 doi:10.20944/preprints202209.0177.v1

Article

Combined Fungicide Treatment Affects Soil Microbial Community and Incidences of Fungal Diseases on Wheat

Alexey S. Vasilchenko[1]*, Darya V. Poshvina[1], Mikhail V. Semenov[1,2], Vyacheslav N. Timofeev[3], Artyom A. Stepanov[1], Arina N. Pervushina[1] and Anastasia V. Vasilchenko [1]

[1]Laboratory of Antimicrobial Resistance, Institute of Environmental and Agricultural Biology (X-BIO), Tyumen State University, Tyumen, Russian Federation

[2] Dokuchaev Soil Science Institute, Moscow, Russian Federation

[3] Scientific Research Institute of Agriculture for Northern Trans-Ural Region - Branch of Tyumen Scientific Centre SB RAS

* Correspondence: avasilchenko@gmail.com

Abstract: Pesticides are widely used in agriculture as a pest control strategy. Despite the benefits of pesticides on crop yields, the persistence of chemical residues in soil have an unintended impact on non-targeted micro-organisms. In this study, we evaluated the impact of the combined fungicide (difenoconazole, epoxiconazole, and kresoxim-methyl) on fungal and bacterial communities of Phaeozem. In the fungicide-treated soil, the Shannon index of both fungal and bacterial communities was decreased, while Chao1 index did not differ compared to the control soil. Among bacterial taxa, the relative abundance of *Athrobacter*, *Sphingomicrobium*, and *Sphingomonas* increased in fungicide-treated soil due to their ability to utilize fungicides and other toxic compounds. *Rhizopus* and plant-beneficial *Chaetomium* were the dominant fungal genera, which increased 2-4 times in the fungicide-treated soil, while the relative abundance of *Mortierella* and *Talaromyces* decreased. *Fusarium acuminatum* was the most abundant phytopathogenic fungus that causes root rot disease of wheat, but applied fungicide treatment decreased their diversity in the soil 2 times, which is consistent on the observed plants.

Keywords: non-target action; soil microbiome; pesticide contamination; fungicide; soil quality

1. Introduction

Every year up to 2.5 million tons of pesticides get into the environment, and then pesticide residues are found in soils. Only a small dose of pesticides reaches target organisms [1], while a significant part of them negatively affects beneficial organisms. Exposure of soil to pesticides could lead to negative effects on soil microbial communities and microbial processes, consequently reducing the soil quality [2]. The final effect on the soil microbiome after the application of pesticide may be positive, negative, or neutral depending on soil properties, pesticides chemical nature, and others.

The negative effects of herbicides were showed for *Rhizobium*, *Chlamydomonas*, *Azotobacter*, *Azospirillum*, and heterotrophic S-oxidizing and S-reducing bacteria [3,4]. At the same time, insecticides carbofuran, carbosulfan, thiomethoxam, imidacloprid, chlorpyrifos did not significantly change the abundance of *Rhizobium* and phosphorus solubilizing bacteria [5]. Despite the fact that fungicides contribute to more than 35% of the global pesticide market [6] their impact on ecosystems has received less attention compared to herbicides and insecticides. Among fungicides, triazoles and stroiblurins account for the largest part.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 September 2022 doi:10.20944/preprints202209.0177.v1

Triazoles were introduced into practice in the 70s of last century, since then their market share has been increasing annually. Triazole fungicides disrupt ergosterol biosynthesis in fungal cells by inhibiting the enzyme lanosterol 14α-demethylase, and do not prevent spore germination and early germ-tube growth [7]. In turn, strobilurins were administered in the German market in 1996 [8]. Strobilurins are the Q_oI fungicides which bind to the quinol oxidation (Q_o) site of cytochrome b and inhibit mitochondrial respiration [9].

Although triazoles and strobilurines are well known to be effective for major groups of plant pathogenic fungi, little is known about their effects on prokaryotes. Bacteria do not have sterols or mitochondria, so the usage of triazoles and Q_oI fungicides is assumed to have no effect on them. However, it was found that triadimefon (triazole) had long-term inhibiting effects on soil bacterial community [10]. Triticonazole and tebuconazole increased the number of soil bacteria and stimulated the dehydrogenase activity [11], while two other sterol-targeting fungicides (fenpropimorph and propiconazole) inhibited overall bacterial activity [12]. Metagenomic analysis showed that some soil microbial genera belonging to Proteobacteria and Firmicutes decreased significantly, while the abundance of Actinobacteria increased when strobilurins are present in the soil [13]. Often, pesticides and their decomposition products serve as carbon sources for soil microorganisms. However, there is insufficient data on the predominance of pesticide-degrading microorganisms that affect soil quality. In addition the use of fungicides leads to the problem of the emergence of resistant pathogens [14].

Agricultural practices based on using combined fungicides will minimize resistant pathogen clones. Mixing or rotating fungicides with different modes of action is considered to be an effective approach to slowdown the development of field resistance [15]. Strobilurin and triazole fungicides have been widely used for plant-protection for several decades. These fungicides have a wide spectrum of activity and their combination significantly reduces resistance of phytopathogens.

In this study, we used the fungicide mixture which combined strobilurin kresoxim-methyl and two triazoles (difenoconazole and epoxiconazole). Our primary objective was to assess the post-treatment effects of the combined fungicide on the fungal and bacterial communities of Phaeozem. The questions which were answered are included various aspects of soil health changing under the treatment. Does a fungicide combining strobilurin with triazoles affect the bacterial community? What is the functional potential of the taxa whose abundances have been changed? How has the diversity and the abundance of pathogenic bacterial and fungal taxa changed and is there a relationship with incidences of diseases in wheat?

2. Results

2.1. The shift of bacterial taxa under fungicide treatment

Estimation of amount of dominant bacterial taxa in the soils revealed a remarkable shift in bacterial communities under the fungicide application. The most abundant bacterial phylum in the control soil sample was Actinobacteriota; Proteobacteria and Acidobacteria were the second and the third dominant phyla, respectively (Figure 1). The bacterial community of the fungicide treated soil was represented by the same dominant phyla, but

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 September 2022 doi:10.20944/preprints202209.0177.v1

their abundances changed. The application of the combined fungicide resulted in an increase in the number of Actinobacteriota and Proteobacteria, while a decrease in Acidobacteriota and Verrumicrobiota compared to the control soil. (Figure 1).

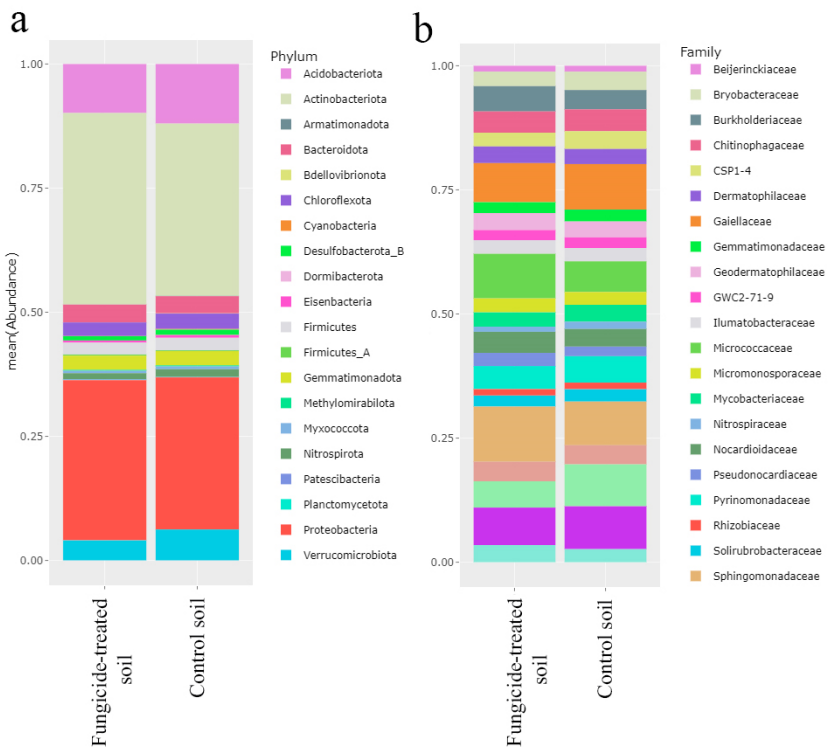

Figure 1. The relative abundances of bacterial phyla (a) and families (b) in the control and the fungicide-treated soils.

At the family level, the most affected taxa were represented by *Micrococcaceae*, *Sphingomonadaceae* and *Nocardioidaceae*, which abundances increased in fungicide-treated soil, while abundances of *Gaiellaceae*, *Xanthomonodaceae* and *Pyrinomonadaceae* decreased (Figure 1)

Five genera, including Arthrobacter, Sphingomicrobium, Sphingomonas, Bradyrhizobium, and a genus of uncultivated bacteria belonging to the Sphingomonadaceae, have been shown to constitute a major group in the soil bacterial community. All of these taxa were found to be significantly more abundant in the fungicide-treated soil compared to untreated one. (Figure 2 a).

Moderately presented genera (500–1000 16S rRNA reads) (Figure 2 b) were composed by 8 genera; they predominated in the soil treated with the fungicide. The minor taxa (< 500 16s rRNA reads) composed by 14 genera, and 5 among them have been less abundant in the fungicide-treated soil (Figure 2 c). Twelve bacterial genera have been found to be unique to the each of studied soils (Table S1), however, most of them are unclassified taxa.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 September 2022 doi:10.20944/preprints202209.0177.v1

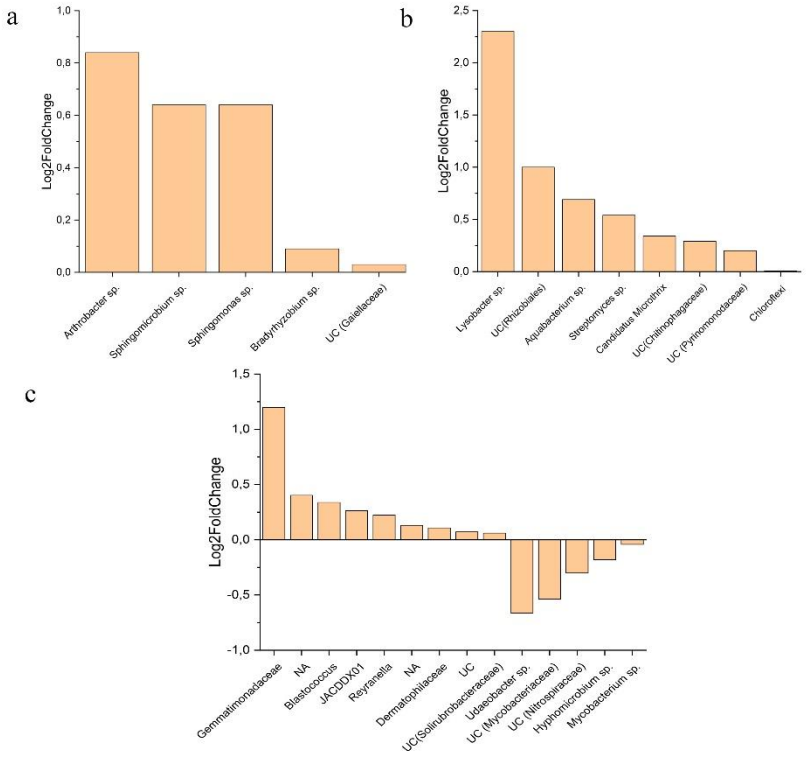

Figure 2. Changes in the number of bacterial taxa in the soil treated with fungicide compared to the control soil. The differences in the abundance of the taxa that comprise the dominant group (a), the moderately presented one (b**),** and the minor group (c).

2.2. Alpha diversity of bacterial community

It has been discovered that the Shannon index in the fungicide-treated soil turned out to be significantly lower than those of the untreated soil (p-value < 0.05) (Figure 3 a). Chao1 index has shown no statistically significant difference between the fungicide-treated and the control soils (p-value 0.8273) (Figure 3 b).

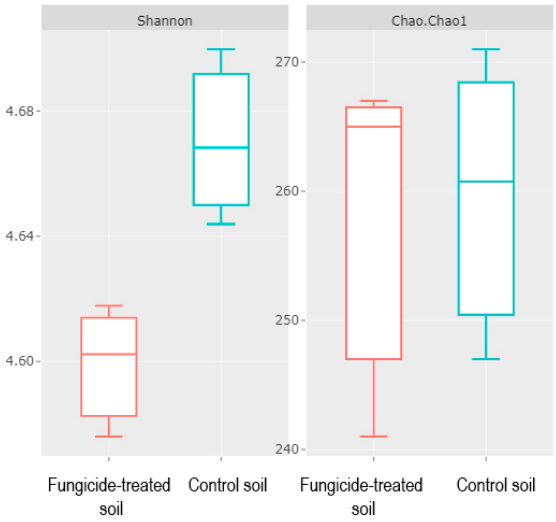

Figure 3. The Shannon and Chao1 alpha-diversity indices of bacterial communities of the fungicide-treated and the control soils

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 September 2022 doi:10.20944/preprints202209.0177.v1

2.3. Functional potential of bacterial community

The reconstruction of metabolic pathways revealed 393 MetaCyc pathways, wherein 31 of them were differentially represented between the control and the fungicides-treated soils. The bacterial community of the fungicides-treated soil has been characterized by the prevalence in relative abundance of the predicted biological pathways (Figure 4). Among them, 12 pathways are associated with biosynthesis of vitamins, carbohydrates, amino acids, while 17 pathways are involved in degradation and assimilation processes.

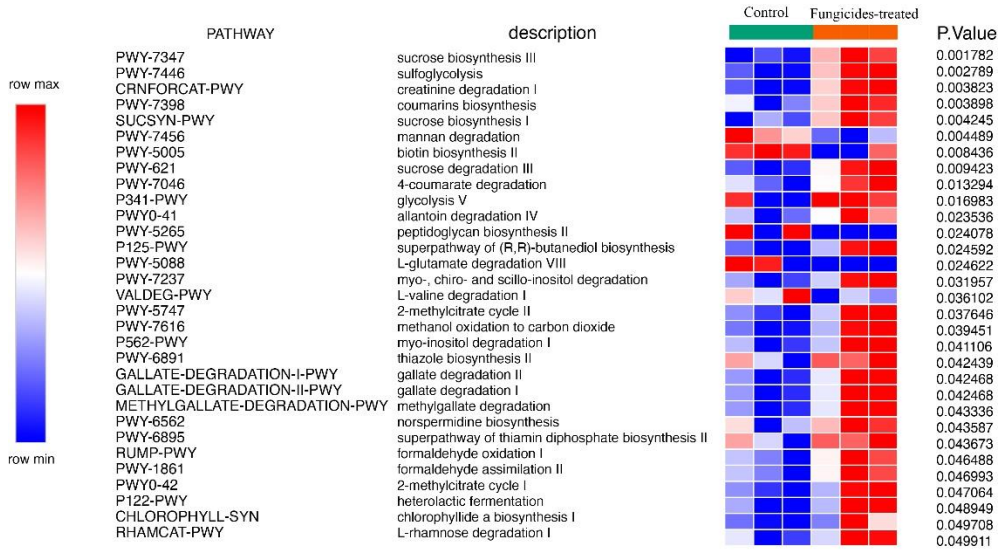

Figure 4. Pathways that were at differential relative abundances between the control and the fungicides-treated soils. Corrected p-values were calculated based on Benjamini–Hochberg FDR multiple test correction. p-value < 0.05 has been considered to be significant. The analysis was based on 16S rRNA sequencing data.

The relative abundances of 5 pathways were significantly increased in the control soil comparing to the fungicides-treated one. These pathways were mannan degradation, L-valine degradation I, L-glutamate degradation VIII, biotin biosynthesis II and peptidoglycan biosynthesis II (Figure 4).

2.4. Shift of fungal taxa under the fungicide treatment

The fungal community in the untreated soil was represented by dominant phyla Ascomycota, Mortierellomycota and Basidiomycota (Figure 5). In the fungicide-treated soil, the dominant phyla were Ascomycota, Mucoromycota, and Mortierellomycota (Figure 5).

At the family level, Rhizopodaceae, Chaetomiaceae, and Aspergillaceae predominated in the fungicide-treated soil (Figure 5), while the abundance of Mortierellaceae, Hypocreaceae, Trichocomacea were lower compared to the control soil.

Changes in the number of fungi at the genera level were estimated by Log2Fold values (Figure 6 a). It has been found that *Rhizopus sp.* was represented moderately in the untreated soil, while in the fungicides-treated soil its abundance has increased by 8 times (+4.22 Log2Fold) (Figure 6 b). In a similar way have increased the abundances of *Chaetomium*

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 September 2022 doi:10.20944/preprints202209.0177.v1

(+2.06 Log2Fold), *Penicillium* sp. (+0.64 Log2Fold), and *Setophoma* (+0.61 Log2Fold) (Figure 6 b).

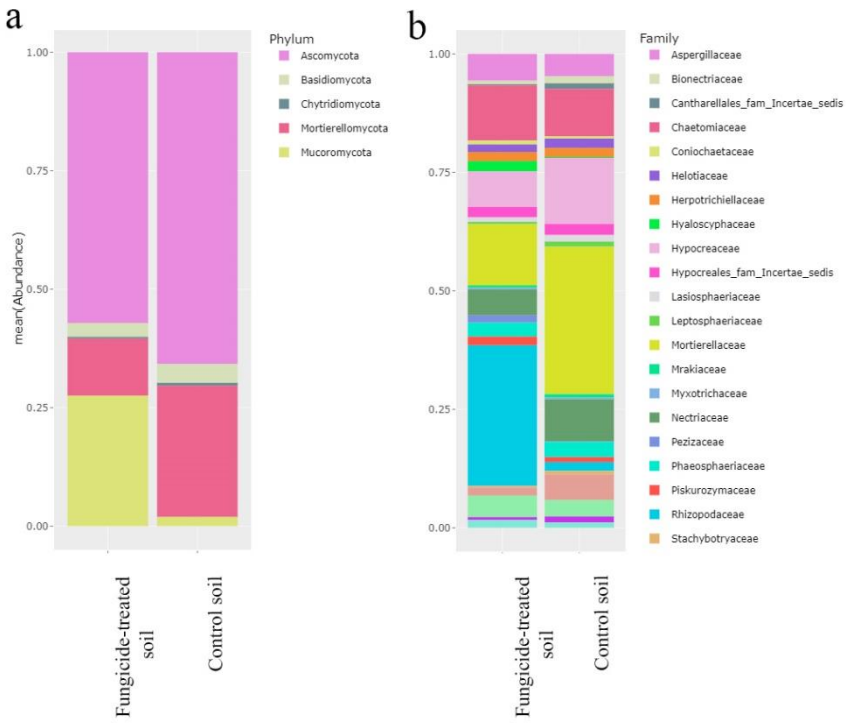

Figure 5. The relative abundance of fungal phyla (a) and families (b) in the control and the fungicides-treated soils.

The most abundant genera in the untreated soil were *Mortierella*, *Talaromyces*, *Fusarium*, *Trichoderma*, *Trichocladium*, and *Monocillium*. However, in the fungicides-treated soil the abundances of *Talaromyces* (-1.43 Log2Fold), *Mortierella* sp (-1.03 Log2Fold), *Trichocladium* (-0.72 Log2Fold), *Trichoderma* sp. (-0.65 Log2Fold), and *Monocillium* sp. (-0.62 Log2Fold) reduced by several times, compared to the intact soil (Figure 6 a). Among the dominant taxa of the control soil, phytopathogenic *Fusarium* genus (subsequently was identified as *Fusarium acuminatum)* has been found, while in the fungicide-treated soil its abundance was less for2times (-0.95 Log2Fold).

The moderately presented part of fungal soil community (500-1000 ITS reads) in the control soil was composed by 5 genera . All these genera have increased their abundance in the fungicide-treated soil (Figure 6 b). Among them, *Lachnum* was the most abundant genus (+3.79 Log2Fold), while the abundance of 4 others genera has increased insignificantly (from + 0.40 to + 0.15 Log2Fold).

The most pronounced shift has occurred with genera that are related to the minor part (<500 ITS Reads) of the fungal community. It has been found that the abundance of 4 genera increased in the fungicide treated soil, and 8 genera have decreased (Figure 6 c). The most pronounced increase in the abundance was undergoing with unidentified genus (related to *Pezizaceae*) (+ 4.7 Log2Fold), while genus *Hypoxylon* was the most reduced in their abundance (- 5.7 Log2Fold) (Figure 6 c).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 September 2022 doi:10.20944/preprints202209.0177.v1

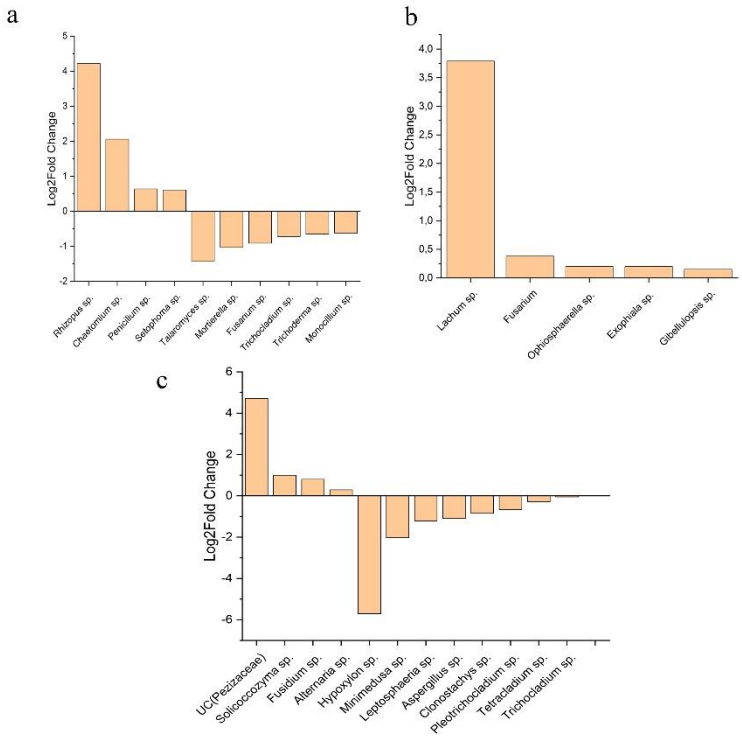

Figure 6. Changes in the number of fungal taxa in the soil treated with fungicides compared to the control soil. The differences in the abundance of the taxa that comprise the dominant group (a), the moderately presented (b**),** and the minor group (c).

Within the minor part of fungal community, the following pathogenic fungi have been found: *Septoriella hirta* (-3.9 Log2Fold), *Aspergillus crustosus* (-1.1 Log2Fold), *Alternaria tricina* (-0.15 Log2Fold), *Alternaria alternata* (0.28 Log2Fold) *Bipolaris sorokiniana* (0.08 Log2Fold).

Six fungal genera were discovered to have completely disappeared following fungicide application, while 2 fungal genera were new for the fungicide-treated soil (Table S.1).

2.5. Alpha Diversity of fungal community

The diversity of fungal community in the control Phaeozem was quite high, while the fungicide application significantly reduced the Shannon index (p-value < 0.05) (Figure 7 a), while the Chao 1 diversity indices were found not to different between the compared soils (p-value 0.8273) (Figure 7 b).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 September 2022 doi:10.20944/preprints202209.0177.v1

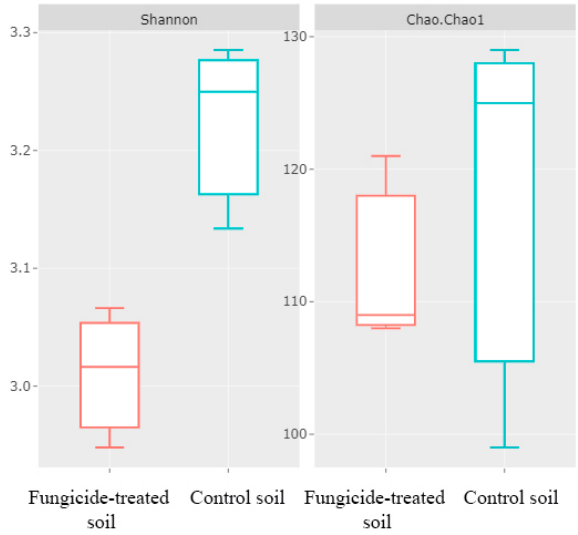

Figure 7. The Shannon and Chao1 alpha-diversity indices of fungal communities in the fungicide-treated and the control soils.

2.6. Functional potential of fungal community

Using the ITS sequencing data, 66 MetaCyc pathways were completely restored. Wherein, 51 were differently represented between groups (p-value < 0.02). Only 7 pathways which are related with ubiquinol and nucleic acids biosynthesis were discovered to be more abundant in the fungicide-treated soil (Figure 8).

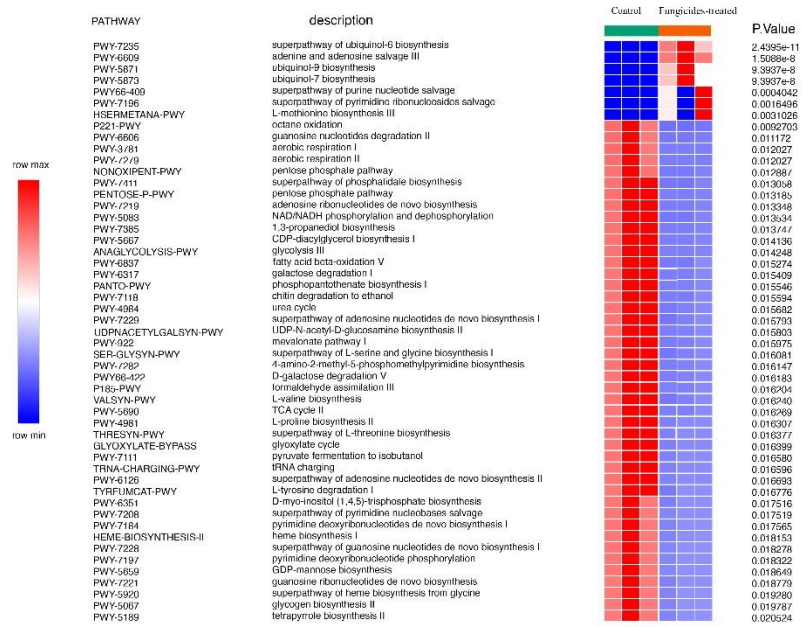

Figure 8. Pathways that were at differential relative abundances between control and fungicides-treated soils. Corrected p-values were calculated based on Benjamini–Hochberg FDR multiple test correction. p-value < 0.05 has been considered to be significant. The analysis was based on ITS sequencing data.

2.7. Effect of soil management on fungal disease manifestation

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 September 2022 doi:10.20944/preprints202209.0177.v1

Analyzing of wheat at the beginning of the growing season, we have found that up to 8.25 % of growing plants which does not protected by the combined fungicide showed symptoms of root rot. At the end of growing season, the number of affected plants increased up to 14.52 ± 0.18 % (Figure 9). At the same time, the applied scheme for fungicide treatment protected 100% of the plants in the tillering phase, and up to 96.8 ± 0.27 % at the end of growing season (Figure 9).

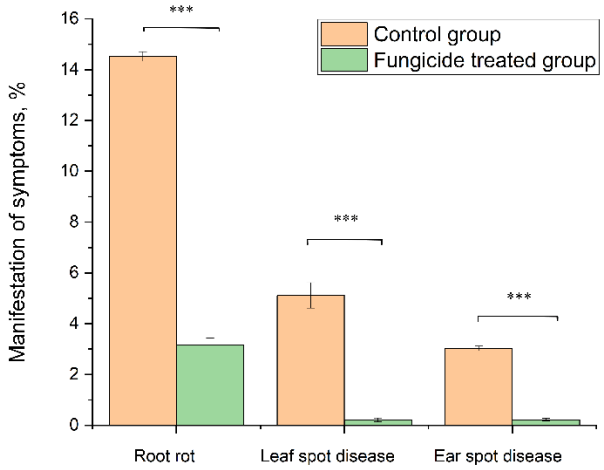

Figure 9. Manifestation of the wheat diseases in the control and the fungicide-treated groups. Data presents mean ± standard deviation (n=50). * p<0.05 (pair sample *t*-test).

The manifestation of leaf spot disease caused by *Septoria trici* was found to be insignificant. The percentages of affected leaf surface were 5.11 ± 0.50 % in the control group and 0.21 ± 0.07 % in the fungicide-treated group. The absence of fungicide in the plant-protection scheme resulted in the appearance of ear spot disease caused by *Stagonospora nodorum*. The manifestation of symptoms in the control group was 3.03 ± 0.10%, and the use of fungicide reduced the percentage of damaged wheat ears to 0.22 ± 0.05% (Figure 9).

3. Discussion

Recent studies have shown that regardless of soil type the overall effect of difenoconazole/epoxiconazole application led to a decrease in soil microbial biomass and soil enzymatic activity [1]. Apparently, the effect on microorganisms depends on soil type, since difenoconazole at concentration of 5 mg/kg did not cause significant changes in microbiological parameters of clay-loam soil [16], while in loamy-sand soil difenoconazole concentration of 0.04 mg/kg reduced microbial biomass [17]. At the same time, there are no available data concerning the effects of kresoxime-methyl on the soil microbial community.

We found that the combined fungicide affected both bacterial and fungal communities. In soil, bacteria and fungi depending on their number were clustered into the three conditional groups: dominant, moderately and minor presented taxa. There were a few

 doi:10.20944/preprints202209.0177.v1

features of microbial response to this treatment. Firstly, bacterial and fungal diversity has decreased under the influence of fungicide. Besides, bacterial and fungal communities differed in their response to the treatment. Finally, when treated with the fungicide, all dominant taxa remained similar to those in the untreated soil, but their abundances changed.

In the fungicide-treated soil, the abundance of *Arthrobacter* genus increased. This bacterial genus is known for its ability to decompose strobilurins as the source of carbon [18]. The numbers of *Sphingomonas* and *Sphingomicrobium* had also increased. The member of *Sphingomonadaceae* are known to have the ability to degrade a variety of aromatic compounds [19]. Many Sphingomonads have been isolated from environments contaminated with pesticides, herbicides, and other xenobiotics, which can be used by bacteria as a sole carbon source [20]. Recently, two strains of *Sphingomonas* spp. were isolated from wheat grain and demonstrated the ability to degrade propiconazole [21]. Thus, at least three bacterial genera found in the studied soils could be biodegrading strobilurins and triazoles. This may explain the increase in their abundance in the fungicide-treated soil.

In the fungicide-treated soil, there was a slight increase in the relative abundance of *Bradyrhizobium* which has a high potential as remediators for fungicide polluted soil [22]. For example, *Bradyrhizobium japonicum* bacteria are able to detoxify hexaconazole, colonize plant tissues and secrete PGP bioactive molecules, even under fungicide pressure [23].

The most pronounced shifts occurred with minor taxa, some of which completely disappeared in the fungicide-treated soil, while others appeared instead. However, most of these taxa belong to non-classified uncultivated genera, and their environmental role is unknown.

The fungal community was affected the most by the fungicide combination. The abundance of *Mortierella* sp. decreased twice; fungal species belonging to *Mortierella* are known to have positive effect on the crop protection, and involved in the reduction of soil contamination by chemical fertilizers and pesticides [24]. The abundance of *Talaromyces* has also been halved, these fungiare known to be effective in preventing the incidence of *Fusarium* wilt disease [25].

Rhizopus was the most abundant taxon of fungal community, and its abundance in the fungicide-treated soil has increased significantly. *Rhizopus* spp. are known as soil saprotrophs, capable to sorption of various toxicants [26]. Since the abundance of this taxon increased by more than 8 times, *Rhizopus* spp. probably play a more specific ecological role which are related with fungicide application.

Another fungal taxon which abundance was significantly increased in the fungicide-treated soil, was *Chaetomium* genus. This genus is known to be plant-beneficial. For example, several *Chaetomium* species are known as biocontrol agents of various phytopathogenic fungi, such as *Fusarium*, *Helminthosporium*, *Pythium*, *Alternaria*, and *Phytophthora* [27-30].

The infection of common root rot of wheat is facilitated by the presence of pathogenes on seeds and in a soil, and the intensity of damage to the root system can vary under the influence of weather conditions (precipitation, temperature), agrotechnical measures for soil preparation.

Phytopathogens such as *Septoriella hirta*, *Myrothecium*, *Alternaria* were found in the fungicide-treated soil in lower shares or were even absent compared to the control one.

 doi:10.20944/preprints202209.0177.v1

Septoriella hirta is considered as an economically important secondary pathogen that increases the cost of harvesting and declines the quality of the grain [31]. However, the relative abundances of all of these fungi were insignificant (less than 100 ITS reads).

On the contrary, *Fusarium acuminatum* was found to be the most abundant phytopathogen (>1000 ITS reads) which was presented in the soil without fungicide. This fungal species colonizes the lower stems (crowns) of bread and durum wheat and mostly related to crown rot [32, 33]. Moreover, *F. acuminatum* produces toxins bowericin, fusarin C, moniliformin [34]. In our study, the relative abundance of *F. acuminatum* has decreased twice when fungicide was applied, and this correlates with twice reduction in root rot incidents.

The description of functional changes in microbialcommunities under the fungicide-treatment could be based on the assessment of their biosynthetic potential. There was an increase in the abundance of bacterial metabolic pathways, which are associated mainly with the conversion of various xenobiotic. The functional change in the fungal community has shown the opposite trend. The abundance of metabolic pathways was decreased in the fungicide-treated soil.

4. Materials and Methods

4.1. Site and sampling

Soils have been sampled on the territory of the experimental field of the Research Institute of Agriculture of the Northern Trans-Urals (Coordinates: 57.094, 65.376). Geographical zone: subtaiga subzone of the Tavda province (Turin subprovince). The relief of the territory is a gently sloping plain with pine-birch and birch grassy forests. The studied plots were located on arable soil under spring wheat crops (*Tríticum durum* Desf.). The soil type under the study was Luvic Phaeozems. Before sowing wheat, the special agrotechnical process was applied, by first plowing in autumn of 2019 and then plowing in early spring of 2020, followed by 3-fold cultivation during the summer of 2020. Sowing of spring wheat was done in May 2021.

The applied fungicide preparation was “Terapevt-Pro”, (Zemlyakoff, Russia). Terapevt-Pro contains (gram per liter): difenoconazole – 80; kresoxim-methyl – 125; epoxiconazole – 125. Terapevt-Pro. The fungicide was applied once during the earing phase of wheat on 7th July 2021 at the rate of 0.7 L per ha. The control (untreated with fungicide) group of soil was located at a distance of 20 m.

Soil sampling was carried out in September 2021 after wheat harvest. Soil samples were collected using the checkerboard sampling method. Three spatially distant plots (1 m^2) were randomly laid in each studied area. Samples were taken from 0-5 cm of the upper humus soil layer at four points in the corners and one in the center of the plot. For each plot, a pooled sample was prepared by mixing incremental samples. The maximum time from the moment of sampling to their arrival at the laboratory was no more than 2 hours. The soil samples were sieved with a mesh size of 2 mm and stored at the temperature of minus 80°C. Thus, the three bags of soil were collected at each area.

The relative humidity of the soils was 13.37±1.65 %, acidity (pH) – 7.06. The texture of the studied soils was presented by clay (28 %), silt (26 %) and sand (clay loam) (46 %). Soil pH

 doi:10.20944/preprints202209.0177.v1

was measured according to the international standard ISO 10390. The pH_{KCl} value was determined potentiometrically in a 1 M KCl solution and the pH_{H2O} value was determined in an aqueous solution at a soil:solution ratio of 1:5 using a pH-meter Orion Star A 111 (Thermo Scientific, US) [35].

4.2. Phytosanitary control of spring wheat

The development of common root rot was determined twice during the growing season in the tillering phase and before harvesting the crop [36].

The development of aerogenic infections (foliar and blotch disease of wheat) was monitored from the tillering phase to milky ripeness. Plant material for analysis was taken from wheat field at three (root rot) and five (aerogenic infections) spatially distant points. The degree of damage to the leaf surface caused by *Septoria trici* (foliar disease of wheat) or damage to the wheat ear caused by *Septoria nodorum* was expressed in percentage using the universal scale [37].

4.3. Total soil DNA isolation and sequencing

Total soil DNA was isolated from 0.5 g of soil using the FastDNA™ Spin Kit for Soil DNA Extraction (MP Biomedicals, USA) according to the manufacturer's protocol. DNA was extracted from three technical replicates per sample to minimize the DNA extraction bias (9 samples of DNA were obtained in total). To assess the yield of total soil DNA , the absorbance was measured at 230, 260, and 280 nm using NanoPhotometer N120 (Implen, USA). Quality of total soil DNA was estimated using absorbance ratio as A260 nm/230 nm (DNA/humic acid) and A260 nm/280 nm (DNA/protein). DNA yield was also quantified fluorometrically with Qubit 4.0 fluorometer (Thermo Fisher Scientific, USA). Both NanoPhotometer N120 and Qubit were used according to manufacturer's protocols. The extracted DNA was stored in a freezer (-80 °C) until further analyses.

The libraries for sequencing were created using "xGen™ Amplicon Core Kit" with primers "xGen™ 16S Amplicon Panel v2" for bacteria identification and "xGen™ ITS1 Amplicon Panel" for fungal identification following the manufacturer's instructions. The amplicon sequencing was carried out using MiSeq Illumina sequencer (Ilumina Inc., San Diego, California, USA) and a set of paired ends v2 Illumina (cluster generation and paired ends sequencing with the power of 2 × 250 bp).

4.4. Data analysis and statistics

The quality of sequenced data was estimated using FastQC v 0.11.9 software [38].

The reads were filtered and trimmed using the Trimmomatic v0.36 program: reads were removed where average quality throughout the entire length was at least 31 (both for forward and reverse reads). After filtering, the data was checked using the FastQC program.

Further processing of the reads was performed using the package dada2 v4.0.3 [39] of the R language. After sequence dereplication the main function of the dada2 algorithm was used - the recovery of the amplicon sequence variant (ASV). The phylogenetic composition of bacterial communities was determined by comparing the v3-v4 16S rRNA nucleotide

 doi:10.20944/preprints202209.0177.v1

sequences with the reference rRNA sequences from the GTDB database using the IdTaxa function of the DECIPHER package [40].

The phylogenetic composition of fungal communities was determined by comparing the ITS2 nucleotide sequences with the reference sequences from the UNITE v 8.3 database 2021-05-10. When necessary, the alignment of nucleotide sequences was carried out using NCBI nucleotide database.

Alpha diversity analysis was carried out using the "estimate_richness" function of the phyloseq v3.14 package [41], Chao1 and Shannon indices were used for calculation. The nonparametric Kruskal-Wallis test was used to determine the influence of the factor on the alpha diversity, The effect was considered significant at $p < 0.05$. Visualization of the alpha diversity indices was performed using the box-and-whisker chart from the ggplot2 v3.3.5 package [42]. Using the deseq2 package R, the Benjamini-Hochberg multiple comparison was applied to identify significant differences in the relative diversity of microorganisms between the compared groups [43]. The difference was considered significant at $p < 0.05$. Dissimilarity between groups at genus level was expressed in log2FoldChange values.

4.5. Functional potential analysis

Using the 16S rRNA or ITS based ASV tables and the reference sequences generated by QIIME2, a functional potential of the bacterial community was predicted using the PICRUSt2 software (version 2.3.0) [44]. The values were converted using the logarithmic transformation Log2. Data visualization and calculation of statistical indicators were performed using the Phantasus web application (version 1.11.0).

5. Conclusions

In general, the metagenomic analysis of the microbial communities in Phaeozem showed that fungicide treatment affected alfa diversity of both fungal and bacterial communities. However, there has been no dramatic decrease in the abundances of plant-beneficial taxa; on the contrary, an increase in the number of prokaryotes capable of biodegrading fungicides was observed. Among fungal taxa, it is worth paying attention to *Rhizopus*. It's ecological role is probably related to the detoxification of fungicides.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table S1: The genera of soil bacteria and fungi that were not detected in 16S rRNA and ITS sequence libraries.

Author Contributions: Conceptualization and methodology ASV; data curation ASV; investigation DVP, VNT, ANP, AAS, AVV; writing original draft ASV; writing review and editing MVS. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Ministry of Science and Higher Education of the Russian Federation within the framework of the Federal Scientific and Technical Program for the Development of Genetic Technologies for 2019-2027 (agreement №075-15-2021-1345, unique identifier RF----193021X0012).

Data Availability Statement: Not applicable.

Acknowledgments: In this section, you can acknowledge any support given which is not covered by the author contribution or funding sections. This may include administrative and technical support, or donations in kind (e.g., materials used for experiments).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Roman DL, Voiculescu DI, Filip M, Ostafe V, Isvoran A.2021. Effects of Triazole Fungicides on Soil Microbiota and on the Activities of Enzymes Found in Soil: A Review. Agriculture 11:893. https://doi.org/10.3390/agriculture11090893.
2. Aktar MW, Sengupta D, Chowdhury A. 2009. Impact of pesticides use in agriculture: their benefits and hazards. *Interdiscip Toxicol*. 2(1):1-12. doi:10.2478/v10102-009-0001-7.
3. Singh G, Wright D. 1999. Effects of herbicides on nodulation, symbiotic nitrogen fixation, growth and yield of pea (*Pisum sativum*). The Journal of Agricultural Science 133(1):21-30.
4. Araújo ASF, Monteiro RTR, Abarkeli RB. 2003. Effect of glyphosate on the microbial activity of two Brazilian soils. Chemosphere, 52(5), 799–804. doi:10.1016/s0045-6535(03)00266-2.
5. Sarnaik SS, Kanekar PP, Raut VM, Taware SP, Chavan KS, Bhadbhade BJ. 2006. Effect of application of different pesticides to soybean on the soil microflora. Journal of Environmental biology 37(2):423-426.
6. Zubrod JP, Bundschuh M, Arts G, Brühl CA, Imfeld G, Knäbel A, Payraudeau S, Rasmussen JJ, Rohr J, Scharmüller A, Smalling K, Stehle S, Schulz R, Schäfer RB. 2019. Fungicides: An Overlooked Pesticide Class? Environ Sci Technol 2, 53(7):3347-3365. doi: 10.1021/acs.est.8b04392.
7. Buchenauer H. 1987. Mechanism of action of triazolyl fungicides and related compounds, in Modern selective Fungicides - Properties, Applications, Mechanisms of Action. LYR H (ed.) VEB Gustav Fischer Verlag, Jena, pp. 205-231.
8. Sauter H, Steglich W, Anke T. 1999. Strobilurins: evolution of a new class of active substances. Angew Chem Int Ed 38:1328–1349. doi: 10.1002/(SICI) 1521-3773(19990517)38:103.0.CO;2-1.
9. Isamu Y and Makoto F. 2005. Recent topics on action mechanisms of fungicides. J Pestic Sci 30:67–74. doi: 10.1584/jpestics.30.67.
10. Yen JH, Chang JS, Huang PJ, Wang YS. 2009. Effects of fungicides triadimefon and propiconazole on soil bacterial communities. Journal of Environmental Science and Health Part B 44(7):681–689.
11. Niewiadomska A, Sawińska Z, Maruwka AW 2011. Impact of selected seed dressings on soil microbiological activity in spring barley cultivation. Fresenius Environmental Bulletin 20:1252–1261.
12. Milenkovski S, Bååth E, Lindgren PE, Berglund O. 2010. Toxicity of fungicides to natural bacterial communities in wetland water and sediment measured using leucine incorporation and potential denitrification. Ecotoxicology 19, 2:285–294.
13. Han L, Liu Y, Fang K, Zhang X, Liu T, Wang F, Wang X. 2020. Azoxystrobin dissipation and its effect on soil microbial community structure and function in the presence of chlorothalonil, chlortetracycline and ciprofloxacin. Environ Pollut., 257:113578.
14. Hobbelen PHF, Paveley ND, van den Bosch F. 2014. The Emergence of Resistance to Fungicides. PLoS ONE 9(3): e91910. https://doi.org/10.1371/journal.pone.0091910.
15. Yang LN, He MH, Ouyang HB. *et al.* 2019. Cross-resistance of the pathogenic fungus *Alternaria alternata* to fungicides with different modes of action. BMC Microbiol **19,** 205. https://doi.org/10.1186/s12866-019-1574-8
16. Da Rocha AG, Pitombo LM, Bresolin JD, da Silva WTL, Espindola ELG, de Menezes Oliveira VB. 2020. Single and combined toxicity of the pesticides abamectin and difenoconazole on soil microbial activity and *Enchytraeus crypticus* population. SN Appl. Sci. 2:1390. https://doi.org/10.1007/s42452-020-3175-4.

17. Feng Y, Huang Y, Zhan H, Bhatt P and Chen S. 2020. An Overview of Strobilurin Fungicide Degradation:Current Status and Future Perspective. Front Microbiol 11:389. doi: 10.3389/fmicb.2020.00389.
18. Clinton B, Warden AC, Haboury S, Easton CJ, Kotsonis S, Taylor MC, et al. 2011. Bacterial degradation of strobilurin fungicides: a role for a promiscuous methyl esterase activity of the subtilisin proteases? Biocatal. Biotransfo. 29:119–129. doi: 10.3109/10242422.2011.578740.
19. Kertesz M.A, Kawasaki A, Stolz A. 2018. Aerobic Hydrocarbon-Degrading Alphaproteobacteria: Sphingomonadales. In: McGenity T. (eds) Taxonomy, Genomics and Ecophysiology of Hydrocarbon-Degrading Microbes. Handbook of Hydrocarbon and Lipid Microbiology. Springer, Cham. https://doi.org/10.1007/978-3-319-60053-6_9-1.
20. Aylward FO, McDonald BR, Adams SM, Valenzuela A, Schmidt RA, Goodwin LA, Woyke T, Currie CR, Suen G, Poulsen M. 2013. Comparison of 26 sphingomonad genomes reveals diverse environmental adaptations and biodegradative capabilities. Appl Environ Microbiol. 79(12):3724-33. doi: 10.1128/AEM.00518-13.
21. Wachowska U, Kucharska K, Pluskota W, Czaplicki S, Stuper-Szablewska K. 2020. Bacteria Associated with Winter Wheat Degrade *Fusarium* Mycotoxins and Triazole Fungicide Residues. *Agronomy* 10(11):1673. https://doi.org/10.3390/agronomy10111673.
22. Shahid M, Khan MS. 2019. Fungicide tolerant *Bradyrhizobium japonicum* mitigate toxicity and enhance greengram production under hexaconazole stress. J Environ Sci (China). 78:92-108. doi: 10.1016/j.jes.2018.07.007.
23. Allef Barbosa dos Santos, Tarcísio Marcos de Souza Gondim, Paulo Ivan Fernandes Júnior, Liziane Maria de Lima. 2021. Effect of fungicides on the symbiosis between Bradyrhizobium strains and peanut. Pesqui. Agropecu. Trop. 51, https://doi.org/10.1590/1983-40632021v5169089
24. Ozimek E, Hanaka A.2021. *Mortierella* Species as the Plant Growth-Promoting Fungi Present in the Agricultural Soils. *Agriculture.* 11(1):7. https://doi.org/10.3390/agriculture11010007.
25. Tian Y, Zhao Y, Fu X, Yu C, Gao K, Liu H. 2021. Isolation and Identification of *Talaromyces* sp. Strain Q2 and Its Biocontrol Mechanisms Involved in the Control of *Fusarium* Wilt. Front Microbiol 12:724842. doi: 10.3389/fmicb.2021.724842.
26. Tsezos M, Volesky B. 1982. The mechanism of uranium biosorption by *Rhizopus arrhizus*. Biotechnol Bioeng. 24(2):385-401. doi: 10.1002/bit.260240211.
27. Dhingra OD, Mizubuti ESG, Santana FM. 2003. *Chaetomium globosum* for reducing primary inoculum of *Diaporthe phaseolorum* f. sp. meridionalis in soil-surface soybean stubble in field conditions. Biological Control 26(3):302-310. 1049-9644, https://doi.org/10.1016/S1049-9644(02)00167-6.
28. Aggarwall R, Tewari AK, Srivastava KD, Singh DV. 2004. Role of antibiosis in the biological control of spot blotch (*Cochliobolus sativus*) of wheat by *Chaetomium globosum*. Mycopathologia. 157(4):369-77. doi: 10.1023/b:myco.0000030446.86370.14.
29. Tomilova OG, Shternshis MV. 2006. The effect of a preparation from *Chaetomium* fungi on the growth of phytopathogenic fungi. Appl Biochem Microbiol **42:**67–71. https://doi.org/10.1134/S0003683806010108.
30. Phong NH, Pongnak W, Kasem S. 2016. Antifungal activities of Chaetomium spp. against Fusarium wilt of tea. Plant Protection Science 52(1):10-17. 10.17221/34/2015-PPS.
31. Crous PW, Carris LM, Giraldo A, Groenewald JZ, Hawksworth DL, Hernández-Restrepo M, Jaklitsch WM, Lebrun MH, Schumacher RK, Stielow JB, van der Linde EJ, Vilcāne J, Voglmayr H, Wood AR. 2015. The Genera of Fungi - fixing the application of the type species of generic names - G 2: Allantophomopsis, Latorua, Macrodiplodiopsis, Macrohilum, Milospium, Protostegia, Pyricularia, Robillarda, Rotula, Septoriella, Torula, and Wojnowicia. IMA Fungus 6(1):163-98. doi: 10.5598/imafungus.2015.06.01.11.

32. Shikur Gebremariam E, Sharma-Poudyal D, Paulitz TC, Erginbas-Orakci G, Karakaya A, Dababat AA. 2018. Identity and pathogenicity of Fusarium species associated with crown rot on wheat (Triticum spp.) in Turkey. Eur J Plant Pathol 150:387–399.
33. Chakroun Y, Oueslati S, Pinson-Gadais L, Abderrabba M, Savoie J-M. 2022. Characterization of Fusarium acuminatum: A Potential Enniatins Producer in Tunisian Wheat. J. Fungi 8:458. https://doi.org/10.3390/jof8050458.
34. Munkvold GP, Proctor RH, Moretti A. 2021. Mycotoxin production in *Fusarium* according to contemporary species concepts. Annu Revi Phytopathol 59:373–402.
35. Pansu M, Gautheyrou J. 2007. Handbook of soil analysis: mineralogical, organic and inorganic methods. Springer Science & Business Media: Springer Berlin, Heidelberg https://doi.org/10.1007/978-3-540-31211-6.
36. Burlakova SV, Vlasenko NG, Chkanikov ND, Khalikov SS. 2020 Influence of Multicomponent Protectors on Seeding Phytopathogens and Spring Wheat Phytocenosis. Agrochemistry, 5:72–79 [in Russian]. DOI: 10.31857/S000218812005004X.
37. Sanin S.S. 2016. Phytosanitary examination of the grain field and making decisions on spraying wheat with fungicides. Theory and practical recommendations. Supplement to the journal Plant Protection and Quarantine. 41 p [In Russian].
38. Andrews, S. FastQC: A quality control tool for high throughput sequence data. Available online: http://www.bioinformatics.babraham.ac.uk/projects/fastqc (accessed on 1 August 2019)
39. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods, 13:581-583. doi: 10.1038/nmeth.
40. *Chaumeil PA, Mussig AJ, Hugenholtz P, Parks DH. 2019*. GTDB-Tk: a toolkit to classify genomes with the Genome Taxonomy Database. *Bioinformatics.* doi:10.1093/bioinformatics/btz848.
41. McMurdie PJ, Holmes S. 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE 8: e61217.
42. Wickham H. 2009. ggplot2: elegant graphics for data analysis. Springer New York.
43. Love MI, Huber W, Anders S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology 15:550. doi: 10.1186/s13059-014-0550-8.
44. Douglas GM, Maffei VJ, Zaneveld JR. *et al.* 2020. PICRUSt2 for prediction of metagenome functions. Nat Biotechnol **38:**685–688 https://doi.org/10.1038/s41587-020-0548-6.