{a}
T1	4.66 ± 0.08^{c}	262.34 ± 0.00^{b}	78.90 ± 0.02 ^{b}	26.29 ± 0.04^{a}	233.94 ± 0.00^{b}	41.61 ± 0.02 ^{b}
T2	3.87 ± 0.20^{c}	243.73 ± 0.01^{b}	65.35 ± 0.01^{b}	24.75 ± 0.01^{a}	412.76 ± 0.08^{a}	55.63 ± 0.01 ^{b}
T3	4.08 ± 0.02^{c}	243.81 ± 0.00^{b}	69.37 ± 0.02 ^{b}	21.30 ± 0.04^{ab}	272.86 ± 0.09^{b}	61.14 ± 0.02 ^{b}
T4	4.30 ± 0.02^{a}	210.14 ± 0.00^{b}	68.36 ± 0.00 ^{b}	16.98 ± 0.02^{b}	199.75 ± 0.03^{b}	55.93 ± 0.02^{b}

C− = negative control; B = biochar; CP = compost; CP + B = biochar + compost; T1 = *S. rochei*; T2 = *S. rochei* + biochar; T3 = *S. rochei* + compost; T4 = *S. rochei* + biochar + compost; C+ = positive control. Concentrations labelled with the same uppercase letters are not significantly different at $p < 0.001$ by Tukey's test. All values are presented as the average of four repetitions. Units: μg p-nitrophenol released h^{-1} g^{-1}soil.

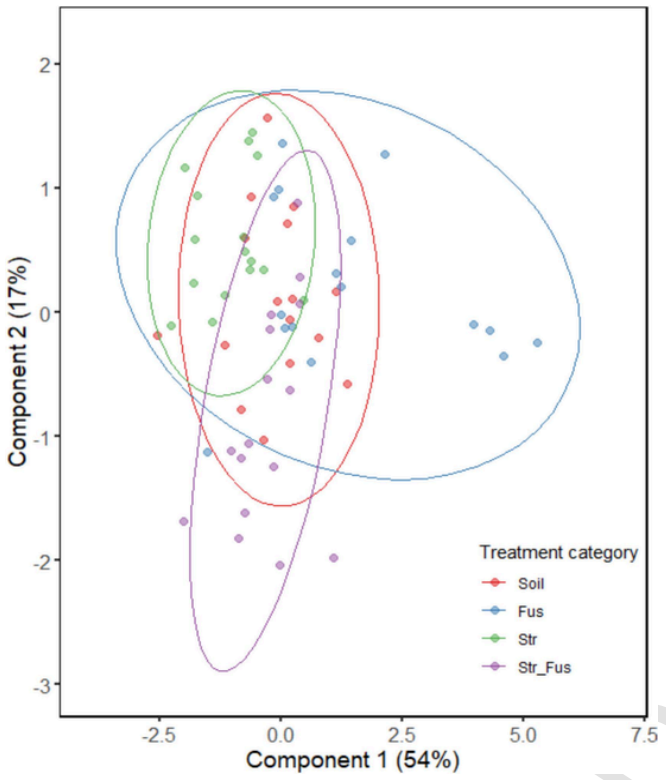


Fig. 4. Principal Component Analysis of the enzymatic activity in soil samples under different treatment categories (Soil: untreated control; Fus: soil inoculated with *F. graminearum*; Str: soil treated with *S. rochei*; and Str_Fus: soil treated with *S. rochei* and inoculated with *F. graminearum*). Each point represents the enzymatic activity profile of a replicate, and the ellipses indicate the confidence intervals for each treatment group.

hibited a unique enzymatic activity profile that was clearly separated from the other groups.

To further explore these differences, a PERMANOVA analysis was conducted. The results confirmed that treatment significantly influenced enzymatic activity profiles ($F = 4.47$; $p = 0.002997$). These results indicate that soil enzyme activities vary significantly among the different treatments.

4. Discussion

4.1. Secondary metabolites and antifungal effect

The GC–MS analysis revealed distinct secondary metabolite profiles in *S. rochei* cultures amended with biochar (T2), compost (T3), or their combination (T4), demonstrating remarkable metabolic plasticity under different substrate conditions. Previous studies have reported that the altered environment created by biochar and compost amendments may induce stress responses in *Streptomyces* spp., leading to the production of different metabolites. For instance, changes in pH, organic matter content, and nutrient availability have been shown to significantly affect microbial community composition (Guo et al., 2019). These environmental changes may indicate adaptive responses in *Streptomyces*, resulting in the synthesis of novel compounds or the modification of existing metabolic pathways.

The antifungal efficacy of the treatments evaluated in this study demonstrated the significant potential of *S. rochei* and its combinations with biochar and compost for controlling *F. graminearum* growth. The dual plate assay revealed an 83 % inhibition of fungal mycelial growth by *S. rochei* alone (S + T1, Fig. 1), similar with previous studies reporting inhibition rates between 71 % and 88 % for other Streptomyces strains (Zhang et al., 2021; Colombo et al., 2020). The incorporation of biochar (S + T2) resulted in a remarkable improvement, achieving 100 % inhibition of sporangia germination, overcoming earlier findings where biochar alone or in combination with other biocontrol agents exhibited lower inhibition rates (Iacomino et al., 2022). This enhanced efficacy can be attributed to the stabilization of volatile organic compounds (VOCs) such as 2,3-butanediol by biochar, which not only inhibits fungal growth but also induces systemic resistance in plants (Mun et al., 2024).

The metabolic plasticity of *S. rochei* under amendment-induced stress played a pivotal role in antifungal activity. Biochar amendment (S + T2) unleashed a 47 % increase in aliphatic compounds, including 2,3-butanediol (52.2 %), through mechanical and biochemical stressors. Biochar's porous structure altered hyphal-surface interactions, limiting direct nutrient uptake and redirecting acetyl-CoA flux towards aliphatic biosynthesis (Mun et al., 2024). Concurrently, its alkaline properties (pH 9.2, Table S1) disrupted cytoplasmic pH homeostasis, activating stress-responsive pathways favoring Volatile Organic Compounds (VOCs) production (Zhang et al., 2023). This acid synergized with biochar's alkaline pH to enhance fungal hyphal membrane permeability, quantified via electrochemical impedance spectroscopy (Ali et al., 2024). These stressors explain the dominance of 2,3-butanediol, a compound recently shown to stabilize under biochar's microporous structure, prolonging its antifungal and plant growth-promoting effects (Gao et al., 2019; Wu et al., 2019).

Compost amendment (S + T3) enriched phenolic derivatives like oxime-methoxy-phenyl (33.6 %), a response to ligninolytic stress. Compost-derived lignin fragments activated Streptomyces' lignocellulose-degrading regulons, stimulating aryl-oxime synthesis (Buzón-Durán et al., 2020; Feng et al., 2021), while humic acids acted as chemical elicitors, upregulating cytochrome P450 monooxygenases involved in oxime biosynthesis (Zhang et al., 2023). This metabolic change is probably related to S + T3's 85% sporangia inhibition.

The combination of *S. rochei* with compost (S + T3) achieved an 85 % inhibition of sporangia germination, while the triple combination of *S. rochei*, biochar, and compost (S + T4) reached 93 %. These results highlight the synergistic effects of compost and biochar in enhancing the antifungal activity of *S. rochei*. Compost likely contributed to this synergy by enriching the production of oxime-methoxy-phenyl, a compound known to disrupt fungal signaling pathways (Al-Garadi et al., 2022). Furthermore, S + T4 (biochar + compost) results highlight the importance of combining organic amendments to optimize biocontrol efficacy. When combined, compost and biochar create a favorable environment for plant growth while suppressing pathogenic fungi. These elements act as substrates for *Streptomyces* colonization, potentially enhancing their antifungal activity (Gorovtsov et al., 2020). Nakashita et al. (2002), confirmed that probenazole and its active metabolite 1,2-benzisothiazole-1,1-dioxide induce systemic acquired resistance by triggering signaling at a point upstream of salicylic acid accumulation in tobacco. Tris (*tert*-butyldimethylsilyloxy) arsane has been found in 2 treatments: T4 (*S. rochei*, compost and biochar) (6.2 %) and in T3 (*S. rochei* + biochar) (2.0 %). In addition to having antifungal properties against Rhizoctonia and Macrophomina, this compound was effective in increasing the germination percentage and the fresh and dry weights of roots and shoots (Kalaivani et al., 2023).

Compared to previous studies, the treatments tested in this study demonstrated superior antifungal activity. For instance, while Streptomyces pratensis S10 achieved an 88 % inhibition rate against *F. graminearum* (Zhang et al., 2021), *S. rochei* + biochar (S + T2) in this study almost depleted sporangia germination. Similarly, other studies using multiple Streptomyces strains with biochar reported inhibition rates exceeding 90 % but did not achieve complete suppression (Jin et al., 2023). The results obtained suggest that the specific combination of *S. rochei* with biochar and/or compost enhances the production of key sec-

ondary metabolites that contribute to pathogen suppression and highlights the synergy between organic amendments and microbial metabolism. The results observed in this study showed the potential of integrating *S. rochei*, biochar, and compost as a sustainable strategy for managing fusariosis. The ability of these treatments to inhibit fungal growth through multiple mechanisms (ranging from VOC stabilization to nutrient competition) positions them as promising alternatives to chemical fungicides for sustainable agriculture.

4.2. Impact in plant growth

The treatments evaluated in this study demonstrated significant improvements in triticale growth, particularly under pathogen stress caused by Fusarium graminearum. Soils in presence of pathogen, suffered near total biomass loss (leaf: 87.5 %; root: 77.1 %). Among the treatments, T2 (*S. rochei* + biochar) exhibited the most pronounced effects, with a 160 % increase in root biomass, a 75.8 % increase in leaf biomass and a 15.6 % in leaf length, compared to the negative control (C−) (Figs. 2 and 3). At this point it is worth mentioning that the error bars in Figs. 2 and 3 can be explained by the inherent biological and experimental variability, which is common in studies involving soil-plant-microbe interactions. First, plant growth responses to microbial treatments often exhibit high variability due to genetic differences among plants and uneven colonization of roots by beneficial microbes such as Streptomyces. This variability is further amplified in non-sterile soil systems, as the ones used in this study, where native microbial communities interact dynamically with introduced microbes, creating micro-niche competition that leads to differences in plant responses across replicates (Nonthakaew et al., 2022; Abbasi et al., 2021). Second, the use of biochar and compost as soil amendments introduces further physical and chemical heterogeneity in the soil. These amendments vary in their pore structure and nutrient release patterns, which can influence microbial activity and root exploration differently across replicates. Such spatial variability is particularly pronounced in treatments combining both biochar and compost, as seen in CP + B and T4 (Lehmann et al., 2011). Third, the interaction between *S. rochei* metabolites and F. graminearum is inherently nonlinear, as the biocontrol efficacy depends on the timing of metabolite production relative to pathogen activity (Köhl et al., 2019). This threshold-dependent response contributes to increased data dispersion, particularly in treatments where both biochar and compost are present (Fitzpatrick et al., 2019).

These results obtained for root and leaves biomass and length of leaves highlight the synergistic benefits of combining biochar with microbial inoculants like *S. rochei*, which not only suppress pathogens but also promote plant growth through mechanisms such as systemic resistance induction and improved nutrient uptake. The ability to mitigate pathogen-induced damage through synergistic mechanisms. Sharma et al. (2025), demonstrated that biochar enhances microbial colonization and suppresses pathogens indirectly by altering soil chemistry and microbial dynamics. Biochar's porous structure provides favorable habitats for beneficial microbes, which promote nutrient cycling and plant growth while suppressing fungal pathogens (Yang et al., 2025). This remarkable enhancement is attributed to biochar's dual role as a microbial scaffold and soil conditioner, which stabilizes volatile organic compounds (VOCs) like 2,3-butanediol, a metabolite known for its plant growth-promoting properties. Recent studies have demonstrated that 2,3-butanediol induces systemic resistance in plants, promotes root development, and enhances nutrient uptake by stimulating jasmonic acid and ethylene pathways (Mun et al., 2024)(Ryu et al., 2003). Furthermore, biochar's porous structure prolongs the stability of VOCs like 2,3-butanediol in the soil, ensuring sustained stimulation of plant physiological processes (Zhang et al., 2023). The observed resilience of biochar treated soils against *F. graminearum*, suggesting that biochar not only improves soil health but also enhances plant immune responses. A recent review on biochar microbe interactions, explain that the stability of compost treatments across conditions further supports their buffering capacity and ability to enrich beneficial microbes (Jatuwong et al., 2025). These results highlight the importance of optimizing amendment-microbe combinations to address the specific requirements of individual pathosystems. Future research should focus on long-term field studies to evaluate biochar aging effects on microbial communities and its sustained efficacy in pathogen suppression.

T4 (*S. rochei* + biochar + compost) also showed balanced root and leaf growth under pathogen stress, with the highest soil organic matter content (3.7 %, Table 1) among all treatments. The synergistic effects of biochar and compost improved soil structure and microbial activity while promoting the production of secondary metabolites such as butanoic acid (40.7 %), which suppressed ethylene overproduction by 29 % in infected plants (Santos et al., 2023). Compost-enriched phenolic derivatives like oxime-methoxy-phenyl, further contributed to lignin deposition in root exodermis, physically blocking fungal penetration (Al-Garadi et al., 2022). Furthermore, the promotion of crop productivity is facilitated by soil Streptomycetes, which aid in the decomposition of organic materials (Khan et al., 2023).

The results highlight the importance of amendment-specific responses. Biochar alone improved water-use efficiency by 22 %, creating optimal conditions for microbial activity and plant growth (Liu et al., 2021). Compost enhanced soil conductivity and organic matter content while promoting microbial activity essential for nutrient transformation (Bello et al., 2023; Adugna, 2016). The combined application of biochar and compost amplified these effects, providing a favorable environment for *S. rochei* colonization and secondary metabolite production (Agegnehu et al., 2017).

These results suggest that amendment-induced stress reshapes microbial metabolism to favor metabolites with dual antifungal and plant growth-promoting functions. The combination of *S. rochei*, biochar, and compost represents a promising strategy for improving triticale productivity while mitigating fusariosis through sustainable agricultural practices.

4.3. Soil health improvement

Concerning the soil fertility properties evaluated, the results showed that treatments with biochar and with compost-biochar mixtures significantly increased soil water holding capacity, organic matter content and cation exchange capacity, which improved soil structure and nutrient retention. Bioavailable phosphorus (P) was significantly higher in soils amended with biochar (Table 2). The mixture of compost and biochar improved microbial metabolic activity and microbial community evenness, although overall microbial diversity remained stable. In addition, they enhance the effects of carbon sequestration. These results are consistent with the results obtained in recent studies (Gao et al., 2025; Yang et al., 2025).

The treatments evaluated in this study demonstrated varying effects on plant growth and soil health, highlighting the potential of combining *Streptomyces rochei* with biochar and compost to improve crop productivity and soil fertility. T1 (*S. rochei*) showed moderate root and leaf biomass increases (Fig. 2), attributed to the production of secondary metabolites such as 5-hydroxymethylfurfural (HMF). This secondary metabolite produced by *S. rochei* in this study enhances soil health by promoting plant growth through root biomass accumulation and organic matter deposition. HMF stimulates root exudation, which enriches carbon pools soil and fosters beneficial microbial communities, as observed in treatments with *S. rochei* alone (T1) (Kaushal and Sharma, 2022; Singh et al., 2023). Additionally, HMF exhibits mild antimicrobial properties that suppress soil-borne pathogens, indirectly improving soil biodiversity and nutrient cycling efficiency (Świątek et al., 2022).

The combination of *S. rochei* and biochar (T2) exhibited notable improvements in soil health, emphasizing its potential as an eco-friendly amendment. Biochar's porous structure provided a favorable habitat for microbial colonization and stabilized key volatile organic compounds (VOCs), such as 2,3-butanediol, which are known to enhance plant growth and suppress fungal pathogens (Mun et al., 2024; Wu et al., 2019). This stabilization likely contributed to the observed increase in enzymatic activities, particularly acid phosphatase, which plays a critical role in phosphorus cycling and nutrient availability (Hamdali et al., 2012). Furthermore, biochar improved soil water retention and cation exchange capacity, creating optimal conditions for nutrient transformation and microbial activity (Agegnehu et al., 2017; Liu et al., 2021).

In addition to enhancing plant growth, the treatments influenced nutrient dynamics in pathogen-infected soils. The presence of F. graminearum increased Olsen phosphorus levels by 71.4 %, likely due to pathogen-induced hyphal acidification and enzymatic hydrolysis (Yan et al., 2023). Concurrently, *S. rochei* increased phosphorus availability by 67.6 %, attributed to its phosphate-solubilizing activity and siderophore production (Hamdali et al., 2012; (Ali et al., 2017). These results highlight the potential of combining biochar and compost with Streptomyces to enhance nutrient cycling and improve soil fertility under pathogen pressure.

T3 (*S. rochei* + compost) enhanced leaf length under pathogen-free conditions and balanced root/leaf growth under pathogen stress. This effect is associated with the enrichment of oxime-methoxy-phenyl, which promotes lignin deposition in roots, physically blocking fungal penetration. Additionally, compost increased soil organic matter and nutrient availability (Al-Garadi et al., 2022; Feng et al., 2021). The primary biological activity of compost's microbiota, which generally interacts with soil organic matter and the host plant via controlling the rhizosphere microbial community, is linked to the mechanisms behind the suppressive effects of compost. These mechanisms included: microbiostasis and fungistasis; (ii) competition for the nutrients and host plant infection sites; (iii) antibiosis, hyperparasitism, and plant pathogen predation through the synthesis of lytic enzymes, antibiotics, and non-volatile antifungal compounds; (iv) activation of genes that resist disease; and (v) enhanced nutrition and robustness of the entire plant (De Corato, 2020).

Finally, T4 (*S. rochei* + biochar + compost) achieved the highest organic matter content (3.7 %, Table 2) among all treatments. This improvement is attributed to the synergistic effects of biochar and compost in enhancing soil structure and microbial activity. Furthermore, butanoic acid production suppressed ethylene overproduction in infected plants, contributing to improved stress tolerance (Santos et al., 2023; Guimarães et al., 2018).

Furthermore, the results demonstrate that the enzymatic activity profiles in soil are significantly influenced by the interaction between *S. rochei* and *F. graminearum*, as evidenced by the distinct clustering observed in the PCA (Fig. 4). The combined treatment (Str_Fus) exhibited unique enzymatic patterns, likely due to synergistic interactions between the two microorganisms. This aligns with previous studies showing that microbial co-cultures can modulate enzymatic activity through competitive or cooperative mechanisms, such as enhanced production of hydrolytic enzymes or resource sharing (Guo et al., 2024; Jatuwong et al., 2025). Furthermore, the positive impact of Streptomyces on these enzyme activities is attributed to their ability to produce various extracellular enzymes and their role in promoting overall soil microbial activity. Streptomyces can also help mitigate the negative effects of soil pollutants on enzyme activities, thereby improving soil health and functional diversity (Ali et al., 2017; Yan et al., 2023).

5. Conclusions

Gas chromatography-mass spectrometry analyses evidenced that *S. rochei* produces different bioactive compounds depending on the substance (biochar, compost, or both) with it is inoculated during the process. All these compounds presented antimicrobial properties, promoted plant growth, induced systemic resistance and could significantly improve biomass plant growth. The most abundant bioactive compounds were: 5-hydroxymethylfurfural, 2–3 butanediol, oxime-, methoxy-phenyl and butanoic acid. In the in vitro mycelial growth inhibition tests, *S. rochei* can inhibit 83 % of the growth of *F. graminearum*. The presence of biochar mixture with *S. rochei* enhanced the activity of *S. rochei* alone and can inhibit 100 % of the sporangia germination. Micropot trials showed that biochar and the mixture of biochar and *Streptomyces* spp. had a positive effect on the protection of triticale against fusariosis. Soil microbial activity (*Streptomyces* spp.) is fundamental not only for an increase in biomass production but also for protection against crop diseases. This work highlights the importance of the soil microbiome stimulated by the presence of a carbon source (biochar) in the production of biomass and crop protection against cereal fusariosis. These promising results support the idea that organic residues can be valorized and combined with biocontrol agents to potentially serve as a source for both pathogen removal and enhanced crop development and soil health.

CRediT authorship contribution statement

Laura Buzón-Durán: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Beatriz Molinuevo-Salces:** Writing – original draft, Supervision, Resources, Funding acquisition. **Mari Cruz García-González:** Writing – review & editing, Project administration. **Mercedes Sánchez-Báscones:** Resources, Funding acquisition. **Claudia Vitoria:** Investigation. **Carmo Horta:** Visualization, Supervision, Software, Resources, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Funding sources

This work was supported by three International Projects: LIFE GREEN AMMONIA [LIFE 20 ENV/ES/00858], CSTO2NE [HORIZON-TMA-MSCA-SE] and CERNAS-IPCB [UIDB/00681/2020], and the grant: RYC-2020-029030-I/AEI/10.13039/501100011033.

Uncited reference

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Beatriz Molinuevo-Salces reports financial support was provided by Spanish Scientific Research Council. Reports a relationship with that includes:. Has patent pending to. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Laura Buzón Durán thanks the “Research Staff Mobility Plan Grant. Call 2023-University of Valladolid” for the support in her first period stay in Portugal. Laura also wants to express her sincere gratitude to Prof. Carmo Horta and her team – Claudia, Angela, and Elena – for their invaluable assistance. B. Molinuevo-Salces thanks AEI for funding, through RYC-2020-029030-I/AEI/10.13039/501100011033.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2025.179595.

References

Abbasi, S., Spor, A., Sadeghi, A., Safaie, N., 2021. *Streptomyces* strains modulate dynamics of soil bacterial communities and their efficacy in disease suppression caused by *Phytophthora capsici*. Sci. Rep. 11, 9317. https://doi.org/10.1038/s41598-021-88495-y.

Adugna, G., 2016. A review on impact of compost on soil properties, water use and crop productivity. Acad. Res. J. Agric. Sci. Res. 4, 93–104. https://doi.org/10.14662/ARJASR2016.010.

Agegnehu, G., Srivastava, A.K., Bird, M.I., 2017. The role of biochar and biochar-compost in improving soil quality and crop performance: a review. Appl. Soil Ecol. 119, 156–170. https://doi.org/10.1016/j.apsoil.2017.06.008.

Alam, K., Mazumder, A., Sikdar, S., Zhao, Y.M., Hao, J., Song, C., Li, A., 2022. *Streptomyces*: the biofactory of secondary metabolites. Front. Microbiol. 13, 968053. https://doi.org/10.3389/fmicb.2022.968053.

Al-Garadi, M.A., Qaid, M.M., Alqhtani, A.H., Pokoo-Aikins, A., Al-Mufarrej, S.I., 2022. In vitro phytochemical analysis and antibacterial and antifungal efficacy assessment of ethanolic and aqueous extracts of *Rumex nervosus* leaves against selected bacteria and fungi. Vet. World 15, 2725. https://doi.org/10.14202/vetworld.2022.2725-2737.

Ali, Q., Ali, M., Jing, H., Hussain, A., Manghwar, H., Ali, M., Raza, W., Mundra, S., 2024. Power of plant microbiome: A sustainable approach for agricultural resilience. Plant Stress 14, 100681. doi: 10.1016/j.stress.2024.100681.

Ali, A., Guo, D., Mahar, A., Ma, F., Li, R., Shen, F., Zhang, Z., 2017. *Streptomyces pactum* assisted phytoremediation in Zn/Pb smelter contaminated soil of Feng County and its impact on enzymatic activities. Sci. Rep. 7, 46087. doi: 10.1038/srep46087.

Bello, A., Liu, W., Chang, N., Erinle, K.O., Deng, L., Egbeagu, U.U., Babalola, B.J., Yue, H., Sun, Y., Wei, Z., Xu, X., 2023. Deciphering biochar compost co-application impact on microbial communities mediating carbon and nitrogen transformation across different stages of corn development. Environ. Res. 219, 115123. https://doi.org/10.1016/j.envres.2022.115123.

Buzón-Durán, L., Pérez-Lebeña, E., Martín-Gil, J., Sánchez Báscones, M., Martín Ramos, P., 2020. Applications of *Streptomyces* spp. enhanced compost in sustainable agriculture. In: K. Meghvansi, M., Varma, A. (Eds.), Biology of Composts. Springer, pp. 257–291.

Colombo, E.M., Kunova, A., Gardana, C., Pizzatti, C., Simonetti, P., Cortesi, P., Pasquali, M., 2020. Investigating useful properties of four *Streptomyces* strains active against *Fusarium graminearum* growth and deoxynivalenol production on wheat grains by qPCR. Toxins 12, 560. https://doi.org/10.3390/toxins12090560.

De Corato, U., 2020. Disease-suppressive compost enhances natural soil suppressiveness against soil-borne plant pathogens: a critical review. Rhizosphere 13, 100192. https://doi.org/10.1016/j.rhisph.2020.100192.

De Vrieze, M., Germanier, F., Vuille, N., Weisskopf, L., 2018. Combining different potato-associated *Pseudomonas* strains for improved biocontrol of *Phytophthora infestans*. Front. in microbiol. 9, 2573. https://doi.org/10.3389/fmicb.2018.02573.

Dinolfo, M.I., Martínez, M., Castañares, E., Stenglein, S.A., 2020. Presence of *Fusarium graminearum* sensu stricto associated with triticale (× *Triticosecale* Wittmack) in Argentina. Span. J. Agric. Res. 18, 1002. https://doi.org/10.5424/sjar/2020181-14796.

dos Santos Gomes, A.C., da Silva, R.R., Moreira, S.I., Vicentini, S.N.C., Ceresini, P.C., 2021. Biofungicides: an eco-friendly approach for plant disease management. In: Zaragoza, Ó., Casadevall, A. (Eds.), Encyclopedia of Mycology. Elsevier, UK, pp. 641–649.

Eivazi, F., Tabatabai, M.A., 1988. Glucosidases and galactosidases in soils. Soil Biol. Biochem. 20, 601–606. https://doi.org/10.1016/0038-0717(88)90141-1.

Feng, J., Wang, B., Zhang, D., 2021. *Streptomyces griseorubens* JSD-1 promotes rice straw composting efficiency in industrial-scale fermenter: evaluation of change in physicochemical properties and microbial community. Bioresour. Technol. 321, 124465. https://doi.org/10.1016/j.biortech.2020.124465.

Fitzpatrick, C.R., Mustafa, Z., Viliunas, J., 2019. Soil microbes alter plant fitness under competition and drought. J. of Evol. Biol. 32 (5), 438–450. https://doi.org/10.1111/jeb.13426.

Food and Agriculture Organization of the United Nations (FAO). GLOSOLAN – Global Soil Laboratory Network, 2021. FAO. https://www.fao.org/global-soil-partnership/glosolan-old/soil-analysis/standard-operating-procedures/en/#c763834.

Gao, S., DeLuca, T.H., Cleveland, C.C., 2019. Biochar additions alter phosphorus and nitrogen availability in agricultural ecosystems: a meta-analysis. Sci. Total Environ. 654, 463–472. https://doi.org/10.1016/j.scitotenv.2018.11.124.

Gao, S., Medina, M., Gonzalez-Ospina, L., Burce, K., Burce, K., Melbourne, A., 2025. Boosting soil health and crop nutrients with locally sourced biochar and compost in Sacramento urban agriculture. Front. Sustain. Food Syst. 9, 1546426. https://doi.org/10.3389/fsufs.2025.1546426.

Góral, T., Wiśniewska, H., Ochodzki, P., Walentyn-Góral, D., 2016. Higher *Fusarium* toxin accumulation in grain of winter triticale lines inoculated with *Fusarium culmorum* as compared with wheat. Toxins 8, 301. https://doi.org/10.3390/toxins8100301.

Ghanem, G.A.M., Gebily, D.A.S., Ragab, M.M., Ali, A.M., Soliman, N.E.-D.K., El-Moity, T.H.A., 2022. Efficacy of antifungal substances of three *Streptomyces* spp. against different plant pathogenic fungi. Egypt. J. Biol. Pest Control 32, 112. doi: 10.1186/s41938-022-00612-9.

Góral, T., Wiśniewska, H., Ochodzki, P., Twardawska, A., Walentyn-Góral, D., 2020. Resistance to fusarium head blight, kernel damage, and concentration of *Fusarium* mycotoxins in grain of winter triticale (x *Triticosecale* Wittmack) lines. Agronomy 11, 16. https://doi.org/10.3390/agronomy11010016.

Guimarães, A., Venancio, A., Abrunhosa, L., 2018. Antifungal effect of organic acids from lactic acid bacteria on *Penicillium nordicum*. Food Addit. Contam. Part A 35, 1803–1818. https://doi.org/10.1080/19440049.2018.1500718.

Guo, D., Ren, C., Ali, A., Li, R., Du, J., Liu, X., Guan, W., Zhang, Z., 2019. *Streptomyces pactum* combined with manure compost alters soil fertility and enzymatic activities, enhancing phytoextraction of potentially toxic metals (PTMs) in a smelter-contaminated soil. Ecotoxicol. Environ. Saf. 181, 312–320. https://doi.org/10.1016/j.ecoenv.2019.06.024.

Guo, L., Xi, B., Lu, L., 2024. Strategies to enhance production of metabolites in microbial co-culture systems. Bioresour. Technol. 406, 131049. https://doi.org/10.1016/j.biortech.2024.131049.

Hamdali, H., Bouizgarne, B., Hafidi, M., Lebrihi, A., Virolle, M.J., Ouhdouch, Y., 2012. Screening for rock phosphate solubilizing Actinomycetes from Moroccan phosphate mines. Appl. Soil Ecol. 38, 12–19. https://doi.org/10.1016/j.apsoil.2007.08.007.

Harir, M., Bendif, H., Bellahcene, M., Fortas, Z., Pogni, R., 2018. *Streptomyces* secondary metabolites. In: Enany, S. (Ed.), Basic Biology and Applications of Actinobacteria. IntechOpen, UK, pp. 99–122.

Iacomino, G., Idbella, M., Laudonia, S., Vinale, F., Bonanomi, G., 2022. The suppressive effects of biochar on above-and belowground plant pathogens and pests: a review. Plants 11, 3144. https://doi.org/10.3390/plants11223144.

Imboden, L., Afton, D., Trail, F., 2018. Surface interactions of *Fusarium graminearum* on barley. Mol. Plant Pathol. 19, 1332–1342. https://doi.org/10.1111/mpp.12616.

Jatuwong, K., Aiduang, W., Kiatsiriroat, T., Kamopas, W., Lumyong, S., 2025. A review of biochar from biomass and its interaction with microbes: enhancing soil quality and crop yield *in Brassica* cultivation. Life 15 (2), 284. https://doi.org/10.3390/life15020284.

Jin, L., Feng, S., Tang, S., Dong, P., Li, Z., 2023. Biological control of potato late blight with a combination of *Streptomyces* strains and biochar. Biol. Control 183, 105248. https://doi.org/10.1016/j.biocontrol.2023.105248.

Kalaivani, K., Senthil-Nathan, S., Stanley-Raja, V., Vasantha-Srinivasan, P., 2023. Physiological and biochemical alterations in *Vigna rdiate* L. triggered by sesame derived elicitors as defense mechanism against *Rhizoctonia* and *Macrophomina* infestation. Sci. Rep. 13, 13884. https://doi.org/10.1038/s41598-023-39660-y.

Kaushal, S., Sharma, P., 2022. Antimicrobial and antioxidant potential of banana (*Musa sapientum*) peel extracts of variety grand naine, major compound 5-hydroxymethylfurfural in methanolic peel extract and its derivatives. Agric. Res. J. 59. https://doi.org/10.5958/2395-146X.2022.00134.X.

Khan, S., Srivastava, S., Karnwal, A., Malik, T., 2023. *Streptomyces* as a promising biological control agents for plant pathogens. Front. Microbiol. 14, 1285543. https://doi.org/10.3389/fmicb.2023.1285543.

Köhl, J., Kolnaar, R., Ravensberg, W.J., 2019. Mode of action of microbial biological control agents against plant diseases: relevance beyond efficacy. Front. Plant Sci. 10, 845. https://doi.org/10.3389/fpls.2019.00845.

Le, K.D., Yu, N.H., Park, A.R., Park, D.J., Kim, C.J., Kim, J.C., 2022. *Streptomyces* sp. AN090126 as a biocontrol agent against bacterial and fungal plant diseases. Microorganisms 10, 791. https://doi.org/10.3390/microorganisms10040791.

Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C., Crowley, D., 2011. Biochar effects on soil biota – a review. Soil Biol. Biochem. 43, 1812–1836. https://doi.org/10.1016/j.soilbio.2011.04.022.

Liu, D., Ding, Z., Ali, E.F., Kheir, A.M., Eissa, M.A., Ibrahim, O.H., 2021. Biochar and compost enhance soil quality and growth of roselle (*Hibiscus sabdariffa* L.) under saline conditions. Sci. Rep. 11, 8739. https://doi.org/10.1038/s41598-021-88293-6.

Logrieco, A., Manka, M., Altomare, C., Bottalico, A., 1990. Pathogenicity of *Fusarium graminearum* chemotypes towards corn, wheat, triticale and rye. J. Phytopathol. 130, 197–204. https://doi.org/10.1111/j.1439-0434.1990.tb01168.x.

McLaughlin, M.S., Roy, M., Abbasi, P.A., Carisse, O., Yurgel, S.N., Ali, S., 2023. Why do we need alternative methods for fungal disease management in plants? Plants 12, 3822. https://doi.org/10.3390/plants12223822.

Mun, B.G., Hussain, A., Park, Y.G., Lee, I.J., Yun, B.W., 2024. The PGPR *Bacillus aryabhattai* promotes soybean growth via nutrient and chlorophyll maintenance and the production of butanoic acid. Front. Plant Sci. 15, 1341993. doi: 10.3389/fpls.2024.1341993.

Nakashita, H., Yoshioka, K., Yasuda, M., Nitta, T., Arai, Y., Yoshida, S., Yamaguchi, I., 2002. Probenazole induces systemic acquired resistance in tobacco through salicylic acid accumulation. Physiol. Mol. Plant Pathol. 61, 197–203. https://doi.org/10.1006/pmpp.2002.0426.

Nonthakaew, N., Panbangred, W., Wisuwat, S., Bungonsiri, I., 2022. Plant growth-promoting properties of *Streptomyces* spp. isolates and their impact on mung bean plantlets' rhizosphere microbiome. Front. Microbiol. 13, 1–17. https://doi.org/10.3389/fmicb.2022.967415.

Ons, L., Bylemans, D., Thevissen, K., Cammue, B.P., 2020. Combining biocontrol agents with chemical fungicides for integrated plant fungal disease control. Microorganisms 8, 1930. https://doi.org/10.3390/microorganisms8121930.

Pazhanimurugan, R., Radhakrishnan, M., Shanmugasundaram, T., Gopikrishnan, V., Balagurunathan, R., 2016. Terpenoid bioactive compound from Streptomyces rochei (M32): taxonomy, fermentation and biological activities. World J. Microbiol. Biotechnol. 32, 161. https://doi.org/10.1007/s11274-016-2121-5.

Qian, S., Zhou, X., Fu, Y., Song, B., Yan, H., Chen, Z., Sun, Q., Ye, H., Qin, L., Lai, C., 2023. Biochar-compost as a new option for soil improvement: Application in various problem soils. Sci. Total Environ. 870, 162024. doi: 10.1016/j.scitotenv.2023.162024.

Renuka, G., Ugandhar, T., 2024. Unveiling the mechanisms of actinobacteria in biocontrol against fungal phytopathogens: exploring potential biocontrol. J. of Biotech. and

Biochem. 10 (2), 24–37. https://doi.org/10.9790/264X-1002012437.
Ryu, C.M., Farag, M.A., Hu, C.H., Reddy, M.S., Wei, H.X., Paré, P.W., Kloepper, J.W., 2003. Bacterial volatiles promote growth in Arabidopsis. Proc. Natl. Acad. Sci. 100, 4927–4932. doi: 10.1073/pnas.0730845100.
Sadigh-Eteghad, S., Dehnad, A., Shanebandi, D., Khalili, I., Razmarayii, N., Namvaran, A., 2011. Identification and characterization of a *Streptomyces* sp. isolate exhibiting activity against multidrug-resistant coagulase-negative Staphylococci. Vet. Res. Commun. 35, 477–486. https://doi.org/10.1007/s11259-011-9491-9.
Santos, J.E.D.Á., de Brito, M.V., Pimenta, A.T.Á., da Silva, G.S., Zocolo, G.J., Muniz, C.R., da Silva, C.D.F.B., 2023. Antagonism of volatile organic compounds of the *Bacillus* sp. against *Fusarium kalimantanense*. World J. Microbiol. Biotechnol. 39, 60. doi: 10.1007/s11274-022-03509-9.
Sharma, M., Kaushik, R., Pandit, M.K., Lee, Y.-H., 2025. Biochar-induced microbial shifts: advancing soil sustainability. Sustainability 17 (4), 1748. https://doi.org/10.3390/su17041748.
Singh, A.K., Chhatpar, H.S., 2011. Combined use of Streptomyces sp. A6 and chemical fungicides against *Fusarium* wilt of *Cajanus cajan* may reduce the dosage of fungicides required in the field. Crop Prot. 30, 770–775. https://doi.org/10.1016/j.cropro.2011.03.015.
Singh, P., Dhankhar, J., Kapoor, R.K., Sharma, A., 2023. A comparative study on GC-MS analysis and antimicrobial activity of bioactive compounds present in aerial parts (leaf and fruit) of *Ficus benghalensis* L. J. Appl. Nat. Sci. 15, 870–883. https://doi.org/10.31018/jans.v15i2.4618.
Świątek, K., Olszewski, M.P., Kruse, A., 2022. Continuous synthesis of 5 hydroxymethylfurfural from biomass in on-farm biorefinery. GCB Bioen. 14 (6), 681–693. https://doi.org/10.1111/gcbb.12938.
Tabatabai, M.A., Bremner, J.M., 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol. Biochem. 1, 301–307. https://doi.org/10.1016/0038-0717(69)90012-1.
Wu, Y., Zhou, J., Li, C., Ma, Y., 2019. Antifungal and plant growth promotion activity of volatile organic compounds produced by *Bacillus amyloliquefaciens*. MicrobiologyOpen 8, e00813. https://doi.org/10.1002/mbo3.813.
Xu, M., Wang, Q., Wang, G., Zhang, X., Liu, H., Jiang, C., 2022. Combatting Fusarium head blight: advances in molecular interactions between *Fusarium graminearum* and wheat. Phytopathol. Res. 4, 1–16. https://doi.org/10.1186/s42483-022-00142-0.
Yan, X., Guo, S., Gao, K., Sun, S., Yin, C., Tian, Y., 2023. The impact of the soil survival of the pathogen of *Fusarium* wilt on soil nutrient cycling mediated by microorganisms. Microorganisms 11, 2207. https://doi.org/10.3390/microorganisms11092207.
Yang, W., Zhang, L., Wang, Z., Zhang, J., Li, P., Su, L., et al., 2025. Effects of biochar and nitrogen fertilizer on microbial communities, CO_2 emissions, and organic carbon content in soil. Sci. Rep. 15, 9789. https://doi.org/10.1038/s41598-025-94784-7.
Zambrano, E.C., Parra, A.S., Ortiz, Á.M.M., 2021. Biocontrol of rice sheath blight with microorganisms obtained in rice cultivated soils. Bragantia 80, e0921. https://doi.org/10.1590/1678-4499.20200356.
Zhang, J., Chen, J., Hu, L., Jia, R., Ma, Q., Tang, J., Wang, Y., 2021. Antagonistic action of *Streptomyces pratensis* S10 on *Fusarium graminearum* and its complete genome sequence. Environ. Microbiol. 23, 1925–1940. https://doi.org/10.1111/1462-2920.15282.
Zhang, X., Song, Y., Yang, X., Hu, C., Wang, K., 2023. Regulation of soil enzyme activity and bacterial communities by food waste compost application during field tobacco cultivation cycle. Appl. Soil Ecol. 192, 105016. https://doi.org/10.1016/j.apsoil.2023.105016.
Gorovtsov, A. V., Minkina, T. M., Mandzhieva, S. S., Perelomov, L. V., Soja, G., Zamulina, I. V., Rajput, V. D., Sushkova, S. N., Mohan, D., Yao, J., 2020. The mechanisms of biochar interactions with microorganisms in soil. Environ. Geochem. Health. 42, 2495–2518. https://doi.org/10.1007/s10653-019-00412-5.