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# High-throughput sequencing metabarcoding and network analysis elucidate the effects of soil fumigation and biostimulant on potato yield, rhizoctonia canker, and fungal community

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This study investigated the impact of chloropicrin fumigation and a *Bacillus* species-based biostimulant applied alone or in combination in soil of potato crop over five key time-points in season. Rhizoctonia canker incidence, potato yield and the effect on the fungal community have been monitored. Quantitative PCR and high throughput metabarcoding sequencing were used to assess soil fungal diversity, specifically biomass, richness, composition and network interactions. Potato gross yield increased by 7.06% (23.5 cwt. acre<sup>-1</sup>) with fumigation alone and by 8.41% (28 cwt. acre<sup>-1</sup>) with biostimulant applied to fumigated soil in comparison to non-fumigated control treatment. Net yield losses caused by rhizoctonia canker exceeded 20% in control plots, while both treatments reduced rhizoctonia canker index by 30% and yield losses to less than 5.6%. Fumigation alone reduced fungal abundance during the season, but the biostimulant slightly increased the mean and variability of fungal abundance. Shannon and Evenness diversity indices decreased in fumigated plots, while the biostimulant increased the variability of Chao1 and Observed ASVs indices throughout the season. Biostimulant application promoted rapid late-season fungal recolonization, enlarged connectivity among taxa in non-fumigated soil, and, when combined with fumigation, eliminated the late-season resurgence of *Rhizoctonia solani* detected in fumigation-only plots. Fumigation also delivered early-season knock-downs of *Fusarium* spp. (10-fold reduction) and *Colletotrichum coccodes* ( $\geq 3$ -fold), yet these both opportunists partially rebounded by harvest while the biostimulant had little effect on these genera. *Alternaria* spp. and *Verticillium nubilum* stayed sporadic (<0.5 % reads) but were consistently lowest in fumigated soils. Overall, integrated fumigant-biostimulant management delivered the greatest season-long suppression of pathogenic

fungi, the most resilient and functionally connected fungal network, and the highest tuber yield, demonstrating that coupling targeted chemical fumigation with microbial stimulation can reconcile disease control with soil microbiome monitoring in intensive potato systems.

#### KEYWORDS

chloropicrin, bacillus species-based biostimulant, *Rhizoctonia solani*, fungal community, MiSeq metabarcoding, machine-learning analysis, network interaction

## Introduction

Potato (*Solanum tuberosum* L.) is one of the world's most important staple crops, providing a substantial portion of global food security. However, the cultivation of potato crops is constantly threatened by various biotic and abiotic stresses, among which rhizoctonia canker (black scurf) caused by the soilborne fungal pathogen *Rhizoctonia solani* Kühn (*Thanatephorus cucumeris*), a fungus prevalent in potato-growing regions worldwide (1, 2). *Rhizoctonia* disease manifests as foliar and stem rot, tuber decay, and damping-off in seedlings. Furthermore, tubers frequently exhibit the presence of rhizoctonia canker (black scurf), which consists of irregular black sclerotia of *R. solani* accumulated on the tuber surface (2). The economic implications of this disease are profound, as it not only reduces yield quantity but also affects tuber quality, leading to market rejection and financial losses throughout the supply chain (1). The ease with which *Rhizoctonia solani* can persist in soil and its capacity to infect a wide range of crops further compounds the complexity of managing this pathogen effectively. Traditional methods of disease management, such as chemical fumigation and rotation, have shown limited success due to the pathogen's ability to form resilient resting structures and its wide host range (3). Additionally, increasing concerns about the environmental impact and long-term sustainability of chemical interventions necessitate the exploration of alternative strategies for disease management. This context underscores the urgency of developing innovative and sustainable approaches that not only mitigate the disease's impact but also promote the overall health of the agroecosystem. Although biological control methods have proven effective in some cases, the outcomes are not always consistent (3, 4). Soil microorganisms play a vital role in maintaining plant health and productivity, and changes to the soil microbiome resulting from farming practices can affect soil functions (5, 6) and alter *R. solani* disease incidence (7).

Previous authors found that soil fumigation altered the microbial communities when compared to non-fumigated soils (8). In Canadian potato crop systems, the most commonly used chemical fumigants are chloropicrin and metam-sodium (9). In a study on tomato production systems, authors explored the potential of chloropicrin as an alternative fumigant to methyl bromide (10). They found that chloropicrin was highly effective at reducing soil

microbial populations, with more than 85% of bacteria, fungi, and actinomycetes killed according to a bioassay method that estimated microbial inoculum survival on potato dextrose agar petri dishes. Although chemical fumigation effectively reduces soil-borne diseases, it can have negative consequences, including reduced soil microbial biomass and diversity, enzyme activities, and carbon/nitrogen cycling and mineralization (11–13). Repeated soil fumigation with chloropicrin could lead to a decline in microbial richness and diversity, but to an increase in the abundance of *Actinobacteria* and *Saccharibacteria* (14).

While fumigation with chloropicrin has a significant and persistent impact on microbial diversity (9), other fumigation methods and biofumigation with mustard plant residues also lead to transient decreases in microbial diversity (3, 9, 15). These changes in the soil microbiome may impair the functions and persistence of potato beneficial microorganisms which can result in dysbiosis, creating opportunities for soilborne plant pathogens to invade or recolonize the soil and negatively impacting agricultural productivity (16, 17). Since the use of chloropicrin to control soil-borne pests and diseases may destroy beneficial soil microorganisms, it is necessary to promote rapid microbial repopulation of the soil using microorganisms that stimulate plant growth (18, 19).

Biostimulants based on seaweed extracts contain plant hormones and polysaccharides, as those based on humic substances promote phytohormone-like activity that have been shown to enhance soil nutrient availability and increase mineral content in plant-based food (20). The *Bacillus* genus is a well-known source of phytostimulants, biopesticides and biofertilizers products. Indeed, mixes of *Bacillus* species-based biostimulant are available commercially. These biostimulants increase microbial activity leading to increased nutrient availability that affect plant growth and enhance plants' tolerance to biotic and abiotic stresses (21). A better understanding of the impact of inoculated bacterial mixed species-based biostimulants on plant-growth and soil microbial community may lead to efficient strategies to promote sustainable management farming practices to mitigate the effects of soil fumigation on soil microbial diversity, structure, and functions (19, 22, 23).

To address this gap, our study aims to use high throughput sequencing (HTS) amplicon-based Illumina MiSeq and network inference analysis to assess the effect of Chloropicrin-based

fumigant and mix of five *Bacillus* species-based biostimulant, applied alone or in combination, on soil fungal community and their interactions in potato production field.

Nowadays, machine learning techniques such as penalized regression, support vector machine, random forest, artificial neural network, and deep neural networks are increasingly being used to explore the complex relationships between microbiome and host phenotypes (24, 25). Our study will employ the artificial neural network technique to contribute to a better understanding of the interactions among different fungal species within a community, while also providing insights into the use of biostimulant and fumigant to promote plant growth and control rhizoctonia canker disease.

We hypothesize that fumigation will increase potato yield and reduce yield losses caused by *Rhizoctonia solani*, while altering interactions within the soil fungal community. The combination of biostimulant and fumigation will further increase potato yield, promote rapid fungal recolonization which will increase species richness and reduce soil persistence of potato fungal pathogens in comparison to control and fumigated soils. Our specific objectives were (1) to evaluate the agronomic performance of potato plants and the incidence of rhizoctonia canker on potato yield in soil treated with Chloropicrin-based fumigant and mix *Bacillus* species-based biostimulant, applied alone or in combination (2), to compare the effects of the treatments on soil fungal community, specifically its quantity, diversity, richness, and composition, and (3) to assess the relationships and interactions between the soil fungal populations.

## Materials and methods

### Field experiment design

Experiment was carried out on 16 plots of 6 rows of 6 m in length, planted in a field located in Saint-Ubalde (46.729085, -72.266851) (QC, Canada). Experiments were conducted in a randomized complete block design with four repetitions, installed in an area of 532.53 m<sup>2</sup> in 2018. Soil physicochemical properties and nutrients content are described in Table 1. Planting was done at a density of 33,300 plants ha<sup>-1</sup>. The two rows in the center of each plot were used to determine the potato yield, the next two rows were used for soil composite and plant biomass sampling, while the outer rows represent a treatment buffer zone. Main plots involved four treatments: T1 (non-fumigated soil without biostimulant application), T2 (non-fumigated soil with biostimulant application), T3 (fumigated soil without biostimulant application) and T4 (fumigated soil with biostimulant application).

The fumigation of plots T3 and T4 was done by a well-trained soil fumigation service provider using Chloropicrin Pic Plus fumigant (Synagri, QC, Canada) injected under pressure with shank-injectors penetrating the first 15 cm of the soil. The fumigation process took place on May 19<sup>th</sup>, 2018. This method involved applying the fumigant to the soil to control soil-borne pests and diseases. Regarding the *Bacillus* species-based

TABLE 1 Soil properties and nutrients content of the soil's experimental site.

Elements	Values
Al (ppm)	1599.0
B (ppm)	0.5
Cu (ppm)	8.6
Fe (ppm)	171.7
Mn (ppm)	10.1
Zinc (ppm)	10.6
P (Kg.ha <sup>-1</sup> )	481.0
Ca (Kg.ha <sup>-1</sup> )	1721.0
Mg (Kg.ha <sup>-1</sup> )	220.0
Na (Kg.ha <sup>-1</sup> )	21.0
K (Kg.ha <sup>-1</sup> )	156.0
Organic Matter (%)	5.5
Saturation -K+Mg+Ca (%)	25.8
Saturation -Ca (%)	20.0
Saturation -K (%)	0.9
Saturation - Mg (%)	4.4
PSI (%)	13.4
Estimated CEC	18.8
Lime index	60.0
pH water (1:1)	5.1
pH SMP	6.0

Soil properties and nutrient contents were measured as described in Materials and Methods.

biostimulant application, Alpine Bio20 (AgBrima, QC, Casnada) is a mix of eight-isolates from five *Bacillus* species: *Bacillus subtilis*, *Bacillus amyloliquefaciens*, *Bacillus licheniformis*, *Bacillus pumilus*, and *Bacillus velezensis* applied at around  $5 \times 10^5$  CFU.mL<sup>-1</sup>. These strains were selected based on their plant growth-promoting traits, including indole-3-acetic acid (IAA) production (>20 µg mL<sup>-1</sup>), siderophore production, and phosphate solubilization. The application was done by inoculating the *Bacillus* species-based biostimulant at 30 L. ha<sup>-1</sup> to the furrow on the potato seed pieces at plantation. This process occurred on May 29<sup>th</sup>, 10 days after the soil fumigation and during the plantation (10 DAF and 0 DAP). Indeed, certified seed tubers of 'Russet Burbank' were sourced from a single commercial lot (Patates Dolbec Inc., QC) and processed under SOP PB-DA-FT-2018-01 ("Seed Tubers Preparation Planting"). Tubers were mechanically graded to 6.4–7.6 cm diameter and cut into single-eye pieces weighing 50 g ± 5 g to ensure uniform size and vigor. Uniform seed piece size and weight promotes consistent emergence and root development, reducing variability in plant vigor and soil-tuber contact. Smaller or uneven pieces can delay canopy closure and increase soil exposure, creating more infection sites for *Rhizoctonia solani*. By standardizing seed

characteristics, we minimized this source of variability in disease occurrence. The Table 2 presents the milestones of the project.

## Soil sampling and analysis

Composite of four soil cores (4.5 cm diameter and 0–20 cm depth) were sampled, one core in each corner of the rows adjacent to center rows of a plot at five time-periods during the growing season (Table 2). Soil samples were stored at 4°C for no more than a week until being processed for soil analysis. The soil was air-dried and sieved to 2 mm. A subsample was ground to 100 mesh to determine total carbon and nitrogen content using dry combustion analysis at 950°C (Leco-CNS) (26). The percentage of organic matter (% M.O.) was obtained by multiplying the C values by 1.724, as mentioned in the Fertilization Reference Guide (27). The water pH was determined on air-dried soils with an electrode in a soil and water solution in a 1:1 ratio. The buffer pH (SMP) was determined in a 1:2 ratio after adding the SMP buffer solution at pH 7.5 and after stirring for 15 minutes (27). The contents of P, K, Ca, Mg, Na, B, Al, Cu, Fe, Mn and Zn elements were extracted with Mehlich-3 solution in a soil:solution ratio of 1:10 (3 ml of weighed soil, 30 ml of solution) (28) and then determined by argon plasma emission spectrometry (ICP) (29, 30). The CEC was estimated with the sum of exchangeable bases and estimated exchangeable acidity (27).

## Assessing plant agronomic performance and incidence of *Rhizoctonia* spp.

Two plants per row adjacent to the two centered rows were randomly sampled for a total of four plants per plot one hundred one

days after planting. The roots were shaken to remove all adhering soil and placed in a paper bag, while the aerial parts of the plants were placed in a paper bag to determine plant root and plant aerial biomass respectively. Potatoes were harvested over 2 x 5 m ( $\pm$  32 plants) targeting the two central rows of each plot. Following the harvest of the tubers from each of the two central rows (2 x 5 m), the number of plants and the number and weights of the tubers were recorded to calculate total gross yield. Tubers were sorted into five size classes based on maximum diameter: < 2 in (< 5.1 cm), 2  $\frac{1}{8}$ –2  $\frac{1}{2}$  in (5.4–6.4 cm), 2  $\frac{1}{2}$ –2  $\frac{3}{4}$  in (6.4–7.0 cm), 2  $\frac{3}{4}$ –3 in (7.0–7.6 cm), and > 3 in (> 7.6 cm) to determine gross yield per class. For disease assessments, 25 tubers from the 2  $\frac{1}{2}$ –2  $\frac{3}{4}$  in (6.4–7.0 cm) class and 15 tubers from the > 3 in (> 7.6 cm) class were examined per plot. Tubers were washed, air-dried, and rated for rhizoctonia canker lesion coverage on a six-point scale: 0 = no lesion; 1 =  $\leq$  1%; 2 = > 1–5%; 3 = > 5–10%; 4 = > 10–25%; 5 = > 25–50%; 6 = > 50%. The rhizoctonia disease indices were determined as the produce of the rate by the number of tubers showing this rate of lesion coverage, as described in Supplementary Table S1. The rhizoctonia index was estimated according to the adapted method of (31) following the formula:  $RI(\%) = \frac{SI}{NTO} \times 100$ , where RI: rhizoctonia index, SI: sum of disease indices, which results by  $\sum_i i$  (with Rate  $i$  X Number of tubers  $i$ ) and NTO: number of tubers observed. For each plot, tubers showing rhizoctonia symptoms on more than 5% of their surface were used to estimate the gross yield losses, the remaining harvested tubers representing the marketable yield.

## Soil DNA extraction

The DNA extraction was carried out as described (32). In order to collect and prepare soil samples for DNA extraction, a 200 g

TABLE 2 Time-periods for treatment application, soil sampling and potatoes harvest.

Date	Period	Treatment	Days After Fumigation (DAF)	Days After Plantation (DAP)	Activity
2018-05-17	P1	T1	-2 DAF	-12 DAP	Soil sampling before applying treatments
2018-05-19		T3	0 DAF	-10 DAP	Fumigation is applied to soil
2018-05-25	P2		6 DAF	-4 DAP	Soil sampling 6 DAF and 4 days before the biostimulant inoculation at plantation.
2018-05-29		T2 + T4	10 DAF	0 DAP	Plantation and Biostimulant inoculation
2018-07-13			55 DAF	45 DAP	Hilling to ensure the shape of the soil ridge of potato rows
2018-07-19	P3		61 DAF	51 DAP	Soil sampling 61 days after fumigation and 51 days after biostimulant inoculation
2018-08-13	P4		86 DAF	76 DAP	Soil sampling after tuber initiation, 86 days after fumigation and 76 days after biostimulant inoculation
2018-09-07	P5		111 DAF	101 DAP	Plant and roots biomass and Soil sampling 6 days before top killing potato plants, 111 days after fumigation and 101 days after biostimulant inoculation
2018-09-24	P6		128 DAF	118 DAP	Potatoes harvest

Period indicates the date when the soils had been sampled or the tubers had been harvested. Treatment indicates type of plot: T1 no fumigation and no biostimulant; T2 no fumigation but biostimulant inoculated in furrow at planting May 29th; T3 chloropicrin fumigation May 19th and no biostimulant; T4 chloropicrin fumigation May 19th, then biostimulant inoculated in furrow at planting May 29th.

aliquot of each soil sample was manually homogenized and sieved to 6 mm, then a 0.5 g subsample of the 6 mm sieved soil was added to 2 mL tubes containing 1.4 g of matrix E beads and 1 mL of lysis buffer that were supplied with the FastDNA™ SPIN Kit for Soil (MP Biomedicals, Solon, OH, USA), as described by (33). The homogenization step was done using a FastPrep-24™ instrument (MP Biomedicals, Solon, OH, USA). Quality control of DNA extracts was evaluated by spectrophotometry (Biophotometer, Eppendorf, Mississauga, ON, Canada) with a G1.0 microcuvette (mCuvette, Eppendorf) at 230, 260, and 280 nm.

## Quantitative PCR analysis

The FF390 (5'-CGATAACGAACGAGACCT-3') and FR1 (5'-AACCATTCAATCGGTAAT-3') primers from (34) were used to amplify a partial 18S rRNA sequence (approximately 330 bp) from fungal DNA. qPCR was performed on a CFX96 instrument (Biorad, Hercules, CA) using SYBR green qPCR mix (Qiagen, Toronto, ON) as described by (33). Briefly, the thermal cycling conditions consisted of an initial activation step of 15 minutes at 95°C, followed by 40 cycles of 95°C for 1 minute, 30 seconds at the annealing temperature (51°C for fungi), and 72°C for 1 minute. Standard curves were generated from a known quantity of amplified DNA fragments, diluted over a 4-log range. This method was used to quantify the total fungal content in the soil samples, expressed as the number of amplified units per gram of dry soil (AU g<sup>-1</sup> dry soil). The data were normalized, and a log transformation was applied.

## Library construction and sequencing

The eukaryotic fungal rRNA Internal Transcribed Spacer 1 (ITS1) region was amplified from soil DNA extracts to prepare amplicons for Illumina sequencing, following the protocol described by (33). Briefly, the ITS1 region was amplified using the primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') (35) and ITS2 (5'-GCTGCGTTCCTCATCGATGC-3') (36). A two-step dual-indexed PCR approach was employed, specifically designed for Illumina sequencing platforms by the Plateforme d'analyses génomiques (IBIS, Université Laval, Quebec City, QC, Canada).

In the first PCR step, the ITS1 region was amplified using the ITS1F and ITS2 primers with overhang adapters compatible with Illumina index and sequencing adapters. The PCR conditions were as follows: initial denaturation at 95°C for 3 minutes; 25 cycles of 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 30 seconds; followed by a final extension at 72°C for 5 minutes.

The PCR products were then purified using AMPure XP beads (Beckman Coulter, Mississauga, ON, Canada) to remove excess primers and nucleotides. In the second PCR step, dual indices and Illumina sequencing adapters were added using the Nextera XT Index Kit (Illumina, San Diego, CA, USA). The indexing PCR

conditions consisted of initial denaturation at 95°C for 3 minutes; 8 cycles of 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 30 seconds; followed by a final extension at 72°C for 5 minutes.

The indexed PCR products were purified again using AMPure XP beads, quantified using a Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA, USA), and pooled in equimolar amounts. The pooled library was validated using an Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) to confirm the size and purity of the amplicons. Sequencing was performed on an Illumina MiSeq platform using the MiSeq Reagent Kit v3 (600-cycle) to generate paired-end reads of 2 × 300 bp.

## Statistical and bioinformatic analyses

The agronomic data collected were subjected to one-way analysis of variance (ANOVA) using SAS (Statistical Analysis System) software, version 9.3, following the mixed linear model procedure. Treatment, time, repetition and the interactions mean were compared by performing *post hoc* Duncan's test at the p-value threshold of  $P \leq 0.05$ .

Sequence analysis was performed under QIIME2 (37) using the DADA2 filtration approach (38) for determining the Amplicon Sequence Variants (ASV). The taxonomic assignment of ASVs was performed using a pre-trained classifier based on Unite (version 8) reference database (39) and a classification approach (40, 41).

R Studio software (version 4.1.3) was used to analyze the diversity indices, and the relative abundance of the most abundant taxa with treatment, sampling time points and blocks were considered as fixed factors. Non-normal data were normalised and scaled. Each of the inferred single DNA sequences recovered from a high-throughput analysis of marker genes, namely ASV, were subjected to several analyzes. Indeed, fungal alpha-diversity (Shannon), richness (Chao1), evenness (Pielou's evenness), and observed number ASVs were estimated via Phyloseq package (42) using an Anova, following by a *post hoc* Duncan's test at the p-value threshold of  $p \leq 0.10$ , based on the selected ASV table at a depth of 4,890 sequences per sample. Non-metric multidimensional scaling (NMDS) and hierarchical classification analysis were performed using the Bray–Curtis distance matrix, using microeco package (43). Analysis of the  $\beta$ -diversity of the fungal community, as well as the multilevel pairwise comparisons between treatment and sampling periods were done using a permutational multivariate analysis of variance (PERMANOVA) via the Adonis function (with 999 permutations) as described by (44).

We analyzed the ASV sequences database obtained from each of four soil samples per treatment (T1, T2, T3 and T4) and per sampling periods (P3, P4 and P5) to assess the detection of fungal pathogens. For each of the targeted pathogens, the total number of sequences detected in the total soil samples of a treatment at the three sampling-periods P3, P4 and P5 has been calculated. This total number of sequences was then used to calculate the relative abundance of reads associated with a pathogenic fungus by dividing this total by the total number of sequences obtained

from all the soils sampled for that treatment. The analysis of the evolution of the percentages per soil sampling-period, and of the total number of sequences per treatment as well as the proportion of sequences associated with the pathogen in the treatment soils allows to estimate the effectiveness of the treatments at reducing the presence of each pathogen at each time-period and at reducing their persistence in the treated soil during the season.

In order to analyse the changes in ASVs abundance and composition over time, we also used Venn diagram via the package *microeco* (43).

To investigate the relationships between fungal populations within the community in soil, we utilized an artificial network to examine co-occurrence patterns, by calculating all possible Spearman's rank correlations between ASVs (860 ASVs) at the correlation threshold ( $P = 0.001$ ). Indeed, Spearman correlation based on WGCNA package through the RMT theory (45) was applied to select the correlation threshold ( $COR\_p\_thres = 0.01$ ,  $COR\_cut = 0.8$ ) to define robust associations within the fungal community. This previous filtering step was applied to remove poorly represented ASVs and reduced network complexity, facilitating the determination of the core soil community, as described by (46) via the *microeco* (43) and *igraph* (47) packages. To summarize the variation in relative abundance of ASVs within a module (as recommended by (25), we used the module eigen-gene, following the method described by (45) We identified communities or modules within the network using modularity, as described by (48). To assign each node's role based on its within-module and among-module connectivity, we followed the approach outlined by (49). Various common network properties were measured to describe the network's topological properties. This included connectedness, which is one of the most important measurements for summarizing hierarchical structures (50). Node degree, also known as connectivity, is the most commonly used concept for describing the topological property of a node in a network (51). Clustering coefficient, which describes how well a node is connected with its neighbors (52). Density, which is closely related to the average connectivity (53). Betweenness, which is used to describe the ratio of paths that pass through the  $i^{th}$  node (a high betweenness node can serve as a broker similar to stress centrality (54). Centralization of degree, which is close to 1 for a network with a star topology and close to 0 for a network where each node has the same connectivity (53).

Then, we imported the data in Gephi's program (55) where all statistical analyses were carried out. Therefore, the resulting network with node colors indicating calculated modules were visualized via the ForceAtlas2 algorithm (56) with the overlapping parameter's speed 3.0, ratio (1.2) and margin 5.0. To estimate the number of nodes connected between different taxa or within one taxon in the soil, the function *plot\_sum\_links* was used to present the positive links from the function *cal\_sum\_links* via the *chorddiag* package (<https://github.com/mattflor/chorddiag>).

## Results

### Agronomic performance of potato plants and incidence of rhizoctonia canker disease

Various parameters (root biomass, aboveground biomass, tuber biomass, percentage of tubers showing rhizoctonia canker disease lesions, rhizoctonia canker disease index, gross yields and marketable yields) were measured to evaluate the effects of the fumigant chloropicrin and the *Bacillus* species-based biostimulant on agronomic performance of potato plants and rhizoctonia canker (black scurf) disease incidence caused by *Rhizoctonia solani* Kühn (*Thanatephorus cucumeris*). The results showed that the treatments had a significant impact on parameters variability. The application of biostimulant (T2) and fumigant (T3) alone led to a substantial increase in root biomass and tuber biomass measured at time-period P5 (Table 3). The application of biostimulant in fumigated soil (T4) showed the lowest aboveground biomass.

The incidence of rhizoctonia canker disease varied among the plots treated with or without chloropicrin, as measured by the percentage of tubers showing disease lesions at harvested time P6. The control plots (T1) and the biostimulant inoculated in non-fumigated plots (T2) showed respectively 20.00% and 22.50% infected tubers, while the fumigated plots (T3) and the biostimulant inoculated in fumigated plots (T4) showed respectively 5.63% and 7.50% of diseased tubers (Table 3). The fumigation treatment, either alone or in combination with biostimulant, significantly reduced the rhizoctonia canker disease index (DI) values. Furthermore, DI values of treatments T3 and T4 are not different and are significantly smaller

TABLE 3 Physio-agronomic performance of potato and incidence of rhizoctonia canker caused by *Rhizoctonia solani* in soil treated with fumigant and biostimulant alone or in combination.

Variables	T1	T2	T3	T4	R <sup>2</sup>	CV
BR	6.67 ± 1.65 b	9.59 ± 5.92 a	7.89 ± 2.20 ab	6.25 ± 3.06 b	0.15	47.41
BA	55.03 ± 17.47 a	54.38 ± 25.23 a	56.92 ± 16.19 a	42.90 ± 17.56 b	0.10	37.17
BT	211.31 ± 48.98 bc	239.13 ± 63.15 a	231.34 ± 34.88 ab	192.25 ± 50.11 c	0.15	23.01
TDL (%)	20.00 ± 23.89 a	22.50 ± 27.23 a	5.63 ± 6.57 b	7.50 ± 10.61 b	0.17	137.77
DI	0.40 ± 0.48 a	0.48 ± 0.60 a	0.12 ± 0.14 b	0.13 ± 0.18 b	0.18	140.70

Treatments T1: Non-fumigated soil without biostimulant, T2: Non-fumigated soil with biostimulant, T3: Fumigated soil without biostimulant, T4: Fumigated soil with biostimulant. Statistics R<sup>2</sup>, R-squared; CV, Coefficient of variation; Variables BR, Root Biomass (in dry g plant<sup>-1</sup>), BA, Aboveground Biomass (in dry g plant<sup>-1</sup>), BT, Tuber Biomass (in dry g plant<sup>-1</sup>), TDL, Percentage of tubers showing *rhizoctonia canker* disease lesions; DI, rhizoctonia canker disease index; BR, BA and BT were determined by sampling at P5 period, while TDL and DI parameters were measured at P6 period; Values (mean ± standard deviation) in the same row followed by different letters are significantly different at  $P \leq 0.05$ , Duncan's test.

than the DIs of treatments T1 and T2 (Table 3). The T2 treatment increased the biomass values (root and tuber biomass) when applied to control soils, whereas the inoculation of the *Bacillus* species-based biostimulant in fumigated soils reduced the values of the three biomasses compared to fumigated soils without biostimulant inoculation (Table 3).

The results from the study on the agronomic performance of potato plants and incidence of rhizoctonia canker disease showed that there was a significant difference in the average gross yields among the four treatments. As depicted in Figure 1, the potato gross yields were 332.98, 317.23, 356.43, and 360.43 cwt. acre<sup>-1</sup>, respectively, for T1, T2, T3, and T4 treatments. The fumigated plots (T3 and T4) had a significantly higher yield compared to the non-fumigated plots, with an increase of 23.5 cwt. acre<sup>-1</sup> (7.06%) and 28 cwt. acre<sup>-1</sup> (8.41%), respectively, when compared to the average gross yield of T1 plots.

The intensity of rhizoctonia canker disease also had a significant impact on the marketable yield of potatoes. Control plots (T1) and non-fumigated soil with biostimulant, (T2) plots showed respectively a 20.0% and 22.5% loss in marketable yield due to the disease, while treated plots T3 and T4 had significantly lower losses of 5.6% and 5.0%, respectively. The losses were calculated based on the presence of rhizoctonia canker lesions symptoms covering more than 5% of the tuber surface.

## Absolute fungi abundance in soil throughout the growing season

To investigate the effect of chloropicrin fumigation and *Bacillus* species-based biostimulant on the absolute fungi abundance in soil during the growing season, a qPCR protocol was used with specific primers targeting the ITS1 regions of fungi. The results showed that

the number of fungi in soil varied depending on the fumigation of the plots (Figure 2). Prior to treatment (P1), the average quantity of fungi was estimated at  $8.11 \pm 0.14$  Log AU g<sup>-1</sup> dry soil across all experimental plots. At P2 (6 days after fumigation) the average number of fungi in non-fumigated T1 and T2 plots was  $8.21 \pm 0.17$  Log AU g<sup>-1</sup> dry soil, compared to  $7.92 \pm 0.08$  Log AU g<sup>-1</sup> dry soil in fumigated T3 and T4 plots. The small 6-day gap between fumigation and the P2 sampling-period of the composite soils may have allowed the extraction of DNA from fungal propagules killed by fumigation but whose DNA had not been completely destroyed. At P2, the assessment of absolute fungal abundance by qPCR could thus be overestimated and may reduce the perception of fumigation effects. The highest median numbers of fungi were observed in T1 and T2 soils during P4 and P5 time-periods, while the lowest median numbers of fungi were observed in T3 plots during P3, P4, and P5 time-periods (Figure 2). This indicates that the effect of chloropicrin fumigation treatment on reducing the number of fungi in soil may take a few weeks to manifest itself in qPCR results, but it can persist throughout the growing season.

## Assessment of alpha-diversity in soil fungal community

Several alpha-diversity indices were utilized to evaluate the effects of treatments and sampling time-periods on the richness and diversity of fungal communities (Figure 3). A significant interaction between treatments and time-periods was observed for the Shannon diversity, Pielou's evenness, Chao1 richness, and unique ASV count (at  $P \leq 0.05$ ).

Results revealed that fumigated-treated soil (T3) and the biostimulant inoculation in fumigated soil (T4) exhibited lower Shannon diversity and Pielou's evenness values at P3 and P4 time-

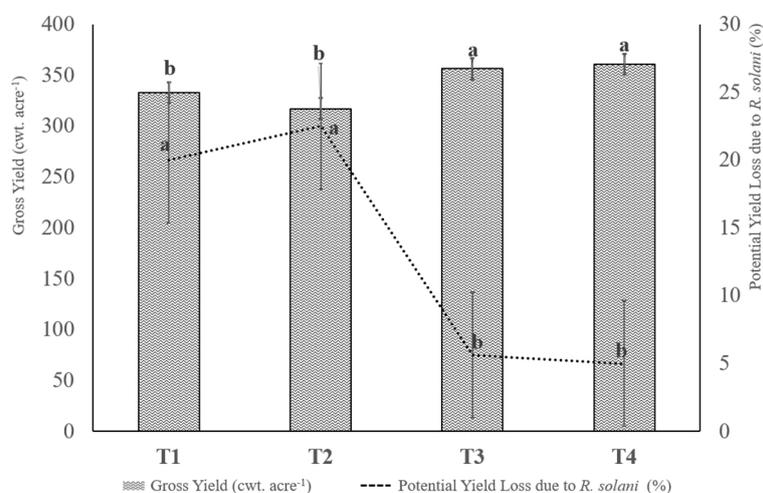
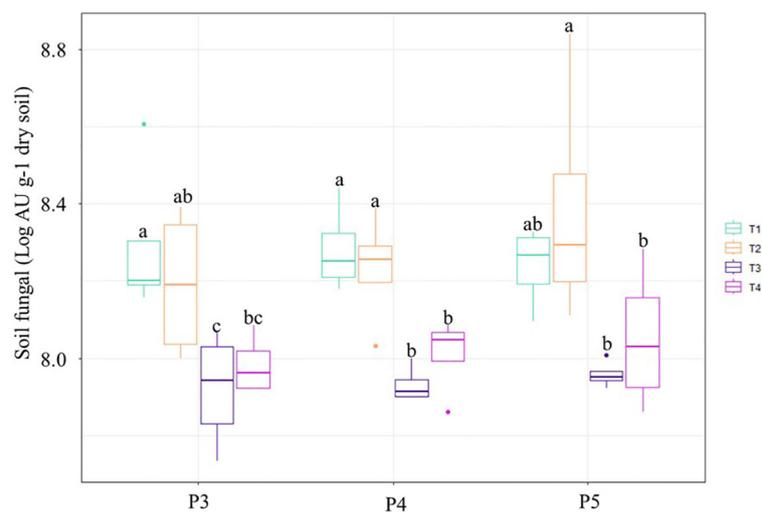
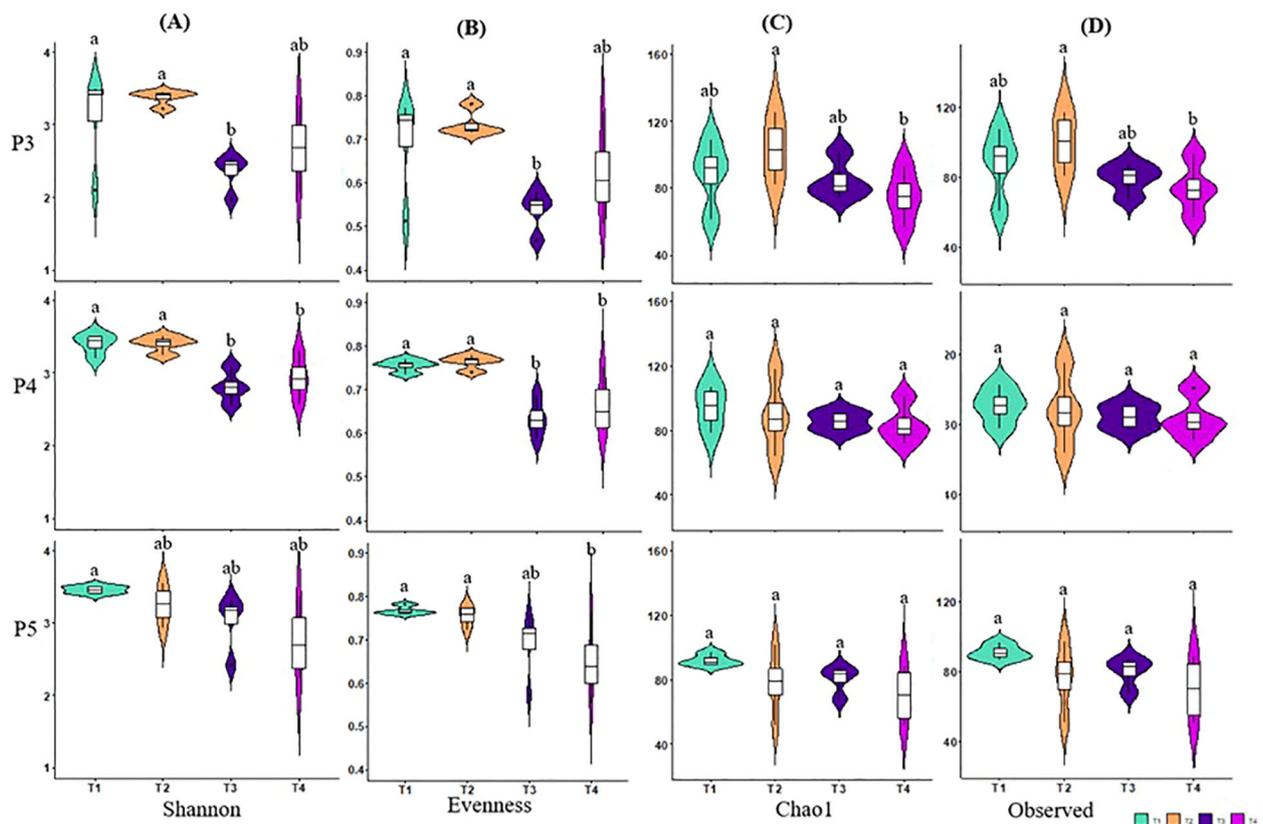


FIGURE 1

Effects of the treatments on potato gross yield and on relative proportion of potential yield losses due to the severity of *Rhizoctonia solani* disease (superficial lesions coverage of tuber surface  $\geq 5\%$ ). Treatments not sharing a letter are significantly different at  $P \leq 0.05$  according to one-way analysis of variance (ANOVA) among treatments. T1: Non-fumigated soil without biostimulant, T2: Non-fumigated soil with biostimulant, T3: Fumigated soil without biostimulant, T4: Fumigated soil with biostimulant.



**FIGURE 2** Box and whisker plots showing the quantitative abundances of soil fungi for each treatment and sampling-period points P3 to P5. The line inside the box represents the median, while the whiskers represent the lowest and highest values within the 1.5 interquartile range (IQR). Treatments comprised: non-fumigated soil without biostimulant (T1), Non-fumigated soil with biostimulant (T2), Fumigated soil without biostimulant (T3), Fumigated soil with biostimulant (T4). Treatments not sharing a letter above the boxes at any time-period are significantly different (Duncan test,  $P \leq 0.05$ ).



**FIGURE 3** Violin plots of soil-fungal  $\alpha$ -diversity indices across treatments (T1–T4) and sampling periods (P3–P5). (A) Shannon diversity; (B) Pielou’s evenness; (C) Chao1 richness; (D) Observed ASV count. Boxes show inter-quartile range, central lines indicate medians, and letters above violins denote significant differences between treatments at each period (Duncan test,  $P \leq 0.05$ ).

periods than those of the control soil (T1) and the biostimulant-treated soil (T2) (Figures 3A, B). Additionally, the violin plots of Figure 3 showed bimodal distributions of fungal species as a function of time-periods for the four alpha-diversity indices variables in control (T1), biostimulant (T2), fumigant (T3) and combine biostimulant and fumigant -treated soils (T4).

This indicates that the T3 and T4 treatments had a transient effect on fungal Shannon diversity and Pielou's evenness during most of the growing season and the effect did not persist at P5 time-period. Figure 3 showed systematically higher variability among the values of Chao1 and Observed ASVs indices in biostimulant-treated non-fumigated soils (T2) from P3 to P5 time-periods, while during the same time-periods higher variability among the values of Shannon and Pielou's evenness index was also observed in biostimulant-treated fumigated soils (T4).

Moreover, at P3 time-period only, the biostimulant-treated fumigated soil (T4) had significantly lower Chao1 richness and Observed index values compared to those of all other treatments (Figures 3C, D) ( $P \leq 0.001$ ). Additionally, the violin plots in Figure 3 showed bimodal distributions of fungal species, as a function of soil sampling-periods, for the four alpha diversity index variables in the control (T1), biostimulant (T2), fumigant (T3) or biostimulant-fumigant combination (T4) soils. This would suggest that specific groups of fungi were able to thrive in the fungal community at soil sampling-period because they were favored by the ecosystem conditions imposed by one, few, or all the treatments.

## Assessing fungal diversity global composition under different treatments over time

Results from a permutational multivariate analysis of variance showed highly significant differences between the fungal diversity composition among the different treatments and time-periods (Table 4).

A non-metric multidimensional scaling (NMDS) analysis also revealed an evolution in the composition of fungal diversity in plots of potato production over time (Figure 4). Fungal communities among the treatment plots at time-period P2 were less variable, indicating that the short-term effect of fumigation was not yet

noticeable. However, from time-periods P3 to P5, the composition of fungal communities in treatments applied to non-fumigated soils (T1 and T2) were significantly different from those applied to fumigated soils (T3 and T4) (Figure 4). Interestingly, the biostimulant treatment also significantly altered the composition of fungi community in non-fumigated plots but it much less affected the composition in fumigated soils (Table 5, Supplementary Table S2). At P5, the composition of the soil fungal community became more variable among each treatment, with their fungal composition becoming less significantly different. Thus, our findings demonstrate a clear effect of the fumigant Chloropicrin and of the *Bacillus* species-based inoculant, which was particularly noticeable during time-periods P3 to P5 (Figure 4).

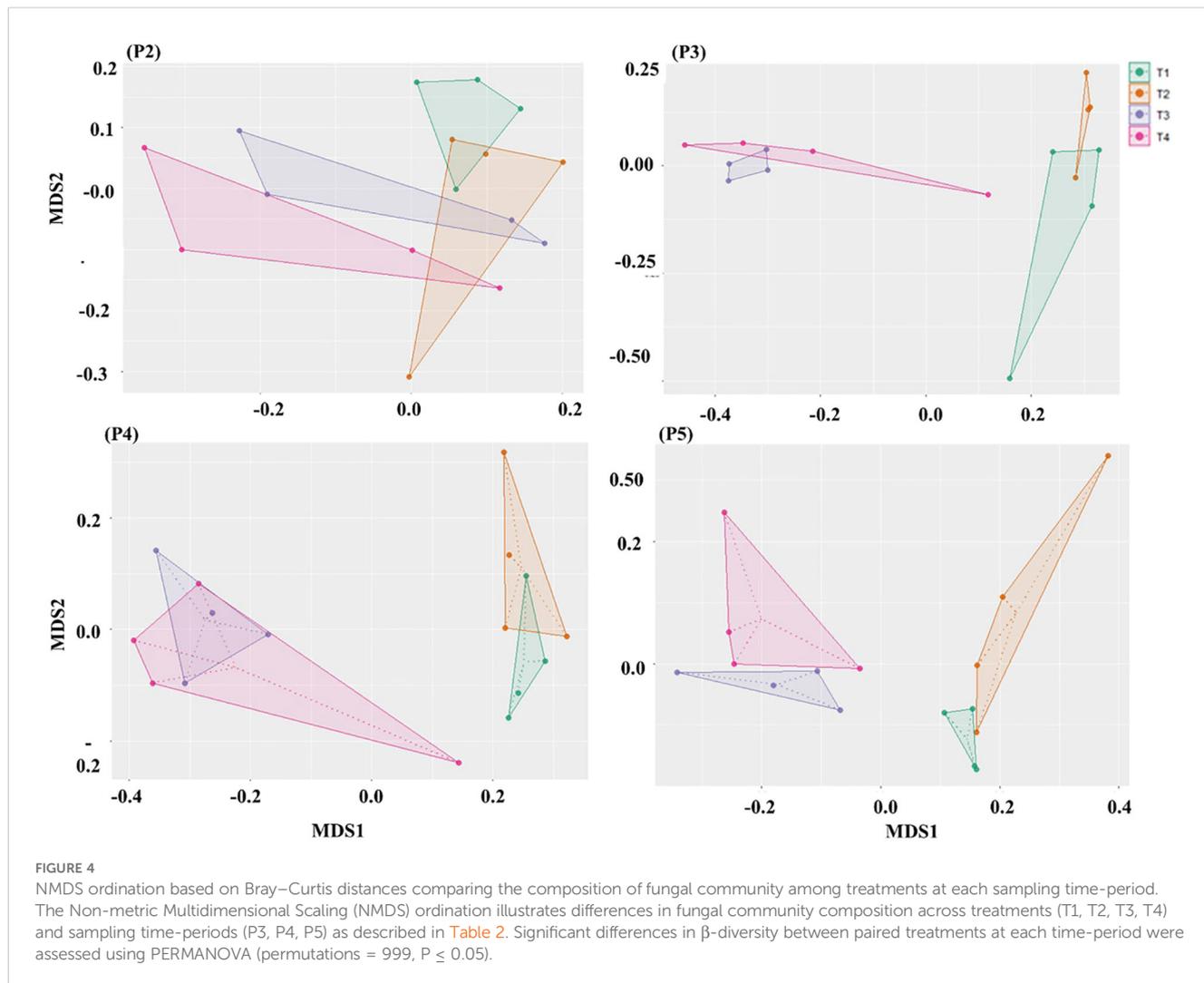
## Fungal community composition

The taxonomic analyses of the fungal community composition provide insightful details into community shifts induced by soil treatments during the growing season. When the treatment fumigation alone (T3) was compared to the control (T1), the fungal family *Hypocreaceae*, and the genus and specie *Trichoderma evansii*, showed a noticeable high relative abundance in T3 plots throughout the growing season and the abundance of the family *Aspergillaceae* increased slightly at time-period P5. The relative abundance of several fungal families, genus and species in T3 plots decreased in comparison to those of T1 plots during time-periods P3-P4, while this decrease trend was less noticeable through P5 time-periods (Figure 5, Supplementary Table S2). When the treatment biostimulant alone (T2) was compared to the control (T1), the relative abundance of fungal family, genus and specie (*Aspergillaceae*, *Aspergillus*, *A. caninus*) increased at time-periods P4 and P5, while families (*Chaetomiaceae*, *Plectosphaerellaceae*, *Nectriaceae*), genus (*Humicola*, *Penicillium*) and species (*Plectosphaerella oratosquillae*, *Humicola olivacea*) increased at P5. The biostimulant alone (T2) also decreased the relative abundance of families (*Didymellaceae*, *Microascaceae*, *Hypocreaceae*, *Lasiosphaeriaceae*), genus (*Trichoderma*, *Cladorrhinum*, *Schizothecium*, *Nectria*, *Lophotrichus*) and species (*T. evansii*, *C. flexuosum*, *N. ramulariae*, *L.fimeti*) (Figures 5). When the biostimulant inoculated in fumigated soil (T4) was compared to

TABLE 4 Permutation test for adonis under reduced model of composition of fungal community composition in soils sampled at P3 through P5 periods.

SV	Df	SumOfSqs	R <sup>2</sup>	F	Pr(>F)	Significance
Treatment (T)	3	2.789	0.362	8.848	0.001	***
Periods (P)	2	0.512	0.066	2.435	0.007	**
(TxP)	12	1.341	0.023	9.214	0.002	**
Residual	42	4.412	0.572			
Total	47	7.713	1.000			

SV, Source of variation, Df, Degrees of freedom, SumOfSqs, Sum of Squares, R<sup>2</sup>, Proportion of variance explained, F, F-statistic, Pr(>F), p-value. Significance levels:  $P \leq 0.05$  (\*),  $P \leq 0.01$  (\*\*),  $P \leq 0.001$  (\*\*\*). Treatments (T1, T2, T3, and T4) and sampling periods (P3, P4, and P5) are as described in Table 2. The analysis was based on 999 permutations.



the treatment T3, only the families *Chaetomiaceae* and *Microaeceae* at time-period P3 and family *Plectosphaerellaceae* at time-period P5 showed an increased relative abundance (Figure 5A). The comparison of treatments T4 vs T3 showed an increase variability of the effect of the biostimulant measured on the relative abundance of the families, genus and species among the four T4 plots throughout the growing season (Figures 5).

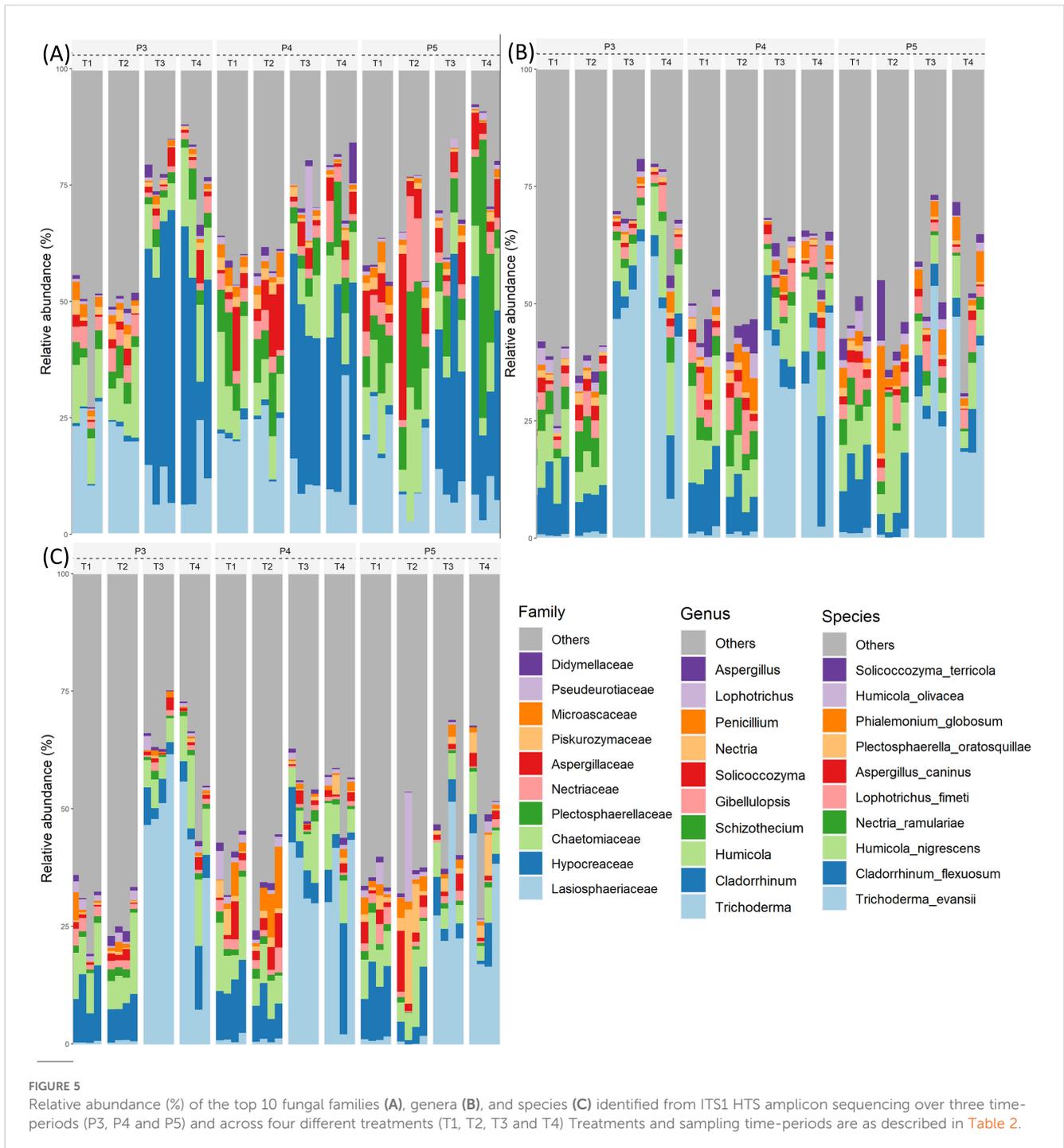
### Identification of fungal pathogens

We analysed our ITS1 ASV sequences database to provide taxonomic perspective where we observed many ASVs classified as Fungi – Basidiomycota – Agaricomycetes – Cantharellales, with no finer taxonomic assignment beside “NA” at Family/Genus level. The Cantharellales order includes *Ceratobasidiaceae* family known to

**TABLE 5** PERMDISP test implemented to check multivariate homogeneity of treatments dispersions (variances) of fungal community compositions in soils sampled over all P3, P4 and P5 periods.

Pair No.	Groups			Measure	F	R <sup>2</sup>	p-value	p-adjusted	Significance
1	T4	vs	T3	bray	0.99	0.04	0.43	0.43	ns
2	T4	vs	T2	bray	8.39	0.28	0.00	0.00	**
3	T4	vs	T1	bray	12.29	0.36	0.00	0.00	**
4	T3	vs	T2	bray	11.49	0.34	0.00	0.00	**
5	T3	vs	T1	bray	17.02	0.44	0.00	0.00	**
6	T2	vs	T1	bray	1.67	0.07	0.04	0.04	*

R<sup>2</sup>, R-squared; Significant differences (PERMDISP, permutations = 999,  $P \leq 0.05$ ) in fungal community among paired treatments at each sampling time-period are represented by stars. \*\*\* significant at 0.001, \*\* significant at 0.01, \* significant at 0.05, ns, non-significant. Treatments (included T1, T2, T3 and T4) and sampling time (included P3, P4 and P5, as described in Table 2).



harbor plant pathogenic fungi such as *Rhizoctonia solani*, a soil-borne pathogen causing stem canker and black scurf on potato tubers. Given its taxonomic placement in our database, the ASV 3b9531f47e78eff3daec652cc86ec07f likely represents a putative *Rhizoctonia*-like fungus. This putative *Rhizoctonia* spp. ASV was detected only in Basidiomycota sequences groups, suggesting that no Ascomycete pathogen (like *Fusarium* or *Alternaria*) shares this ASV's sequence. The dynamics of ASV 3b9531f47e78eff3daec652cc86ec07f total number of sequences varied across treatments T1 to T4, and sampling-periods (P3-early, P4-mid, and P5-late season).

Indeed, in our ASVs database, a grand total of 91–507 sequences were detected in cumulative soil samples from treatment T1 while the grand total of reads cumulated in soil samples from T2, T3 and T4 were respectively 98 483, 91–870 and 85 605 (Table 6). A total of 821 reads from a putative *Rhizoctonia* spp. ASV were detected in all three sampling-periods of the control (T1) and among these sequences, 27% were detected at P3, 4% at P4 and 68% at P5 (Table 6). For the biostimulant inoculated in non-fumigated soil (T2), almost all the 786 total sequences were detected in P3 time-period. Finally, 74 reads were detected in fumigated soil T3 at time-

TABLE 6 Occurrence of potato pathogens across treatments and time-periods.

Pathogens	Periods/Reads	T1	T2	T3	T4
<i>Rhizoctonia</i> spp.	P3	27 %	99 %	0 %	0 %
	P4	4 %	1 %	0 %	0 %
	P5	68 %	0 %	100 %	0 %
	Reads	821	786	74	0
<i>Fusarium</i> spp	P3	29 %	27 %	19 %	13 %
	P4	23 %	15 %	29 %	56 %
	P5	49 %	58 %	53 %	31 %
	Reads	800	1 906	700	854
<i>Verticillium</i> spp.	P3	24 %	45 %	24 %	24 %
	P4	41 %	30 %	33 %	28 %
	P5	35 %	25 %	43 %	48 %
	Reads	46	89	79	96
<i>Alternaria angustiovoidea</i>	P3	23 %	73 %	18 %	27 %
	P4	35 %	10 %	23 %	28 %
	P5	42 %	17 %	58 %	45 %
	Reads	177	526	190	185
<i>Colletotrichum coccodes</i>	P3	0 %	0 %	79 %	0 %
	P4	0 %	20 %	4 %	60 %
	P5	100 %	80 %	17 %	40 %
	Reads	16	144	161	432
Total Reads (all taxa)		91 507	98 483	91 870	85 605

Treatments (included T1, T2, T3 and T4) and soil sampling-periods (included P3, P4 and P5), are as described in Table 2. Values indicate the percentage fraction of the total number of reads detected at the sampling-period in all 4 plots per sampling-period per treatment. "Reads" row gives the cumulative number of reads of all amplicon-sequence variants (ASVs) attributed to that pathogen across P3–P5 within each treatment. The final "Total Reads" row reports the total cumulative number of reads, obtained per treatment after DADA2 denoising explained in M&M section. Treatments are defined in Table 2: T1 = non-fumigated control, T2 = biostimulant only, T3 = chloropicrin only, T4 = chloropicrin + biostimulant. Sampling periods correspond to 61 (P3), 86 (P4) and 111 (P5) days after fumigation. ITS1, internal transcribed spacer 1; ASV, amplicon-sequence variant.

period P5 while none read was detected in T4 at any time-period (P3 to P5). These observations indicate that the chloropicrin fumigation reduced drastically the detection of the putative *Rhizoctonia* spp. ASV and the effect of fumigation alone (T3) seems to vanish late in season (Table 6).

Our ITS metabarcoding analysis among ASV sequences database identified other fungal taxa associated with potato diseases. A number of ASV associated with known potato pathogenic fungi were detected, the majority of them at low relative abundance in the soil fungal community (Table 6). These included ASVs phylogenetically linked to *Fusarium* spp., or *Verticillium* spp., or *Alternaria* spp. or *Colletotrichum coccodes* (Table 6).

In our database, ASVs linked to genus *Fusarium* spp. showed 800, 1906, 700 and 854 total number of reads in respectively T1, T2, T3 and T4 plots (Table 6). In control soil (T1), among the 800 reads detected, the proportion of relative abundance varied from 29% (P3), 23% (P4) to 49% (P5). In biostimulant applied in non-fumigated soil (T2), among the 1906 reads detected, the percentage of relative abundance varied from 27% (P3) to 58% (P5). Meanwhile, a total of 700 and 854 reads were detected in

fumigated soil (T3) and biostimulant applied in fumigated soils (T4) plots respectively showing the proportion of relative abundance of 19% and 13% at P3 sampling-time, of 29% and 56% at P4 and of 53% and 31% at P5 (Table 6). Globally, over a dozen *Fusarium* spp. are notorious causes of potato dry rot and other diseases. Interestingly, the biostimulant alone (T2) did not suppress *Fusarium*; in fact, T2 showed slightly higher reads of *Fusarium* ASVs at P5 than T1. This suggests that the *Bacillus* species-based biostimulant did not antagonize soil *Fusarium* spp. ASVs and may have indirectly favored their proliferation.

ASVs linked to genus *Verticillium* spp. were detected at a very low total number of reads, 46, 89, 79, 96 in respectively T1 to T4 treatments (Table 6). In all treatments, *Verticillium* spp. ASVs were detected in only a few samples (generally <5% of plots) but the percentage of the abundance was relatively similar among the three time-periods yielding negligible total number of reads for each treatment. Overall abundance was so low that no clear treatment trends emerged (all read values per treatment <0.05% total reads for the treatment).

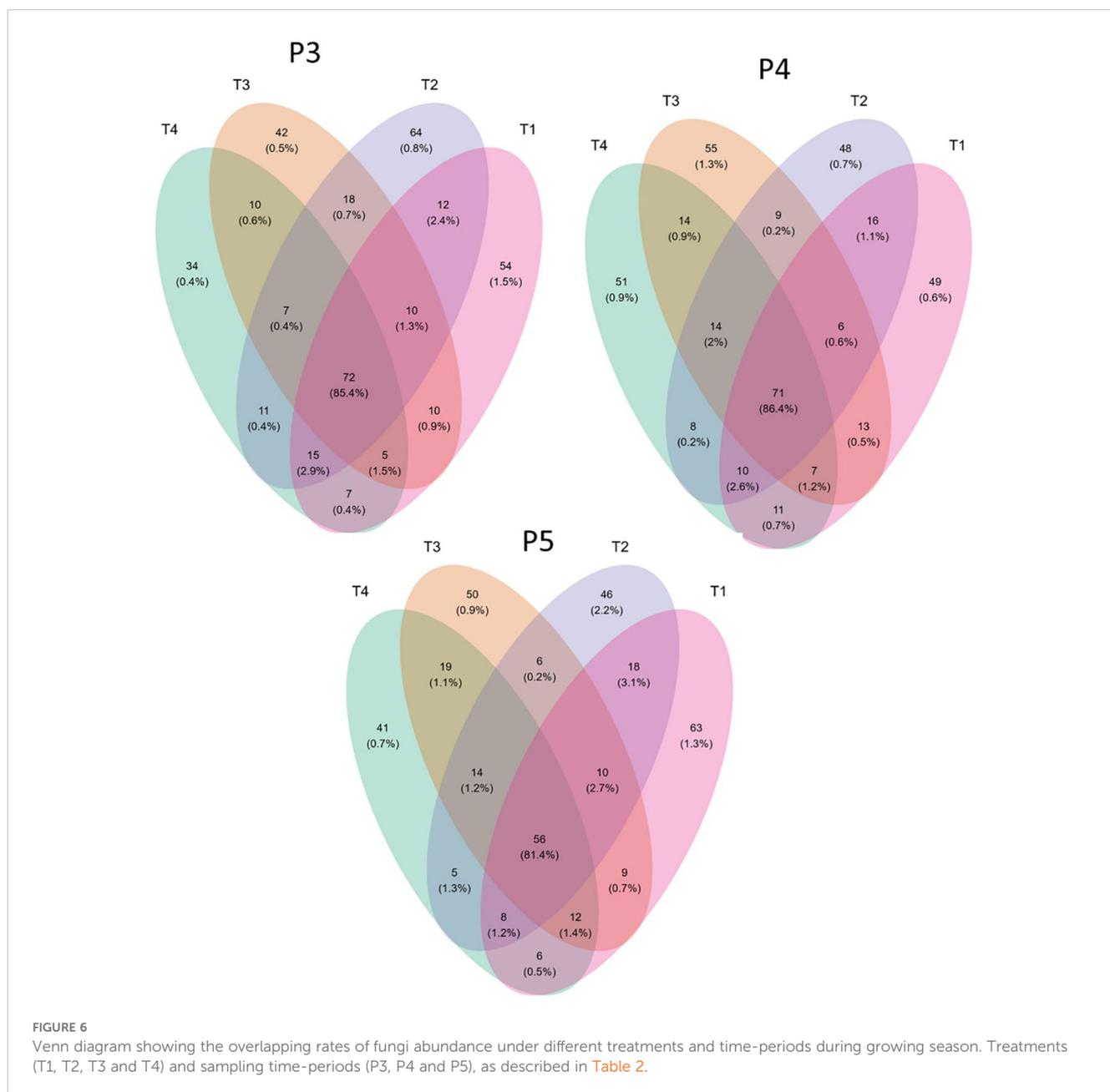
ASVs linked to genus *Alternaria* spp. (including *A. angustiovoidea*) showed 177, 526, 190 and 185 total number of

reads in respectively T1, T2, T3 and T4 plots (Table 6). In control soil (T1), among the 177 reads detected, the proportion of relative abundance varied from 23% (P3), 35% (P4) to 42% (P5). Among the 526 reads detected in T2 treatment, the percentage of relative abundance varied from 73% (P3) to 17% (P5). Meanwhile, a total of 190 and 185 reads were detected in fumigated soil (T3) and biostimulant applied in fumigated soils (T4) plots respectively showing the proportion of relative abundance of 18% and 27% at P3 sampling-time, of 23% and 28% at P4 and of 58% and 45% at P5 (Table 6). Overall, the total number of reads per treatment for *Alternaria* spp. was lower than those detected for *Fusarium* spp., but both genus showed similar trend when T1 and T2 treatments were compared to T3 and T4. By the end of the season at time-period P5, the proportion of relative abundance increased at the time when effect of the fumigation was fading.

ASVs linked to *Colletotrichum coccodes* were detected sporadically across treatments and sampling periods (Table 6). In non-fumigated soils T1 and T2 much of the reads have been detected at time-period P5, while in fumigated soil T3 the higher proportion of relative abundance was detected at P3 and at P4 in biostimulant applied in fumigated soil T4.

### Venn diagram analysis of treatments effect on fungal ASVs repartition

Furthermore, we investigated the impact of biostimulant and chloropicrin fumigation treatments on the abundance of global Amplicon Sequence Variants (ASVs) in the soil fungal community. Our analysis focused on examining changes in ASV abundance and composition over time, as illustrated in Figure 6.



During time-period P3, a total of 194 significant retained ASVs were identified. Specifically, 54 ASVs were exclusively found in untreated control soils (T1), while 42 ASVs were exclusively present in fumigated soil (T3). Additionally, 10 ASVs were shared between T1 and T3. 64 ASVs were exclusively found in biostimulant-treated plots (T2), with 12 ASVs overlapping between T1 and T2. In contrast, 34 ASVs were exclusively found in plots treated with both biostimulant and fumigation (T4), with 7 ASVs overlapping with T1.

In time-period P4, a total of 203 retained ASVs were identified. Among these, 49 ASVs were exclusively found in untreated control soils (T1), while 55 ASVs were exclusively present in fumigated soil (T3). Additionally, 13 ASVs were shared between T1 and T3. Moreover, 48 ASVs were exclusively found in T2 plots, with 16 ASVs overlapping with T1. Similarly, 51 ASVs were exclusively found in T4 plots, with 11 ASVs overlapping with T1.

For time-period P5, a total of 200 significant retained ASVs were identified. Among these, 63 ASVs were exclusively found in untreated control soils (T1), while 50 ASVs were exclusively present in fumigated soil (T3). Furthermore, 9 ASVs were shared between T1 and T3. Additionally, 46 ASVs were exclusively found in T2 plots, with 18 ASVs overlapping between T1 and T2. Similarly, 41 ASVs were exclusively found in T4 plots, with 6 ASVs overlapping with T1.

We observed a significant reduction in the number of ASVs in both T3 and T4 plots compared to T1 and T2 plots across the sampling dates from P3 to P5. This reduction can be attributed to the fumigation treatment. Of particular interest is the impact of the biostimulant application on the fungal community in both non-fumigated (T2) and fumigated (T4) soils. The application of *Bacillus* species-based biostimulant in T2 plots increased more the number of fungal ASVs at time-period P3, but less at time-periods P4 and P5 when compared to the impact of fumigated-only plots (T3).

Among the retained ASVs, 72, 71, and 56 were shared across the plots in P3, P4, and P5, respectively. These shared ASVs represented a significant proportion (85.4%, 86.4%, and 81.4% for P3, P4, and P5, respectively) of the cumulative relative abundance of ASVs.

In summary, our findings demonstrate that both biostimulant and fumigation treatments, as well as the time-periods, significantly influenced the composition of the soil fungal community, including the ASVs linked to potato's pathogens, such as *Ceratobasidiaceae* family. Additionally, the application of biostimulant exhibited a positive impact on the fungal community in both non-fumigated or fumigated soils.

## Effects of treatments on network of interactions within the soil fungal community

The effect of *Bacillus* species-based biostimulant on the network of interactions within the soil fungal community in non-fumigated or fumigated soil was investigated by assessing the number of linkages (edges) and nodes connected within and between different fungal taxa (Figure 7). Each treatment showed notable changes in the network of interactions observed among several

fungal classes. Notably, our network analysis revealed marked alterations in the interactions among fungal taxa within the Agaricomycetes—which include *Rhizoctonia solani*, the principal pathogen causing rhizoctonia canker.

As shown in Figures 7A, B, inoculating the biostimulant in non-fumigated soil (T2) increased the number of connections within and between several fungal classes in comparison to the treatment control T1 (Figure 7B). The increase effect was predominantly observed in the *Tremellomycetes*, *Pezizomycetes*, *Dothideomycetes*, *Eurotiomycetes*, *Leotiomycetes*, and *Sordariomycetes* classes. Conversely, in T2 plots, the number of connections of *Agaricomycetes* and *Saccharomycetes* decreased over the course of the season. In T2, this decrease in *Agaricomycetes* connectivity albeiting to a lesser degree, which may reflect competitive suppression or antagonistic interactions promoted by the biostimulant.

On the other hand, as shown in Figures 7A, C, soil fumigation alone (T3) induced a significant reduction in the number of connections within and between several fungal classes in comparison to the non-fumigated soil control (T1). This reduction effect was particularly noticeable in the *Agaricomycetes* class, which is one of the most abundant. The number of network connections within the *Agaricomycetes* was substantially reduced relative to the control (T1), suggesting that chloropicrin fumigation effectively suppresses populations of this pathogenic group. As well, this reduction was compensated by a substantial increase in connections within the *Pezizomycetes*, *Dothideomycetes*, *Eurotiomycetes*, *Leotiomycetes*, and *Sordariomycetes* classes (Figure 7C).

The combined use of the biostimulant and fumigant (T4) induced a reduction of the effect caused by the soil fumigation alone (T3) treatment, as depicted in Figure 7D. This reduction effect was observed in the *Agaricomycetes*, *Saccharomycetes*, and *C\_Fungi* classes, indicating that adding the biostimulant to fumigated soil could enhanced the impact of fumigant. At the same time, the combined treatment (T4) increased the number of connections in the *Mortierellomycetes*, *Cystobasidiomycetes*, *Tremellomycetes*, *Pezizomycetes*, *Dothideomycetes*, *Eurotiomycetes*, and *Leotiomycetes* classes (Figure 7D). Furthermore, a detailed analysis of the *Sordariomycetes* class revealed interesting patterns. In the control soils (T1), approximately 85 edges were associated with ASVs in *Sordariomycetes*. Out of these, around 13 edges connected ASVs within *Sordariomycetes*, and approximately 35, 15, and 7 edges connected ASVs from *Sordariomycetes* with ASVs from *C\_Fungi*, *Agaricomycetes*, and *Eurotiomycetes*, respectively (Figure 7A). Conversely, fumigated soils without biostimulant application (T3) had around 155 edges associated with ASVs in *Sordariomycetes*, of which roughly 25 edges connected ASVs within *Sordariomycetes*, and approximately 55, 10, and 10 edges connected ASVs from *Sordariomycetes* with ASVs from *C\_Fungi*, *Agaricomycetes*, and *Eurotiomycetes*, respectively (Figure 7C). In biostimulant-treated non-fumigated soil (T2), around 115 edges were associated with ASVs of *Sordariomycetes*, of which roughly 25 edges connected ASVs within *Sordariomycetes*, and approximately 40, 5, and 10 edges connected ASVs from *Sordariomycetes* with ASVs from *C\_Fungi*, *Agaricomycetes*, and *Eurotiomycetes*, respectively (Figure 7B). Finally, in biostimulant-treated fumigated soil (T4 plots), approximately 140

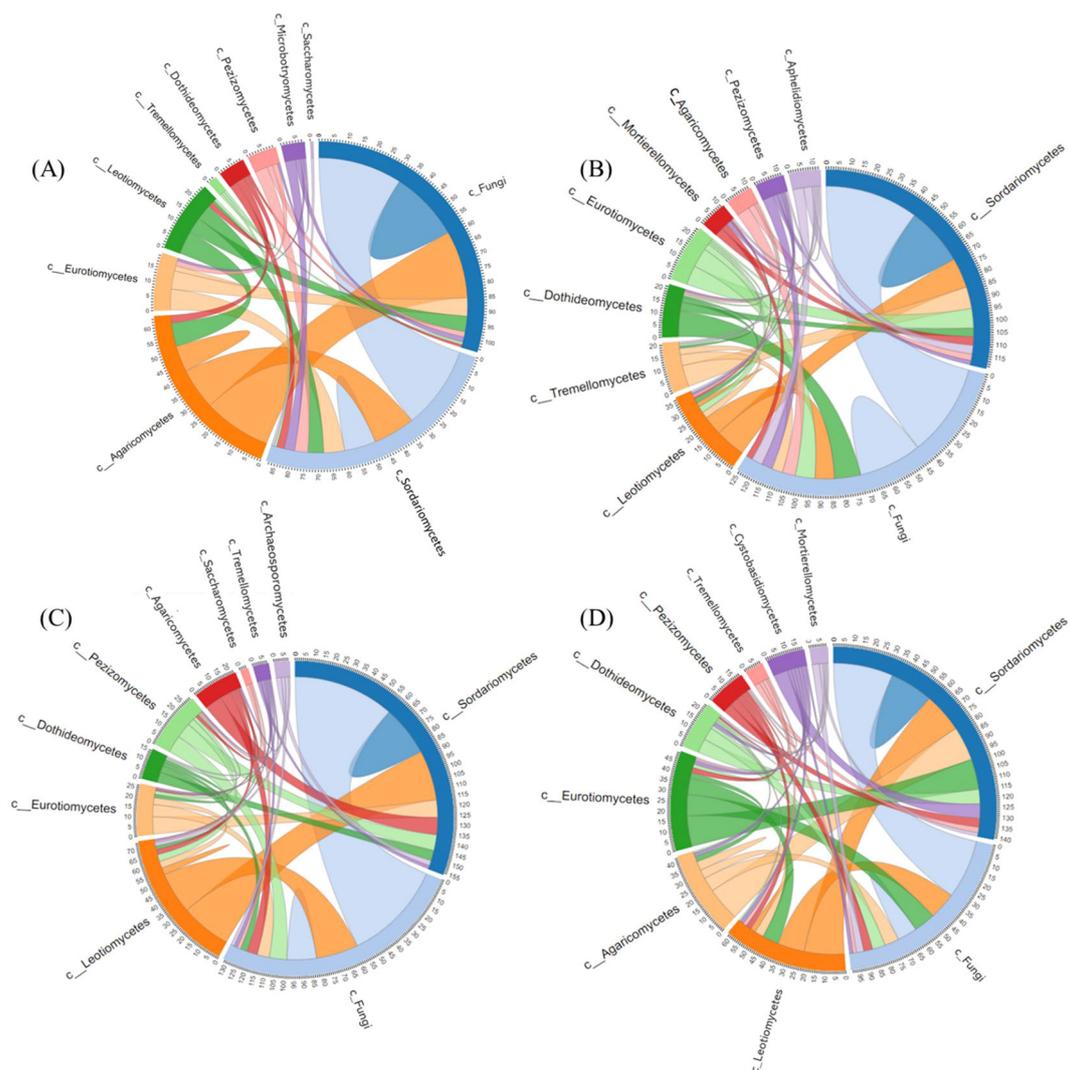


FIGURE 7

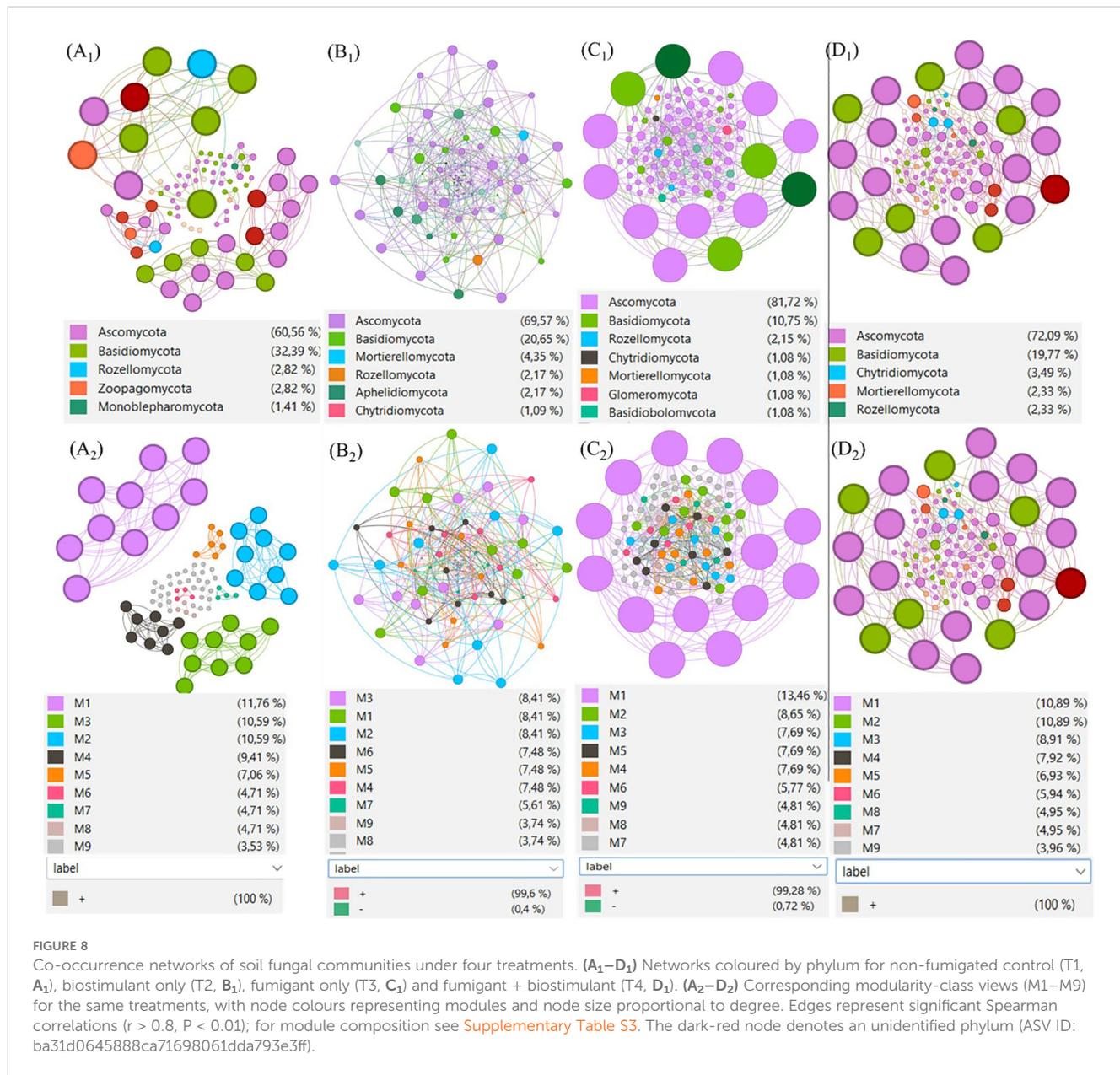
Plots showing the number of edges and nodes connected between different fungal taxa or within one taxon for treatments T1 (A), T2 (B), T3 (C) and T4 (D). The numbers along the outside of the circular plot represent how many edges or linkages are related within a fungal class. Treatments (T1, T2, T3 and T4) and sampling time-periods (P3, P4 and P5) are as described in Table 2. C\_Fungi is a class non identified, corresponding to ASV ID: ba31d0645888ca71698061dda793e3ff.

edges were associated with ASVs in *Sordariomycetes*, of which around 20 edges connected ASVs within *Sordariomycetes*, and around 35, 20, and 18 edges connected ASVs from *Sordariomycetes* with ASVs from *C\_Fungi*, *Agaricomycetes*, and *Eurotiomycetes*, respectively (Figure 7D).

Overall, our findings suggest that chloropicrin fumigation (T3) significantly increased interactions within the soil fungal community, while the application of biostimulant to non-fumigated control (T2) or fumigated (T4) soils favored an increase in the number of nodes connected between different taxa or within one taxon. These findings imply that the treatments modulate the network dynamics of taxa including *R. solani*, and although fumigation suppresses its activity directly, the integration of biostimulant applications may help maintain a more balanced soil fungal community that could indirectly mitigate disease expression.

## Modular structure and eigengene artificial networks inference within the soil fungal community

In this study, we predicted ecological relationships between soil fungal communities treated with fumigant and *Bacillus* species-based biostimulant against potato's rhizoctonia disease by building directed hypergraphs of complex systems from the behavior of their components in fungal communities in soils. Network interactions were constructed for each of the four treatments (Figure 8 A<sub>1</sub>, B<sub>1</sub>, C<sub>1</sub> et D<sub>1</sub>) by combining all significant pairwise relationships. The statistics of modular structure analysis, including the variables connectedness, connectivity, density, betweenness, and centralization, are presented in Table 7. The results showed that the non-fumigated soil treated with a



biostimulant (T2) treatment had the highest number of linkages (57), average path length (1.01), network diameter (3), and modules (25) with a relative abundance varying from 0.01% to 5.02%, presenting 99.6% and 0.4% of positive and negative interactions, respectively. In contrast, the fumigated soil (T3) treatment had the highest number of connectedness/edges (278), average degree (5.35), heterogeneity (0.74), and centralization (0.07), with a relative abundance varying from 0.01% to 3.72%, showing 99.28% and 0.72% of positive and negative interactions, respectively. Indeed, the artificial network analysis revealed that the fumigation (T3) treatment significantly increased interactions within the soil fungal community, whereas non-fumigated soil treated with a biostimulant (T2) favored an increase in the number

of nodes connected between different taxa or within one taxon. The network diameter and density of the associations decreased, but the ratio of positive associations increased with chloropicrin fumigation, indicating a shift in fungal interrelationships from competition to cooperation for utilizing available nutrients after organic material degradation. In plots that received the fumigated soil treated with biostimulant (T4) treatment, we observed a moderate number of connectedness/edges (260), linkage (58), average degree (5.15), average path length (1), network diameter (2.00), clustering coefficient (1.00), density (0.05), heterogeneity (0.66), centralization (0.05), and modules (23) with a relative abundance varying from 0.01% to 1.81% and 100% of positive interactions. The correlations between

TABLE 7 Topological properties of fungal community networks for each treatment.

Variables	T1	T2	T3	T4
Connectedness (edge)	197.00	249.00	278.00	260.00
Connectivity (Linkage/Nodes)	85.00	107.00	104.00	101.00
Relative abundance	11.97	23.73	13.13	6.52
Module	21	26	26	24
Average degree	4.64	4.65	5.35	5.15
Average path length	1.01	1.01	1.00	1.00
Network diameter	2.00	3.00	1.00	2.00
Clustering coefficient	1.00	1.00	1.00	1.00
Density	0.06	0.04	0.05	0.05
Heterogeneity	0.68	0.63	0.74	0.66
Centralization	0.01	0.03	0.07	0.05

Treatments (included T1, T2, T3 and T4) and soil sampling periods (included P3, P4 and P5), are as described in Table 2.

the modules' own genes were used to define the own gene network, and more details about the components of each module are available in Supplementary Table S3 and Figure 8A2, B2 C2 and D2.

## Discussion

### Performance of potato plants and *Rhizoctonia* incidence

Our study aimed to demonstrate the potential of a biostimulant to reduce constraints to the use of chloropicrin fumigation to control losses caused by pathogenic fungi in potato cultivation. Our results indicated that chloropicrin fumigation most effectively reduced rhizoctonia canker symptoms and losses in total and marketable tuber yields. The use of a biostimulant based on five *Bacillus* species in non-fumigated soil improved significantly potato root and tuber biomass. The combination of the biostimulant with the fumigation reduced biomass values compared to those obtained with fumigation alone and it did not improve total and marketable tuber yields obtained with fumigation alone. These findings are consistent with previous studies (13, 59) that have reported soil fumigation as an effective practice for suppressing soilborne plant pathogens. Our findings suggest that fumigation treatment can be a promising method for potato production, especially in regions with a high incidence of bacterial such as the common scab caused by a complex of *Streptomyces* species (60) and of fungal pathogens such as rhizoctonia canker disease caused by *Rhizoctonia solani* (1, 2). Most interestingly, the use of the mix of *Bacillus* species-based biostimulant had significant effects on the fungal community in fumigated or non-fumigated soils during potato cultivation.

### Absolute quantification of soil fungi under different treatments

Our qPCR survey showed a clear two-phase mycobiome response to chloropicrin fumigation. Fungal ITS copies dropped only  $\sim 0.3 \log_{10}$  within six days after fumigation (6 DAF) but diverged sharply thereafter; by 61 DAF fumigated ridges carried  $\approx 1.5 \times 10^6$  fewer copies  $\text{g}^{-1}$  than the untreated control plots, mirroring reports of persistent microbial biomass suppression for 60–90 days after chloropicrin, dazomet or allyl-isothiocyanate applications (61–64). Field studies likewise showed that long-term fumigant or herbicide inputs reshape  $\beta$ -diversity and soil functions (8, 65, 66). Variance in ITS fungal abundance was noticeable in fumigated plots alone at P3, which may reflect patchy chloropicrin dispersion and differential propagule survival (12). Similar heterogeneity has been linked to micro-zones of incomplete volatilisation or soil sealing (61). In-furrow delivery of the five *Bacillus* species consortium (T2) increased both the median and the dispersion of ITS counts at P3 and P5. *Bacillus* species derived lipopeptides and auxin analogues stimulate rhizodeposition, generating diverse C-sources that recruit heterotrophic fungi (67). Similar transient surges have been documented in lettuce and wheat systems (5). Adding the biostimulant to fumigated soil (T4) compressed the recovery window: by 111 DAF fungal ITS counts matched the untreated control, whereas fumigation alone still lagged significantly. This acceleration likely stems from *Bacillus*-mediated exudation and micro-aggregate formation that facilitate fungal ingress and root–fungus contacts (67). Collectively, these results confirm that chloropicrin is a potent—yet ecologically blunt—tool: it delivers sustained pathogen knock-down at the cost of lower fungal biomass and diversity. The *Bacillus* species-based biostimulant mitigates that cost by hastening recolonisation, but in non-fumigated soils it also creates activity “hot-spots” that could favour opportunists and induce greater variability in the dispersion of ITS counts. Interestingly, the application of the biostimulant in fumigated soil (T4) resulted in an increased variability of the number of fungi per gram of dry soil detected at P5 sampling period. This indicates that the T4 treatment may took more time to promote an increase of fungal community in fumigated soil towards the end of the growing season. Balancing chemical and biological inputs therefore remains essential for reconciling pathogen control with soil-function resilience (68).

### Diversity and richness of fungal community in soils under different treatments

Our results indicate that exposure to chloropicrin in fumigated soil (T3) reduced fungal diversity and richness. The altered fungal evenness in chloropicrin-exposed soils may be attributed to varying sensitivities of fungi to the active compound, as reported in previous studies (11, 12, 61). Nonetheless, our findings suggest that

management practices employed on both control methods could mitigate some of the negative effects of fumigation on soil microorganisms (12, 69, 70). Indeed, the biostimulant-treated soil (T2) showed significantly higher Shannon diversity and Pielou's evenness at P3 and P4, while the fumigated-treated soil (T3) exhibited significantly lower Shannon diversity and Pielou's evenness across all growing seasons, suggesting that the treatments may have selected for specific groups of fungi that dominated the soil community in the sampled roots area. Fumigation caused a 18% drop in Shannon diversity, consistent with mesocosm studies where chloropicrin reduced OTU richness by 15–25% and evenness by up to 30% (11). While richness began to recover later in the season, community evenness remained low, indicating proliferation by a limited number of opportunistic taxa. In contrast, the *Bacillus* species-based biostimulant restored evenness and even exceeded control levels by harvest. These results reflect similar findings in lettuce and cucumber systems, where amino-acid-rich exudates from *Bacillus* species-based consortia supported the resurgence of diverse fungal taxa (71). Soil fungal communities in field conditions may exhibit greater stability to chloropicrin perturbation (18). Soil fungi have an advantage in decomposing organic matter rapidly, allowing them to compete for soil niche space created by fumigation (72). However, other abiotic factors or management practices associated with each individual field, such as crop rotation (73), pH (74), soil type (75), and chemical properties of soils (9), can confound the effects of fumigation on soil fungal richness and diversity. Furthermore, community dissimilarity analyses showed that treatment explained 36% of fungal composition variance (PERMANOVA), a higher fraction than reported in legacy soils treated with metam-sodium (19%) (8). NMDS plots indicated early divergence of T2 from the control T1 within 51 DAP, highlighting the rapid and selective footprint of microbial metabolites and root exudates induced by the biostimulant. Indeed, our study found that the short-term effects of fumigation were not yet noticeable in fungal communities at 6 days after the application of fumigation (6DAF, P2). However, from 61 DAF (P3) to 111 DAF (P5), treatments involving non-fumigated soils (T1 and T2) were significantly different from fumigated soils (T3 and T4), indicating that the long-term effects of fumigation on fungal community were pronounced. Although the mechanism of soil microbial resilience to fumigation is not entirely clear, one explanation from (76) is that the relatively short half-life of the effective compound methyl isothiocyanate, which is decomposed from chloropicrin in soil within about 7 days, could play a role. However, chloropicrin fumigation reduced fungal richness and diversity over a period of 61 and 111 DAF (P5), suggesting that chloropicrin remained suppressive to soil fungi. This finding is consistent with other studies that have documented short-term and middle-term reductions in soil microbial populations caused by chloropicrin application (9, 11, 18, 63). Although (11) and (62) reported that fungal diversity and richness recovered after a disturbance, we did not observe such a recovery. This is likely because our sampling time was shorter, and the fungal communities present did not have enough resources to support their recovery. In

general, soil microbial communities can be resistant or resilient to environmental perturbations, either not changing after a disturbance or returning to their original state after a period of time (77). Ultimately, our findings suggest that chloropicrin application negatively affects fungal diversity and richness over a longer period. This highlights the importance of considering the long-term effects of fumigation on soil microbial communities.

## Effect of treatments on fungal community composition

The effect of chloropicrin fumigation on soil microbial composition varied by taxa, as evidenced by the change in relative abundance of fungal taxa depending on fumigation periods. Soil fungal composition was shifted based on fumigation during the growing season periods, with the relative abundance of *Lasiosphaeriaceae*, *Chaetomiaceae*, *Plectosphaerellaceae*, *Nectriaceae*, *Piskurozymaceae*, *Microascaceae*, *Mortierellaceae*, and *Trichocomaceae* decreasing while those of *Hypocreaceae* increasing in soils exposed to chloropicrin. This finding is consistent with the research of (18), who reported an increase in the abundance of genera represented by *Chaetomiaceae*, *Pseudogymnoascus*, *Acremonium*, and *Lectera* with metam-sodium fumigation exposure. The increase in *Chaetomiaceae* abundance following application of dazomet and various isothiocyanates has also been documented in previous studies (62, 78). Furthermore, our detailed taxonomic assessment revealed that soil fumigation (T3) effectively reduced pathogenic fungi across soil sampling-periods (P3–P5), corroborating previous findings on chloropicrin's broad-spectrum pathogen suppression capability (79, 80). Notably, the application of *Bacillus*-species-based biostimulant (T2) and its combined use with fumigant (T4) selectively enriched beneficial fungal taxa such as *Cladorrhinum flexuosum* (*Plectosphaerellaceae*) in T2 and *Trichoderma evansii* (*Hypocreaceae*) in T4 and T3 especially during later soil sampling-periods (P4 and P5). These beneficial fungi are known for their biological control activities against critical potato pathogens, including *R. solani* (81, 82). Furthermore, the marked reduction of pathogenic species such as *Nectria ramulariae* (*Nectriaceae*) and *Phialemonium globosum* (*Cephalothecaceae*) after fumigation treatments (T3 and T4) highlights the effectiveness and potential long-term benefits of integrating soil fumigation and biostimulant application for sustainable disease management practices.

## Dynamics of potato pathogens in response to fumigation and biostimulant treatments

Across all three post-planting soil samplings (P3–P5), the auxiliary ITS-based screen makes it clear that chloropicrin and the *Bacillus* species-based biostimulant did far more than curb rhizoctonia canker. Fumigation provided an early, broad-spectrum “knock-down” of multiple soil- and residue-borne fungi, whereas the biostimulant selectively favoured antagonistic or saprophytic taxa that can further restrain pathogen resurgence. By harvest,

however, several opportunists (notably *Fusarium* species and *Colletotrichum* species) had recolonized fumigated soil, emphasising that a one-off fumigation must be integrated with biological and cultural tactics for season-long protection. Indeed, the putative *Rhizoctonia* ASV (3b9531f47e78eff3daec652cc86ec07f; order *Cantharellales*) surged in untreated plots at P3 ( $\leq 25\%$  of reads) and again at P5 ( $\leq 60\%$ ), mirroring the high disease index and  $\sim 20\%$  marketable-yield loss in T1. Both chloropicrin (T3) and the combined biostimulant and fumigation treatment (T4) suppressed this ASV below the detection limit through P4, with only a minor rebound in one T3 replicate at harvest, confirming the well-documented efficacy of chloropicrin against *R. solani*. Remarkably, the *Bacillus* species-based biostimulant alone (T2) kept the pathogen undetectable at P5, suggesting antagonism by *Bacillus* species via antibiotic production or niche competition (19, 83). Similar *Bacillus*-mediated suppression of *R. solani* has been reported in potato and other crops (84). These dynamics echo field reports that chloropicrin provides an immediate but transient knock-down of *R. solani* inoculum and that recolonisation can occur within one season from surviving sclerotia or airborne sources, while multiple *Bacillus* species antagonise the pathogen via lipopeptides and cell-wall-degrading enzymes, blocking hyphal re-establishment on potato roots; taken together, the sequencing evidence substantiates our conclusion that integrating a *Bacillus* species-based biostimulant with chloropicrin fumigation confers durable season-long exclusion of *R. solani*, whereas either input alone affords only partial or temporal control.

*Fusarium* species (dry-rot and wilt complex) reads were ubiquitous ( $\geq 70\%$  detection in T1 at P5) and are known to cause dry-rot losses of up to 25% in storage (85). Chloropicrin cut early-season abundance by an order of magnitude, yet populations rebounded to  $\sim 1\%$  of reads by harvest, consistent with the long-term survival of chlamydospores and the capacity of fumigated soils to be recolonized. The biostimulant did not curb *Fusarium* species T2 even trended slightly higher than T1 at P5, implying that *Fusarium* species can tolerate or exploit the rhizosphere shifts promoted by *Bacillus* species-based biostimulant enrichment. Hence additional tactics (seed-tuber fungicides, store sanitation) remain essential for dry-rot management. From an agronomic perspective, fumigation appears effective at reducing *Fusarium* species pressure early on, but its eventual recovery signals that one-time fumigation may not fully prevent *Fusarium* species-related tuber rots later in the season. Low but persistent detection of *Alternaria angustiovoidea* in T1 and T2 underscores the difficulty of eliminating foliar inoculum that overwinters in debris or arrives aurally (86). Fumigation temporarily eradicated *Alternaria* species (early blight/brown spot) at P3–P4, yet the pathogen re-appeared in up to 10% of T3 replicates by time-period P5, highlighting rapid recolonization of fumigated ridges from surrounding sources. Because even low soil titers can promote the onset of foliar epidemics (87), integrating residue management and timely fungicide sprays is advisable.

While black-dot prevalence, induced by *Colletotrichum coccodes*, rose steadily in untreated soil ( $\leq 1\%$  reads, 45% detection at harvest), it was not prevented by chloropicrin fumigation (T3) but only delayed since by time-period P5, the detection level caught up to the values measured in T1. This pattern agrees with reports that microsclerotia of *C. coccodes* survive fumigation and recolonise potato roots within a single season (88). The biostimulant had little direct effect, indicating that supplemental measures such as extended rotations or resistant cultivars are required for durable black-dot suppression.

*Verticillium nubilum* was sporadic ( $< 0.1\%$  reads) but detectable in late-season samples from non-fumigated plots. Chloropicrin virtually eliminated it, corroborating evidence that microsclerotia are highly sensitive to soil sterilants (89). Given the low initial inoculum and cool growing season, the verticillium wilt was unlikely to influence yield here; nevertheless, the data show fumigation's collateral benefit against this elusive pathogen. Detection of *Ilyonectria macrodidyma* (root rot fungi) was confined to a few T1/T2 samples at P4–P5. Its near-absence in fumigated soil aligns with studies showing that *Cylindrocarpon conidia* are readily eradicated by fumigation (90). Although current impact appears minimal, metabarcoding alerts us to emerging root-rot threats that warrant surveillance in reduced-fumigant systems.

## Similarity-based artificial network inference within fungal community

The interrelationships among soil microorganisms are complex and essential for soil functions and plant performance (68, 91). In our study, we investigated the biodiversity of fungal communities in soils treated with chloropicrin fumigant and *Bacillus* species-based biostimulant for rhizoctonia disease control, focusing on interactions among species as well as the number and abundance of species. This is crucial because previous research has shown that species interactions can be more critical to ecosystem functioning than species richness and abundance, particularly in complex ecosystems (58, 92). Previous research demonstrated the application of network analysis to describe interactions between microbial community features (46, 60, 93, 94).

By using network analysis, we examined soil fungal co-occurrence patterns and community stability in response to disturbance (95, 96). To our knowledge, this is the first study to investigate the soil fungal co-occurrence in response to chloropicrin fumigation and *Bacillus* species-based biostimulant. Our findings revealed that fumigation significantly increased interactions within the soil fungal community, whereas *Bacillus* species-based biostimulant application favored an increase in the number of nodes connected between different taxa or within one taxon, compared to previous studies (57, 60, 71, 97), which revealed that fumigation and other types of fungicides exposure altered the network structure in bacterial and fungal communities. Specifically,

the network diameter and density of the associations decreased, but the ratio of positive associations increased with chloropicrin fumigation, indicating a shift in fungal interrelationships from competition to cooperation for utilizing available nutrients after organic material degradation (5, 97). Network clusters can represent a group of fungi with similar or related functions (48, 91). Furthermore, we found that the clustering agreements in the fungal networks differed between fumigated and non-fumigated soils, respectively, implying potential functional changes, as described in previous studies with fungicides tebuconazole and boscalid (57, 98).

Network analysis revealed that chloropicrin reduced total edge count by 35% and network diameter to 1, but increased the positive:negative edge ratio to 99:1—an expected outcome in systems undergoing disturbance and reduced niche competition (5). *Mortierellaceae* emerged as keystone taxa in this simplified network, leveraging lysed biomass as nutrient sources and producing phospholipids that support co-resident bacteria (68). The biostimulant application reversed this trend, restoring edge density (+19%), network diameter (to 2), and doubling inter-phylum links (e.g., *Sordariomycetes* ↔ *Tremellomycetes*). Such interconnectivity is associated with higher microbial redundancy and faster recovery from environmental stress (91). Notably, a *Trichoderma*-dominated module was inversely correlated ( $r = -0.78$ ) with *Rhizoctonia* ASV abundance, confirming the suppressive role of the biostimulant community (49). By employing high-throughput sequencing and artificial network inference analysis, our previous study (60) reports that the fumigation with chloropicrin significantly and persistently altered the composition of the soil bacterial community over the growing season, while the biostimulant had a positive impact on the diversity and richness of the bacterial community.

Overall, our study provides insights into the complexity of soil fungal interactions and highlights the importance of considering species interactions in ecosystem functioning. However, due to the limitations of co-occurrence network analysis (99), further studies are needed to characterize these fungal functional profiles in response to chloropicrin fumigation and *Bacillus* species-based biostimulant. This study highlights the importance of network interactions in fungal communities for research in microbial ecology and demonstrates the potential of using modular structure and eigengene artificial networks inference to understand soil fungal community dynamics. This shift indicates a restructured soil mycobiome favoring beneficial over pathogenic taxa, highlighting the potential of biostimulant-fumigant combinations for integrated pathogen suppression.

## Contributions of the study

This research extends our previous focus on bacterial community (60) to explore soil fungal dynamics under chloropicrin fumigation and *Bacillus* species-based biostimulant

applications in potato production. Unlike the earlier work that concentrated on bacteria, this study delves into how these treatments influence fungal communities, crucial for managing *Rhizoctonia solani*. A key innovation here is the detailed examination of shifts in fungal community structure and interactions, illustrating a transformative shift from competition to cooperation in response to soil treatments. This insight enriches our understanding of microbial ecology, offering a new perspective on fostering soil health and sustainability in agriculture.

Employing advanced high-throughput sequencing and network analysis, this work uncovers complex fungal network behaviors and interaction patterns previously unexplored in potato cropping systems. It highlights the potential of integrating fumigation and biostimulants to enhance microbial interactions that benefit soil management and disease suppression.

This study stands out by linking fungal dynamics with bacterial insights, providing a comprehensive view of the soil microbiome's role in enhancing crop production and resilience against diseases.

## Conclusion

Field-scale integration of chloropicrin fumigation with an eight-strains *Bacillus* species-based biostimulant proved to be a double dividend for intensive potato systems: it delivered reliable disease suppression while accelerating microbiome recovery. Fumigation alone cut the rhizoctonia canker index by  $\approx 70\%$  and lifted gross yield by 7%, confirming the long-standing efficacy of chloropicrin against *R. solani* and allied pathogens. Yet this chemical knock-down came at an ecological cost, an 18% drop in Shannon diversity and a collapse of negative (competitive) network edges hallmarks of a perturbed soil mycobiome. Introducing the five species *Bacillus* consortium tried rewiring that trajectory. When applied to unfumigated soil, it boosted plant biomass and selectively enriched antagonistic taxa such as *Trichoderma* species, echoing independent demonstrations of *Bacillus*-mediated growth promotion and pathogen inhibition. When paired with chloropicrin, the biostimulant eliminated the late-season resurgence of *R. solani*, restored network diameter and inter-phylum connectivity, and halved the time needed for fungal abundance to rebound to pre-fumigation levels. The resulting agroecosystem combined the highest marketable yield (+8%) with the most functionally cohesive fungal network, illustrating how targeted chemical control and microbial stimulation can be mutually reinforcing rather than antagonistic. Beyond potatoes, these findings strengthen a broader paradigm: pairing fast-acting fumigants with microbiome-friendly bio-inputs can reconcile short-term phytosanitary goals with long-term soil health.

Future work should (i) refine application timing and dose to minimize diversity losses while maximizing yield gains, (ii) track multi-year legacy effects on carbon–nitrogen cycling and greenhouse-gas fluxes, and (iii) test whether similar network-level

resilience emerges in other fumigated horticultural systems. Such efforts will help translate the present study into a blueprint for sustainable disease management that is both microbiologically informed and agronomically robust.

## Data availability statement

The original contributions presented in the study are publicly available. This data can be found here: NCBI BioProject accession PRJNA1010157.

## Author contributions

HT: Data curation, Formal analysis, Methodology, Software, Conceptualization, Visualization, Writing – original draft, Writing – review & editing. JD'A-P: Data curation, Formal analysis, Methodology, Writing – review & editing. TJ: Resources, Data curation, Methodology, Writing – review & editing. RH: Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Generative AI statement

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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fsoil.2025.1559144/full#supplementary-material>

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