Science of the Total Environment 919 (2024) 170659

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Impact of repeated fumigant applications on soil properties, crop yield, and microbial communities in a plastic-mulched tomato production system

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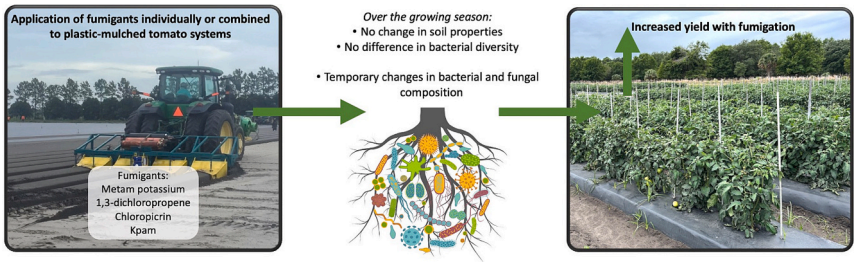
HIGHLIGHTS

- Variations in crop yield were indirectly related to alterations in the soil microbiome.
- Single or fumigant combinations had no major negative impacts on bacterial diversity.
- Fumigants reduced soil fungal diversity.
- Fumigants altered microbial community composition and reduced network complexity.
- Fumigants temporarily reduced soil bacterial and fungal predicted functionality.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Fang Wang

Keywords:
Soil fumigation
Plasticulture
Tomatoes
Plant productivity
Soil microbiome
Microbial diversity

ABSTRACT

Pre-plant soil fumigation is widely applied to control nematodes, soil-borne fungal pathogens, and weeds in vegetable crops. However, most of the research evaluating the effect of fumigants on crop yield and soil microbial communities has been done on single compounds despite growers mainly applying fumigant combinations. We studied the effect of different fumigant combinations (chloropicrin, 1,3-dichloropropene, and metam potassium) on soil properties, crop yield, and the soil bacterial and fungal microbiome for two consecutive years in a plastic-mulched tomato production system in Florida (United States). While combinations of fumigants did not improve plant productivity more than the individual application of these products, application of fumigants with >60 % chloropicrin did significantly increase yield. Fumigant combinations had no significant effect on bacterial diversity, but fumigants with >35 % chloropicrin reduced soil fungal diversity and induced temporary changes in the soil bacterial and fungal community composition. These changes included short-term increases in the relative abundance of Firmicutes and Ascomycota, as well as decreases in other bacterial and fungal taxa. Repeated fumigation reduced network complexity and the relative abundance of several predicted bacterial functions and fungal guilds, particularly after fumigation and at end of harvest (3-months post fumigation). A structural equation model (SEM) showed fumigants not only directly impact crop yield, but they can also indirectly determine variations in plant productivity through effects on the soil microbiome. Overall, this study increases our understanding of the environmental and agricultural impacts of fumigants in a plastic-mulched tomato production system.

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https://doi.org/10.1016/j.scitotenv.2024.170659
Received 22 December 2023; Received in revised form 31 January 2024; Accepted 1 February 2024
Available online 5 February 2024

1. Introduction

Tomato (*Solanum lycopersicum* L.) is an economically important vegetable crop with >200 million tons produced globally and 11 million tons produced in the United States (US) in 2021, making it the third largest producer of tomatoes after China and India (FAOSTAT, 2022). In the US, the state of Florida is the largest producer of fresh market tomatoes with 9307 ha planted and a 323.5 million US dollar farm gate value in 2021 (USDA-NASS., 2022). In the US, tomato production systems in southern states such as Florida often use a plastic production system where plastic mulch is used to cover raised soil beds. Tomato plasticulture production has exponentially increased in the last 10 years (USDA-NASS., 2022), but these agroecosystems face different management challenges compared to non plasticulture production systems (Chitwood-Brown et al., 2021; Tiwari et al., 2022; Boyd et al., 2022). For example, although plastic mulch was designed to effectively suppress weeds, nutsedge species (i.e., *Cyperus* spp.) can emerge from planting holes and penetrate through plastic mulches (Yu et al., 2019, 2021a). Other broadleaf and grass weeds can colonize the row middle (between the plastic-covered beds) and compete with the cash crop for water and nutrients (Boyd, 2016). Tomato production in the southern states of the US is also subjected to plant diseases caused by fungal pathogens of genera such as *Cladosporium*, *Fusarium*, and *Stemphylium* spp., (Yu et al., 2019; Khatri et al., 2021). Both weed pressure and pathogenic fungi can contribute to reduced crop yield in plastic-mulched tomato systems (Yu et al., 2019, 2021b, 2021c).

Pre-plant soil fumigation is a common method used to control soil-borne pests in agriculture. Chloropicrin (Pic), 1,3-dichloropropene (1,3-D), and metam-based products are fumigants widely applied in tomato production systems to control weeds, nematodes, and soil-borne fungi, thus improving crop yield (Khatri et al., 2021; Zhu et al., 2020; Yu et al., 2019, 2021a, 2021b). Although these fumigants are broad-spectrum by design, they do not always control all pests on their own. For example, individual fumigants typically do not adequately control weeds and alternative weed management systems comprised of two or more tactics are often recommended (Yu et al., 2019, 2021a, 2021b). To date, fumigants are rarely applied individually, and growers commonly rely on combinations of 1,3-D and Pic due to strong evidence demonstrating combinations (e.g., 1,3-D, Pic, and metam-based products) are more effective at weed control and provide greater economic benefits (Wu et al., 2020).

The application of fumigants to the soil prior to planting a crop can alter not only the abundance of target microorganisms (mainly soil-borne fungal pathogens) but also the non-target soil microbial communities. Pre-plant soil fumigation can decrease the abundance and diversity of soil bacterial and fungal communities and alter their composition, particularly during the first month after fumigation (Castellano-Hinojosa et al., 2022a). Soil microbial communities are essential components of healthy soils and plants. For example, they can play key roles in several soil processes and functions including cycling of soil nutrients, plant growth promotion, degradation of recalcitrant substances, and resistance against external stresses (Maron et al., 2018; Chen et al., 2020). Beneficial soil microbes of genera such as *Agrobacterium, Pseudomonas, Serratia, Bacillus, Streptomyces*, and *Pantoea* can control soil-borne pests which reduces the incidence of plant diseases (Latz et al., 2012; Wang et al., 2018; Köhl et al., 2019). However, to assess the efficacy and effects on agroecosystems of these products, additional information is needed on the impact of pre-plant fumigants on non-target soil microorganisms. To date, most of the research evaluating the effect of fumigants on the soil microbiome have been done with single compounds whereas growers almost always apply fumigant combinations (Ge et al., 2021; Castellano-Hinojosa et al., 2022a).

Recent work found that repeated fumigation on the same land can have residual effects on soil microbes (Dangi et al., 2017; Zhang et al., 2017; Li et al., 2022). Shifts in soil microbial communities may cause increases or decreases in microbial diversity and the abundance of specific microbial taxa, causing potential alterations in microbiome functionality, plant growth, and crop productivity. Recently, Li et al. (2022) found that repeated fumigation with metam sodium (up to 5 fumigant applications in the same field) reduced soil microbial diversity and altered microbial community composition within 6 weeks after fumigant application. Fumigant effects appear to vary with time (Castellano-Hinojosa et al., 2022a), but the magnitude of these impacts within and after a growing season remains unclear. In addition, crop management practices and environmental conditions (e.g., precipitation and temperature) can alter the degradation rates of fumigants and therefore indirectly affect the soil microbiome (Han et al., 2018; Castellano-Hinojosa et al., 2022a). Multi-year fumigation studies can help further understand the interaction between these products and the soil microbiome under varying environmental conditions.

Here, we examined the impact of different combinations of fumigants on soil properties, crop yield, and the diversity, composition, and predicted functionality of soil bacterial and fungal communities in a Florida tomato production system for two consecutive years at four time points during the growing seasons. We hypothesized that the application of an increased number of fumigants would result in increased crop production compared to the individual application of these products due to broader pest control that would ultimately favor plant growth. We expected an increased number of fumigants would differ in their effect on the soil microbiome. We also hypothesized that the treatment application would result in a short-term decrease in bacterial and fungal diversity (e.g., 1–2 months after fumigation) but these impacts would disappear by the end of the growing season (e.g., 4 months after treatment application). In addition, we expected fumigant impacts on the soil microbiome would result in short-term changes to the relative abundance of specific bacterial functions and fungal guilds but longer-term impacts on the soil microbiome were not expected due to the short half-life of these products in soil.

2. Materials and methods

2.1. Experimental site

The study was conducted at the Gulf Coast Research and Education Center (GCREC) near Balm, FL, USA (27°45′19.46" N, 82°13'55.57" W). The soil type is a Siliceous Hyperthermic Oxyaquic Alorthod (USDA, 2015) (pH of 7.6 and soil organic matter of 0.8 %). The climate of the experimental area is categorized as tropical savanna (Aw), characterized by hot and wet summers and a pronounced dry winter season. Weather parameters were recorded periodically using an Automated Weather Network station located at the GCREC. Mean air temperatures during the experimental period in 2020 and 2021 were 26.4 °C and 25.6 °C, respectively, and ranged from 22.6 °C and 22.0 °C (February 2020 and February 2021, respectively) to 33.4 °C and 32.9 °C (July 2020 and July 2021, respectively). The accumulated rainfall during the experimental period in 2020 and 2021 was 167.2 mm and 156.6 mm, respectively, and ranged from an accumulated rainfall per month of 12.2 mm and 10.9 mm (February 2020 and February 2021, respectively) to 40.4 mm and 37.9 mm (July 2020 and July 2021 respectively).

2.2. Field management, treatments, and soil sampling

Field trials were conducted in two consecutive tomato growing seasons between February and July in 2020 and 2021 at the GCREC near Balm, FL. Six treatments with 4 replicates per treatment were tested and included different fumigant combinations (chloropicrin, 1,3-dichloropropene, and metam potassium) (see abbreviations, formulations, and suppliers in Table 1). The experiment followed a randomized block design, with an untreated soil serving as a control. Raised beds were formed using bed-pressing equipment from Kennco Manufacturing (Ruskin, FL, USA). These beds measured 1.52 m in length, 20.3 cm in height, and had a bed-top width of 81.3 cm. For irrigation, two drip

Table 1
Treatments applied in this study, their trade name, abbreviation, distributor, and active ingredient application rates.

Common name	Trade name	Abbreviation	Distributor	Active Ingredient (kg ai ha^{-1})			
				1,3-d	Pic	MP	Total
Non-treated control	–	Control	–	–	–	–	–
Metam potassium (MP)	K-pam HL	Kpam	Amvac, Newport Beach, CA	0	0	260	260
1,3-dichloropropene (1,3-D) (64 %) + chloropicrin (Pic) (35 %)	Telone C-35	Pic35	TriEst Ag Group, Greenville, NC	160	88	0	248
1,3-D (40 %) + Pic (60 %)	Pic-Clor 60	Pic60	TriEst Ag Group, Greenville, NC	120	183	0	303
Pic(100 %)	Tri-Pic 100	Pic100	TriEst Ag Group, Greenville, NC	0	327	0	327
1,3-D (40 %) + Pic (60 %) + MB	Pic-Clor 60 + K-pam HL	3-way	TriEst Ag Group, Greenville, NC and Amvac, Newport Beach, CA	120	183	260	563

tapes with 30 cm emitter spacing and a flow rate of 0.95 L min^{-1} (Jain Irrigation Inc., Haines City, FL, USA) were placed 2.5 cm below the soil surface on each bed. The beds were covered with a black on white totally impermeable film (TIF) that had a thickness of 1.25 mm (Berry Plastics Corp., Evansville, IN, USA) immediately after fumigation. Each replicated plot consisted of three, 22.8 m raised beds. Tomato plants of the *S. lycopersium* cv. HM1823 variety were transplanted in March 2020 and 2021. Plants were arranged in a single row with a spacing of 60 cm between plants. Fumigant treatments were applied at a depth of 20 cm using a three-shank fumigation rig (Kennco Manufacturing, Ruskin, FL, USA) during the second week of February in 2020 and 2021. Each fumigant followed a different calibration procedure. Metam potassium was applied at a 10 cm depth with a standard fumigant rig equipped with 6 back swept shanks. Application rates are shown in Table 1. The drip tape was used to supply water and nutrients daily during the tomato growing period (March–June). Tomato crops were irrigated and fertilized following Florida industry standards (Yu et al., 2019).

Soil samples (0–20 cm) were collected for soil physicochemical and microbial analyses before the beginning of the experiment (before fumigation in the first week of February in 2020 and 2021), one month after treatment application (after fumigation; second week of March in 2020 and 2021), after final harvest at the end of the growing season (end harvest; first week of June in 2020 and 2021), and approximately one month after the experiment end (residual; second week of July in 2020 and 2021) from six sampled areas (one per bed section, 6 bed sections per replicated plot) a using a soil core, and pooled to provide one soil sample per replicated plot.

2.3. Soil properties and tomato yield

Soil physicochemical analyses were performed by a commercial laboratory (Waypoint Analytical, Memphis, TN, USA) using standard reference methods. The following soil properties were determined: soil pH, cation exchange capacity (CEC), and the concentrations of ammonium (NH_4^+), nitrate (NO_3^-), phosphorus (P), magnesium (Mg), and calcium (Ca). Fruit from 15 tomato plants in each replicated plot were hand harvested in June 2020 and June 2021 and weighed. Tomato yields were expressed in kg/ha.

2.4. Extraction of DNA and microbiome analyses

Extraction of soil DNA, quantification, and sequencing were conducted as described by Castellano-Hinojosa et al. (2022b). DNA was sequenced using a MiSeq sequencer (Illumina, Inc., San Diego, CA, USA) at the DNA Sequencing Center of the University of Illinois Chicago (Chicago, IL, USA). The bacterial community was sequenced using the primers 515Fa and 926R (Parada et al., 2016) that amplify the V4-V5 region of the 16S rRNA gene. The fungal community was sequenced using the primers ITS1F (Gardes and Bruns, 1993) and ITS2R (White et al., 1990) that amplify the ITS1 region. Analysis of the sequence reads was performed following the methods described in Castellano-Hinojosa et al. (2022b). Raw sequence data are available in the NCBI under BioProject PRJNA1012315.

Alpha [number of observed amplicon sequence variants (ASVs) and Shannon and Simpson indices] and beta diversity analyses were conducted as described by Castellano-Hinojosa et al. (2022b) using R version 4.2.3 (http://www.rproject.org/). Differences in beta diversity between treatments were examined using the permutational analysis of variance (PERMANOVA). Differences in beta diversity of the bacterial and fungal community between years for each treatment prior to fumigation were identified by non-parametric analysis (ANOSIM) (Clarke, 1993). For each time point, differentially abundant bacterial and fungal taxa between treatments were detected using the DESeq2 analysis (Love et al., 2014). The PICRUSt2 tool was used to infer the functionality of the bacteria community using the bacterial reads following methods of Douglas et al. (2020). Fungal guilds associated to the fungal ASVs were identified using the FUNGuild tool as described by Nguyen et al. (2016).

2.5. Microbial network analysis

Co-occurrence networks were created to examine the treatment impacts on the interactions and structure of bacterial and fungal taxa at different time points. Networks were created as described by Castellano-Hinojosa et al. (2022b) using Spearman correlations in R and visualized using Cytoscape v3.8.2. The properties of the network with ecological relevance (e.g., nodes, edges, mean degree and density) were estimated using Cytoscape v3.8.2 (Shannon et al., 2003) (see Supplementary Data 1).

2.6. Statistical analyses

All statistical analyses were conducted using R version 4.2.3 (http://www.rproject.org/). A structural equation model (SEM) was used to study how fumigant application (all the five fumigant treatments together vs. a non-treated control treatment) influenced the temporal variation of the alpha (changes in the values of the Shannon index) and beta (first axis of the NMDS) diversity, network complexity (sum of average degree of bacterial and fungal networks) and predicted functionality (changes in the relative abundance of predicted functions and guilds) of bacterial and fungal communities, and how these changes subsequently impacted soil properties (changes in soil pH, CEC, and the concentrations of NH_4^+, NO_3^-, P, Mg, and Ca) and crop production (changes in fruit yield). Data from both tomato growing seasons (2020 and 2021) were used and all time points were considered for all variables included in the SEM model. The SEM model was constructed as described in detail by Castellano-Hinojosa et al. (2023a) in R. Briefly, variables were standardized by Z transformation using the "scale" function in R. The SEM model was fitted using the maximum likelihood method and the "lavaan" package in R. The $\chi 2$-test (Schermelleh-Engel et al., 2003), the goodness-of-fit-index, and root mean square error of approximation (West et al., 2012) were calculated to determine the fit of the SEM model.

3. Results and discussion

3.1. Soil properties at different sampling times

No significant differences in CEC, soil pH, P, Ca, Mg, NO_3^-, and NH_4^+ were detected between treatments in 2020 (Supplementary Table S1A) and in 2021 (Supplementary Table S1B). Previous studies across different crops and environmental conditions have also reported limited impacts of fumigants on soil properties and when they do occur, they often disappear approximately 30 days after fumigation (Dangi et al., 2014; Fang et al., 2018, 2019, 2020). This could be due to the short half-life of these products in soil (Dungan et al., 2003; Castellano-Hinojosa et al., 2022a).

3.2. Tomato yield

Treatment with Pic60, Pic100, and the 3-way combination significantly increased crop yield compared to Pic35 and the control in 2020 (Fig. 1A) and 2021 (Fig. 1B). Crop yield was not significantly different between Kpam, Pic35, and the control treatments for both years (Fig. 1).

We observed that an increased number of fumigants in a mixture did not translate into increases in crop yield compared to fumigant combinations with one or two products. However, treatments with 60 % chloropicrin (Pic) or greater increased crop yield more than the control and Kpam alone. These results suggest that the presence of at least 60 % Pic can increase crop yield in a plastic-mulched tomato production system, an effect that may be due to a greater efficacy to control weeds and soil-borne fungal pathogens compared to the rest of treatments tested in this study. These results are similar to those of Yu et al. (2021c) where combinations of Pic60 with other fumigants (DMDS and 1,3-D) significantly improved tomato yield compared to the nonfumigated control. A previous study also showed that tomato plants in soils fumigated with combinations of Pic60 + DMDS were significantly taller compared to the single application of DMDS (Yu et al., 2019). Although crop yields were not different after fumigation with Pic35 and Pic60 in a previous study carried out in a Florida strawberry production system (Castellano-Hinojosa et al., 2022b), our results show that Pic60 improved crop productivity compared to Pic35 in plastic-mulched tomato production systems. Strawberry, like tomato, is also planted in plastic-covered beds in Florida, but differences in fumigation history, soil type, crop, and other biotic (e.g., pests) and abiotic factors may explain the different results between studies.

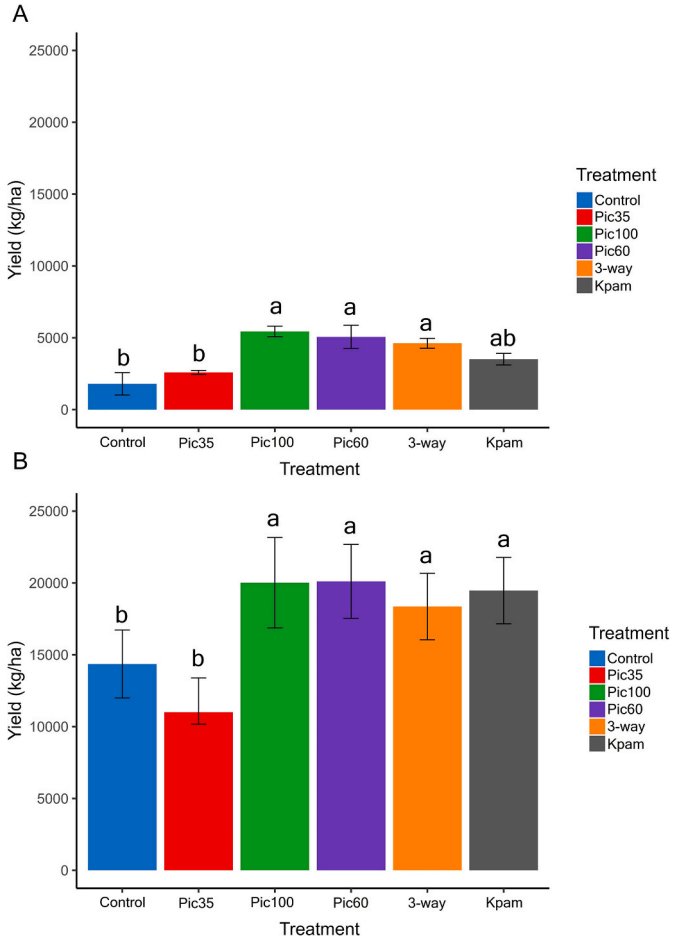

Fig. 1. Average tomato yields (kg/ha) at the end of the growing season in June 2020 (A) and June 2021 (B). Treatments are defined in Table 1. Different letters above the bars indicate significant differences between treatments (Tukey's HSD, $p \leq 0.05$). Values are expressed as means with standard error.

We found that adding Kpam to Pic60 (the 3-way treatment) did not provide any additional benefits on crop productivity compared to Pic60 alone. This could be due to a similar control on pathogenic fungi between the Kpam and Pic60 as shown by similar changes in alpha and beta fungal diversity (Figs. 3 and 4) and differentially abundant fungal taxa (Fig. 6). Nevertheless, the 3-way was the most effective treatment at reducing purple nutsedge species broadleaf and grass weeds in a previous experiment conducted on the same plots with similar fumigation treatments (Yu et al., 2019, 2021c). We acknowledge that the study of repeated fumigant applications on soil pest such as parasitic nematodes and weeds could help understand variations in crop yield among treatments and should be explored in future studies.

3.3. Effects on the diversity and composition of microbial communities

Regardless of the time point, fumigant application had no significant effect on bacterial alpha diversity indices compared to the control in 2020 (Fig. 2A) and 2021 (Fig. 2B). These results show that a single or combination of fumigants had no major impacts on bacterial diversity in these plastic-mulched tomato production systems. Previous work also found no effect of Pic35 on bacterial diversity after one (Dungan et al., 2003; Zeng et al., 2019), two (Liu et al., 2015; Fang et al., 2020), and four months (Zhang et al., 2019). However, other studies reported decreases in bacterial diversity within the first 6 weeks after treatment with Pic35 and Pic60, an effect that was related to increased microbial mortality due to the broad-spectrum activity of these products (Li et al., 2017; Castellano-Hinojosa et al., 2022a). This variation in the effect of pre-plant fumigants on non-target bacterial diversity is likely due to the range of factors that can influence these interactions including the type of fumigant, the amount used, degradation rates, soil properties, application modes, and environmental conditions (Castellano-Hinojosa et al., 2022a).

For the fungal community, significant decreases in the alpha diversity were observed in soils treated with Pic35, Pic60, Pic100, 3-way, and Kpam compared to the control after fumigation and at the end of harvest in 2020 (Fig. 3A). Except for Kpam, these differences in alpha diversity between fumigated and non-fumigated soils remained one month after final harvest in 2020 (Fig. 4A). In 2021, only Pic60, Pic100, and Kpam significantly reduced the alpha diversity indices compared to the control after fumigation but there were no differences between treatments at the end of the growing season (Fig. 3B). We found an increased number of fumigants did not induce greater decreases in fungal alpha diversity compared to the individual application of these products (Fig. 3A, B).

Decreases in fungal diversity were expected to occur within the first weeks after fumigant application (Castellano-Hinojosa et al., 2022a), but our results show these effects can last for several months suggesting fungal communities may take longer to recover. Fungi are known to have lower resilience than bacteria (Rousk and Bååth, 2011) which may explain the significant decreases in alpha diversity in the long-term in

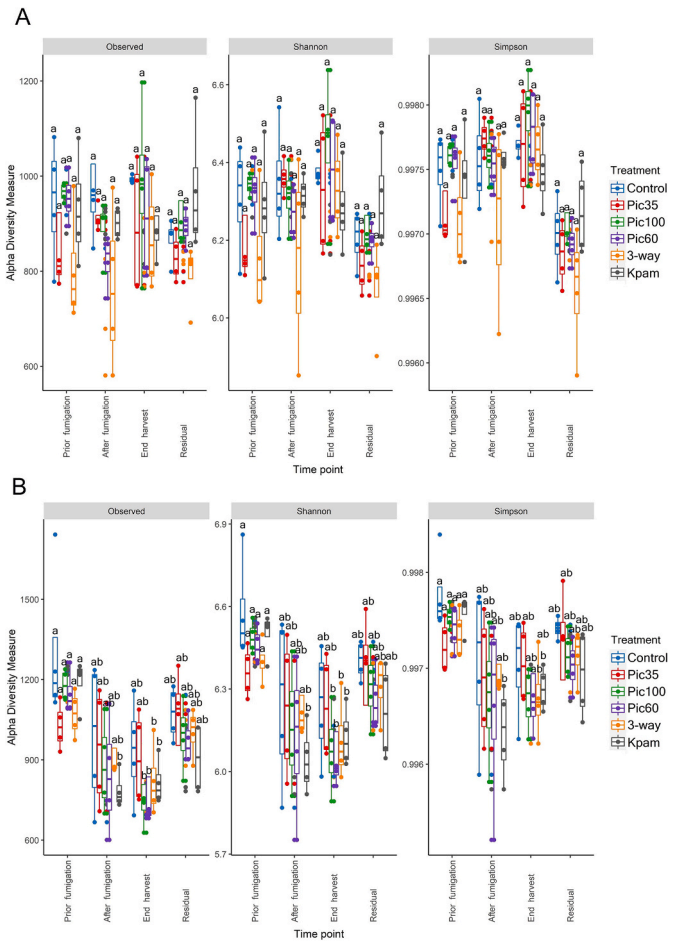

Fig. 2. Number of ASVs, and values of Shannon and inverse Simpson diversity indices for the bacterial community in 2020 (A) and 2021 (B) in non-treated and treated soils. Treatments are defined in Table 1. Samples were collected before the experiment start (prior fumigation), one month after treatment application (after fumigation), after final harvest (end harvest), and one month after final harvest (residual). Different letters above the bars indicate significant differences between treatments and time points (Tukey's HSD, $p \leq 0.05$). Values are expressed as means with standard error.

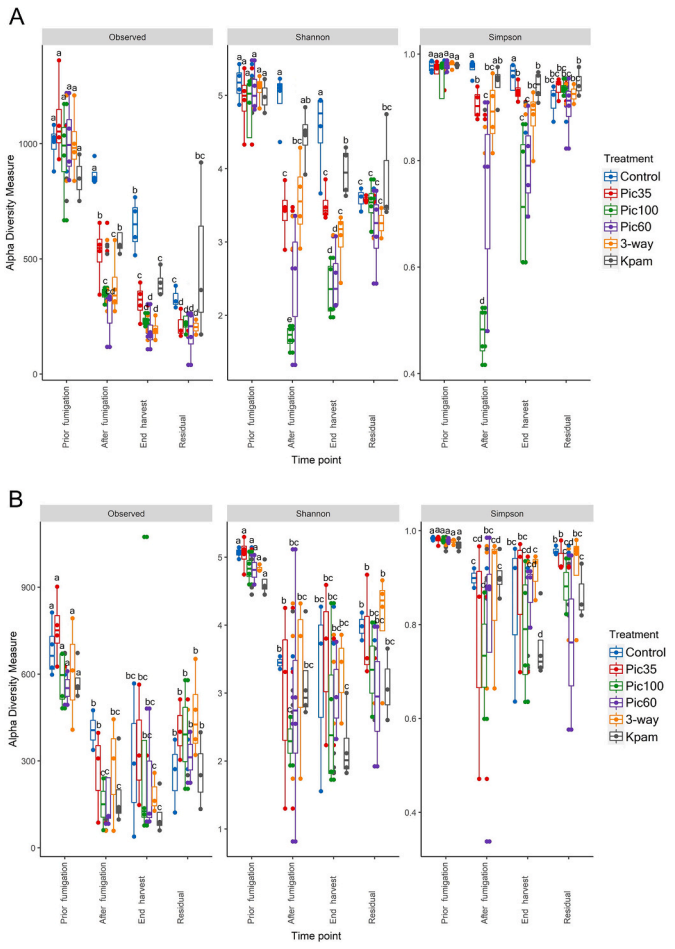

Fig. 3. Number of ASVs, and values of Shannon and inverse Simpson diversity indices for the fungal community in 2020 (A) and 2021 (B) in non-treated and treated soils. Treatments are defined in Table 1. Samples were collected before the experiment start (prior fumigation), one month after treatment application (after fumigation), after final harvest (end harvest), and one month after final harvest (residual). Different letters above the bars indicate significant differences between treatments and time points (Tukey's HSD, $p \leq 0.05$). Values are expressed as means with standard error.

this study. Despite having different spectrums of activity, we found that different combinations of fumigants had similar impacts on fungal alpha diversity which appears to be due to the broad-spectrum activity of these products. This is not surprising as the mode of action of most fumigants remain largely unknown (Castellano-Hinojosa et al., 2022a).

Bacterial (Fig. 4A) and fungal (Fig. 4B) community compositions were significantly different between treatments and time points ($p \leq 0.001$). The composition of the bacterial community of the Pic60, Pic100, and 3-way treatments were not significantly different after fumigation and at the end of harvest in both years (Fig. 4A), whereas there was a significant difference in the composition of the Pic35 treatment. Regardless of the treatment, no significant differences in the composition of the bacterial community were detected prior to fumigation and one month after final harvest both in 2020 and 2021 (Fig. 4A). Treatment with Kpam had no significant impact on the composition of the bacterial community for both years (Fig. 4A; $p > 0.05$). For the fungal community in 2020 and 2021 (Fig. 4B), the composition changed with time and did not return to its prior fumigation state for any of the treatments (Fig. 4B) until prior to fumigation for the next year. However, while the fungal community composition was significantly different from the control, there were no significant differences in the composition of the fungal community among any of the fumigation treatments (Fig. 4B) for any time point. An ANOSIM analysis showed that the bacterial and fungal community compositions were not significantly different prior to fumigation between years for all treatments (Supplementary Table S2; $p > 0.05$). Therefore, the bacterial and fungal community composition was similar at the beginning of both growing seasons suggesting no residual impact of treatments on the soil microbiome composition between years.

In general, we found the individual and combined application of fumigants altered soil bacterial and fungal community composition for four months after fumigation. Recently, we showed that pre-plant fumigants can alter bacterial community composition within the first 1–4 weeks after fumigation in a strawberry production system in Florida (Castellano-Hinojosa et al., 2022b). The composition of the fungal community did not return to prior fumigation levels until prior to fumigation the next year possibly due to slower recovery compared to the bacterial community. Fungi often grow more slowly than bacteria in soil (Rousk and Bååth, 2011), thus potentially taking a longer time to recover after fumigation compared to bacteria.

Our results also showed treatments temporarily altered bacterial and fungal community compositions across all taxonomic levels during the growing seasons (Supplementary Figs. S1-S4). For example, treatments with Pic60, Pic100, and 3-way (which included Pic60) increased the relative abundance of Firmicutes by 51 % and 40 % (on average) in 2020 and 2021, respectively, compared to the control after fumigation but

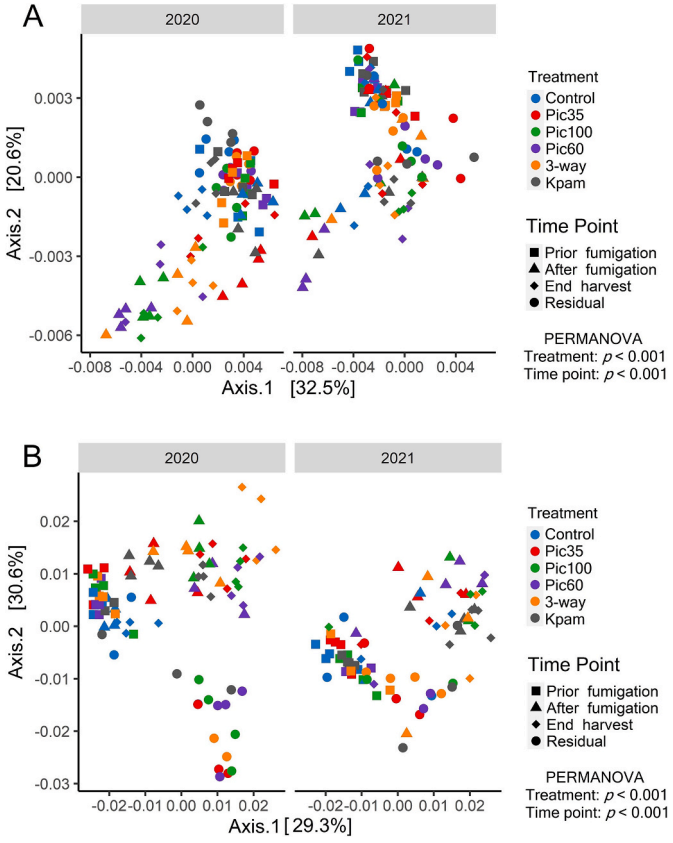

Fig. 4. Non-metric multidimensional scaling (NMDS) plots on unweighted UniFrac distances for the bacterial (A) and fungal (B) communities in non-treated and treated soils in 2020 and 2021. Treatments are defined in Table 1. Samples were collected before the experiment start (prior fumigation), one month after treatment application (after fumigation), after final harvest (end harvest), and one month after final harvest (residual). Differences in community composition between treatments and time points were tested by permutational analysis of variance (PERMANOVA), and p values ≤ 0.01 were considered significant.

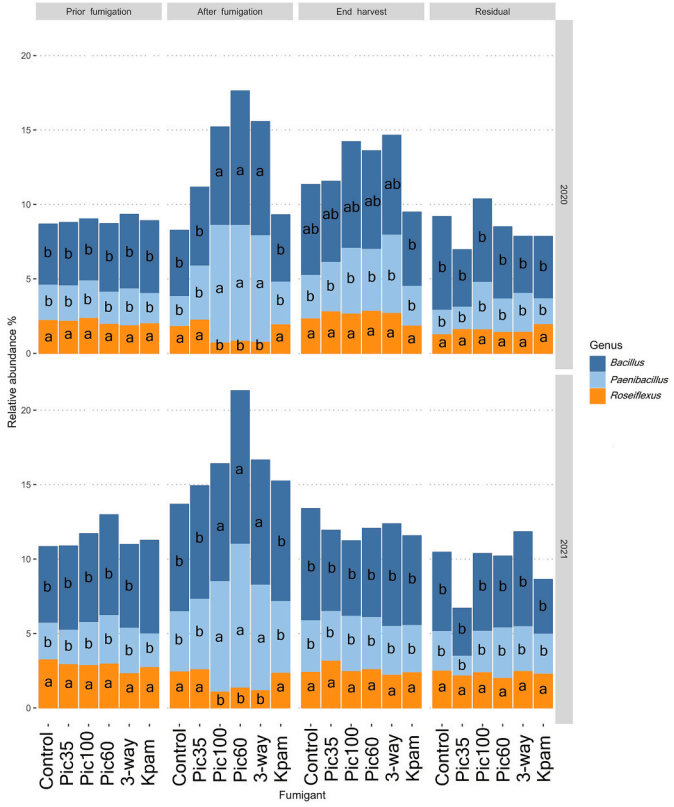

Fig. 5. Differential abundance of bacterial genera across treatment and time points in 2020 and 2021 according to DESeq2 analysis ($p \leq 0.01$). Treatments are defined in Table 1. Samples were collected before the experiment start (prior fumigation), one month after treatment application (after fumigation), after final harvest (end harvest), and one month after final harvest (residual).

values returned to basal levels afterwards (Supplementary Fig. S1). Fungal communities were dominated by Ascomycota (89.5 %) (Supplementary Fig. S2). The application of Pic60, Pic100, and 3-way increased the relative abundance of Chytridiomycota by 50–150 % (on average) in 2020 compared to the control after fumigation but values returned to basal levels afterwards (Supplementary Fig. S2). At the class level for the bacterial community, only Bacilli were temporally enriched (in the order of 20–30 %) after fumigation with Pic60, Pic100, and 3-way in 2020 and 2021, respectively, compared to the control but differences between treatments disappeared afterwards (Supplementary Fig. S3). The fungal classes Rhizophydiomycetes and Eurotiomycetes were enriched in soils treated with Pic60 (20–42 %), 3-way (50–140 %), and Kpam (12–30 %) after fumigation compared to the control in 2020 but no changes were observed in 2021 at this taxonomic level (Supplementary Fig. S4).

It is important to note that soil microbial communities are responsive not only to fumigants but also to other management practices (e.g., cover crops, chemical fertilizers, and compost) in general (Castellano-Hinojosa et al., 2023a, 2023b). Additional research is needed to determine how and to what extent any alterations in the soil microbiome could be translated into increases and/or decreases in plant (e.g., growth and productivity) and soil (e.g., soil quality and nutrient cycling) traits (see Section 3.7. for additional details).

3.4. *Differentially abundant microbial taxa between treated and control soils*

Differentially abundant taxa were identified at the genus level to examine the effect of treatments on specific genera across time points and treatments. In particular, significant increases were found in both bacterial and fungal taxa with the potential to benefit plant growth and/or disease suppression. For example, the application of Pic60, Pic100, and 3-way significantly increased the relative abundance of *Bacillus* and *Paenibacillus* and reduced that of *Roseiflexus* after fumigation compared to the other treatments in 2020 and 2021, but these differences disappeared afterwards (Fig. 5). Members of the genera *Bacillus* and *Paenibacillus* can control soil-borne pests including parasitic nematodes and show different plant-growth promotion (PGP) traits including production of siderophores and phytohormones and the ability to solubilize phosphate (Hussain et al., 2020). *Roseiflexus* spp. are photoheterotrophic bacteria with potential nematicidal activity (Engelbrecht et al., 2021) and the ability to degrade organic compounds, such as lignin, in soils (Schellenberger et al., 2010).

Eight fungal genera (*Aspergillus*, *Curvularia*, *Fusarium*, *Mortierella*, *Talaromyces*, *Thielavia*, *Trichoderma*, and *Zopfiella*) had significant variations in their relative abundance between time points and treatments (Fig. 6). For example, the relative abundance of *Fusarium* after fumigation and at the end of harvest was significantly reduced with the application of any of the treatments, but these differences between treatments disappeared one month after final harvest in 2020 (Fig. 6). However, these variations in the relative abundance of *Fusarium* between treatments only occurred for Pic100 in 2021 (Fig. 6), thus showing fumigant impacts can differ between growing seasons and may depend on both the percentage of Pic in the mixture and colonization-

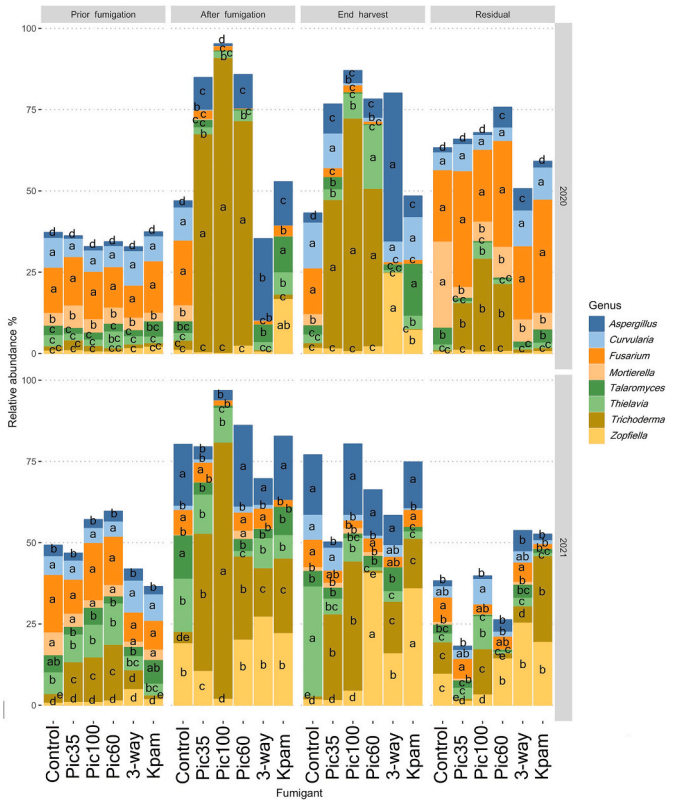

Fig. 6. Differential abundance of fungal genera across treatment and time points in 2020 and 2021 according to DESeq2 analysis ($p \leq 0.01$). Treatments are defined in Table 1. Samples were collected before the experiment start (prior fumigation), one month after treatment application (after fumigation), after final harvest (end harvest), and one month after final harvest (residual).

competition tradeoffs after fumigation (Smith et al., 2018). *Fusarium* contains numerous plant pathogens that cause considerable economic losses, and the efficacy of fumigants against this genus has been widely reported (Castellano-Hinojosa et al., 2022a). There was a significant enrichment of *Trichoderma* in soils treated with Pic60, Pic100, and the 3-way after fumigation and at the end of harvest compared to the control in 2020 and 2021 (Fig. 6). *Trichoderma* spp. can act as biological control agents in soil (Latz et al., 2012;) and increases in its relative abundance after fumigation have been reported (Castellano-Hinojosa et al., 2022b).

For additional insights into the effect of treatments on the bacterial and fungal community structures, pairwise comparisons were run at the ASV level between treated and non-treated soils for each time point and year (Supplementary Figs. S5-S8). These comparisons at the ASV level suggest fumigants can have impacts on soil bacterial and fungal communities that may be species-specific, likely due to their broad-spectrum activity. For example, all treatments had both significant positive and negative impacts on the relative abundance of specific bacterial (Supplementary Figs. S5 and S6) and fungal (Supplementary Figs. S5 and S6) ASVs after fumigation, at the end of harvest, and one month after final harvest, but these effects tended to decrease with time. In fact, over 85 % of the impacts of any of the treatments on the relative abundance of bacterial ASVs were not genus specific as both significantly enriched and depleted ASVs often occurred within a particular genus in 2020 and 2021 (Supplementary Figs. S5 and S6). However, on average, about 74 % of the differentially enriched and depleted fungal ASVs were assigned to specific and different genera showing a more specific genus effect of treatments on the structure of the fungal compared to the bacterial community at the ASV level (Supplementary Figs. S7 and S8). The relative abundances of bacterial and fungal ASVs between treated vs. non-treated soils prior to fumigation for both years were not significantly different ($p > 0.05$).

Our identification of differentially abundant fungal ASVs across time points revealed that these alterations can last at least four months after treatment application, again supporting a potential slower recovery of fungi compared to bacteria after fumigation in the soil microbiome. Interestingly, the application of the fumigant Kpam alone also produced significant variations in the relative abundance of ASVs, highlighting its broad-spectrum activity. Previous studies have shown that fumigants can have multiple activities (e.g., herbicide, fungicide, and nematicide traits) that can go beyond their main expected target effect (Yu et al., 2019; Khatri et al., 2021; Castellano-Hinojosa et al., 2022b). Future studies should aim at isolating differentially abundant taxa and assaying their plant growth promotion traits under controlled conditions.

3.5. *Changes in predicted bacterial functionality and fungal guilds*

Decreases in bacterial functions associated with general metabolic pathways and biogeochemical cycles suggest that fumigants can have broad spectrum effects on bacterial functionality (Supplementary Fig. S9). There was a significant reduction in the relative abundance of 9 predicted KEGG pathways (nitrogen metabolism, carbon fixation, methane metabolism, sulfur metabolism, DNA replication, cell cycle, oxidative phosphorylation, bacterial secretion system, and ABC transporters) after application of Pic60, Pic100, and 3-way and at end of harvest compared to the control in 2020 and 2021 (Supplementary Fig. S9). However, these differences in the relative abundance of predicted pathways between treatments disappeared one month after final harvest (Supplementary Fig. S9). Regardless of the time point, treatment with Pic35 and Kpam had no significant impact on bacterial predicted KEGG pathways compared to the control in 2020 and 2021 (Supplementary Fig. S9). Our results also suggest decreases or losses of soil functions were general across the soil bacteriome and were not group specific as temporal changes in bacterial functionality were not linked to variations in bacterial diversity (which was unaffected in this study). In addition, the predicted bacterial community functionality appeared to return to initial levels when fumigant effects disappeared, as the observed negative impacts of fumigants on bacterial predicted functionality disappeared one month after final harvest. We acknowledge that results from predicted functional tools may have some limitations and should be interpreted with care (Sun et al., 2020) and future studies should explore variations in functionality using RNA and/or cDNA approaches which can better capture variations in microbial functions and metabolic activity. Nevertheless, the accuracy of PICRUSt2 to predict bacterial functions has been demonstrated in different environmental studies (Douglas et al., 2020), and the tool is useful to assess potential functional variations.

The application of Pic35, Pic60, Pic100, 3-way, and to a lesser extent Kpam, significantly reduced the relative abundance of the predicted plant pathogen and arbuscular mycorrhizal fungal guilds after fumigation, at the end of harvest, and one month after final harvest compared to the control in both years (Supplementary Fig. S10). These decreases in the relative abundance of plant pathogen and arbuscular mycorrhizal fungal guilds are likely due to the broad-spectrum impact of these products, particularly on soil fungi (Castellano-Hinojosa et al., 2022a, 2022b). Of note, these effects lasted for several months after fumigation, thus showing these product's high efficacy to reduce the diversity and functionality of fungal communities. However, after fumigation with Pic35, Pic60, Pic100, and 3-way, at the end of harvest, and one month after final harvest compared to the control in both years (Supplementary Fig. S10) there were significantly more undefined saprotrophs. Regardless of the sampling point, no significant effects of Kpam on the relative abundance of undefined saprotrophs were detected compared to the control in both years (Supplementary Fig. S10). Increases in the abundance of saprotrophs after fumigation have been previously

reported and associated with fungi using dead microbial cells as a C source (Nicola et al., 2017; Fang et al., 2018; Huang et al., 2020).

3.6. Effect of treatments on microbial network complexity

Complex ecological networks are formed by soil microbial communities and alterations of these interactions due to the application of soil treatments (e.g., fertilizers and fumigants) is expected to alter their resistance (insensitivity to disturbance) and resilience (the rate of recovery after disturbance) to external stresses (Banerjee et al., 2019; Jiao et al., 2019). A total of 48 microbial networks were created to examine the co-occurrence patterns of bacterial and fungal taxa during the experimental period (prior fumigation, after fumigation, end harvest, and residual). Relevant properties of the co-occurrence networks are presented in Supplementary Data 1.

Our results show that repeated fumigation can temporarily decrease bacterial and fungal network complexity, which was consistent during both growing seasons. This is similar to previous work on fumigant impacts on microbial network complexity in microcosm (Zhang et al., 2021), mesocom (Fournier et al., 2020), and field (Castellano-Hinojosa et al., 2022b) experiments. The number of nodes and edges, mean degree, and density in the bacterial networks from soils treated with Pic60, Pic100, and 3-way were significantly lower after fumigation and at the end of harvest compared to the respective networks from the Pic35, Kpam, and control soils in both years (Supplementary Data 1). However, topological property differences of the bacterial networks disappeared one month after final harvest. The percentage of Pic appeared to impact the magnitude of effects, as bacterial networks from soils treated with >35 % Pic were significantly less densely connected and had fewer nodes and edges compared to networks from the control and Kpam alone after fumigation in both years, and the values gradually increased to reach levels found in the control soils at the end of harvest and one month after final harvest (Supplementary Data 1). Treatment with Kpam had no significant impact on bacterial co-occurrence networks compared to the control during the experimental period in both years.

Fungal networks from soils treated with Pic35, Pic60, Pic100, 3-way, and to a lesser extent Kpam, were significantly less densely connected and had fewer nodes and edges than the respective networks from the control after fumigation, at the end of harvest, and one month after final harvest in both years (Supplementary Data 1). Overall, topological property differences of the fungal networks between treated and the control soils tended to disappear one month after final harvest, but they were still significant (Supplementary Data 1).

3.7. Linkages between treatment application, microbial communities, soil properties, and crop yield

Results from the SEM model suggest that variations in tomato yield in treated soils were not only due to treatment application itself, but to indirect effects of treatments on bacterial and fungal communities. The SEM model showed that treatment application was translated into direct increases in tomato yield in our study (Fig. 7). To a lesser but significant extent, treatment impacts on fungal communities also contributed to increased crop yield (Fig. 7). Interestingly, non-target indirect effects of treatments on bacterial community composition, predicted functionality, and network complexity significantly but negatively influenced crop yield (Fig. 7). These results are of agronomic interest as they suggest increases in crop yield could be achieved by altering the way fumigants impact communities of soil bacteria and fungi. As the percentage of Pic often determined the magnitude of changes in the bacterial and fungal communities, future studies could examine the optimal amount of chloropicrin necessary to have beneficial impacts on pathogens and potentially plant-beneficial taxa but minimize other non-target impacts.

While the SEM model illustrates a link between changes in soil microbial communities and plant productivity, there is still limited direct

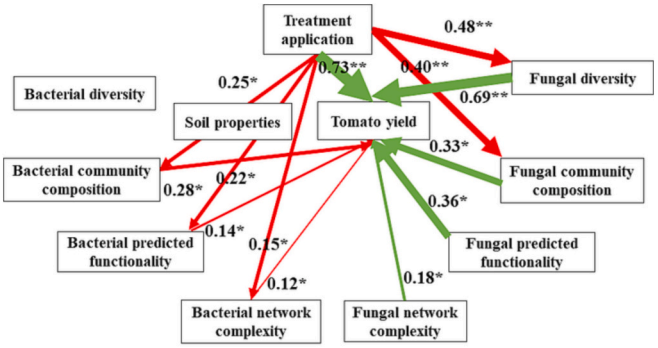

Fig. 7. Structural equation model (SEM) illustrating the effects of treatment application (Pic35, Pic60, PicClor100, 3-way, and Kpam) on the temporal variation of bacterial and fungal community diversity, bacterial and fungal network complexity, and bacterial and fungal predicted functionality, and how these subsequently affected soil properties and crop yield. Data from both seasons (2020 + 2021) were used and all time points were considered for all variables included in the SEM model. Continuous arrows show significant relationships between variables. Non-significant relationships have been omitted for clarity. Green and red arrows indicate positive and negative relationships, respectively. The width of arrows shows the strength of path coefficients. *, **, and *** indicate significance at $p < 0.05$, 0.01, and 0.001, respectively.

experimental evidence for this link in plastic-mulched tomato production system. However, the consistent effect of the single and combined application of fumigants on network complexity, microbial community composition, and abundance of bacterial predicted functions and fungal guilds observed in this study during both growing seasons and across time points suggests these products can have effects on target and non-target microorganisms that may subsequently alter tomato yield.

4. Conclusions

Over two consecutive growing seasons, our study shows that fumigant treatments with at least 60 % chloropicrin alone or in combination with other fumigants can increase tomato yield more than the non-fumigated control in a plastic-mulched tomato production system. While the application of different combinations of fumigants altered the composition of soil bacterial and fungal communities and reduced fungal richness and diversity across two consecutive growing seasons, these effects were temporary and disappeared by the beginning of the next growing season. Our study shows fumigants not only directly impact crop yield, but they can also indirectly determine variations in plant productivity through effects on the soil microbiome. Application of fumigants can reduce network microbial complexity and specific bacterial functions and fungal guilds abundances, an effect that appeared to be controlled by the percentage of chloropicrin and gradually disappeared with time. Future studies should further explore to what extent changes in the soil microbiome after fumigation are linked to long-term variations in crop yield.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.170659.

CRediT authorship contribution statement

Antonio Castellano-Hinojosa: Writing – original draft, Investigation, Formal analysis, Conceptualization. **Elena Karlsen-Ayala:** Writing – review & editing, Investigation, Formal analysis, Conceptualization. **Nathan S. Boyd:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization. **Sarah L. Strauss:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

We would like to thank Brittney Monus for assistance with sample collection and DNA extraction and Emily Witt for assistance with sample and yield collection. We would also like to thank the UF/IFAS Gulf Coast Research and Education Center farm crew for assistance in trial maintenance. This study was supported with funds from the USDA National Institute of Food and Agriculture Research Initiative grant 2022-67019-36958, the USDA National Institute of Food and Agriculture Hatch project 7000945, and the TriEst Ag Group.

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