Pilidium lythri disrupts plant microbiome assembly under climate warming: Evidence for niche-specific community reassembly

Dominika Siegieda[1]
Jacek Panek[1]
S. Emilia Hannula[2]
Magdalena Frąc[1]

[1]Institute of Agrophysics, Polish Academy of Sciences, Doświadczalna 4 street, 20-290 Lublin, Poland

[2]Leiden University, Institute of Environmental Sciences, Department of Environmental Biology, Leiden, The Netherlands

Keywords

Pilidium lythri, emerging fungal phytopathogen, microbial community dynamics, plant holobiont, temperature adaptability, biotic stress in plants, microbial assembly mechanisms

Abstract

Although ongoing global warming is an important driver of phytopathogen spread to new areas, little is known on how fungal pathogens disrupt fragile balance in plant-microbe interactions. Here we demonstrate that thermotolerant *Pilidium lythri* destabilizes microbial communities assembly in soil and plant niches. For our exploratory investigation, we employed high-throughput marker sequencing of bacterial/archeal 16S and fungal ITS markers from 72 samples, combined with mixed-effects modelling, source-tracking, microbiota assembly mechanisms investigation and network analysis. Our analysis revealed that *P. lythri* infection disrupted microbial community assembly processes, transitioning from deterministic to stochastic dynamics in the rhizosphere and bulk soil, aligning with the Anna Karenina Principle. Though bacterial and fungal microbiome diversity did not change under infection, microbial migration patterns highlighted shifts in community interactions. Network analysis underscored *P. lythri*'s role in destabilizing fungal community connectivity, enhancing antagonistic interactions between taxa. This study provides valuable insights into how spreading phytopathogens modulate microbial equilibrium in the plant holobiont, with implications of managing emerging threats in agriculture.

Introduction

Fungal and bacterial microorganisms, together with their host plants, are interconnected through intricate, multi-level interactions that shape ecosystems and sustain plant health and productivity. Based on these relationships, the plant holobiont concept was formed - an integrated system where hosts and microbiota function as one. Plants produce root exudates to modulate microbial assembly of theirs niches and vicinity, while the microbiome, in turn, is able to influence and regulate plant health, improving its resilience against abiotic and biotic stressors (Berg, 2009; Baldrian, 2016; Trivedi *et al.*, 2020; Gupta *et al.*, 2021; Marco *et al.*, 2022; Michl *et al.*, 2023). These finely tuned interactions are widely studied and hold great potential for improving agricultural productivity in a sustainable manner (Vishwakarma *et al.*, 2020; Trivedi *et al.*, 2020; Frąc *et al.*, 2022).

However, this delicate balance between microbiomes and plants is being increasingly threatened by ongoing global warming. The rise of annual global temperature and changed precipitation patterns has created the opportunity for a spread and establishment of invasive phytopathogens into new regions (Bebber *et al.*, 2013; Helfer, 2014; Liu *et al.*, 2019). Despite this urgent challenges, limited attention has been given to understand how these emerging pathogens alter and interact with microbial communities. This issue is particularly concerning for perennial crops like strawberries (*Fragaria x ananassa* Duch.), which depend heavily on stable rhizosphere and soil microbial communities to thrive and are particularly vulnerable to such disruptions.

Pilidium lythri, emerging fungal phytopathogen, causing damage to strawberry plantations, was originally reported in countries located in warmer climate zones, such as India (Phatak & Payak, 1965), Iran (Arzanlou *et al.*, 2013; Sayami *et al.*, 2013) and China (Zhang *et al.*, 2008; Duan *et al.*, 2010). Notably, its occurrence had been recently identified in several temperate climate countries, including Belgium (Debode *et al.*, 2011), Croatia (Milicević *et al.*, 2014), Spain (Aguín *et al.*, 2018), France (Cardin *et al.*, 2009; Caesar *et al.*, 2012) and Poland, although in 2004 only morphologically, but in 2020 we confirmed its presence in symptomatic Polish strawberry fields with molecular methods (Gołębniak & Jarosz, 2004; Malarczyk *et al.*, 2020), and with new isolates collected in present study. Primary targets of this fungal phytopathogen are strawberry plants and fruits (Lopes *et al.*, 2010; Geng *et al.*, 2012; Ayoubi *et al.*, 2016), but it also affects other agriculturally significant plants, including *Olea europaea* L. (Arzanlou *et al.*, 2013), *Cornus mas* (Torbati *et al.*, 2019), *Prunus domestica* (Sayami *et al.*, 2013), *Eucalyptus* spp. (Maússe-Sitoe *et al.*, 2016), *Vitis vinifera* (Aguín *et al.*, 2018), and various ornamental flowers (Zhang *et al.*, 2008; Cardin *et al.*, 2009; Caesar *et al.*, 2012; Bruckart *et al.*, 2013; Garfinkel & Chastagner, 2019). Notably concerning is that *P. lythri* is not host-specific. Strains isolated from olives were able to cause disease symptoms on strawberry fruits (Karimi *et al.*, 2016), further increasing the

severity of the threat posed by this pathogen as it spreads to new regions. Studies conducted in Brazil, Iran and Belgium showed incidence as high as 70% in high humidity and warm temperatures, highlighting its threat in warming climate (Lopes *et al.*, 2010; Ayoubi *et al.*, 2016; Karimi *et al.*, 2016). Pathogenicity test revealed rapid progression of the disease, with symptoms appearing on up to 100% of fruits within days after inoculation (Debode *et al.*, 2011; Fernández-Ortuño *et al.*, 2014). The pathogen is able to infect both wounded and intact grape berries, further underscoring its high virulence (Aguín *et al.*, 2018). The pathogen's ability to adapt and spread under changing environmental conditions makes it an important microorganism to explore relationships between emerging fungal pathogens, and host plants microbiomes. Furthermore, its prompt proliferation and versatile host range makes it an ideal research object to evaluate how emerging pathogens disrupt microbial balance across diverse plant microbiomes.

To explore climate adaptation and microbial interaction in emerging fungal pathogens, we isolated *Pilidium lythri* from symptomatic strawberry plants in a Polish nursery. We compared the metabolic profiles of 3 *P. lythri* strains grown in 24°C and 30°C with BIOLOG PM21-25 plates, identifying its sensitivity to chemical substances in response to different temperatures to imitate climate scenarios. We also investigated the impact of pathogen's infection on the composition and assembly mechanisms of the bacterial and fungal microbiomes of soil (bulk soil, rhizosphere) and strawberry (roots) niches. Current work significantly advances our understanding the microbial dynamics associated with this spreading phytopathogen, offering valuable insights into broader ecological implications of emerging fungal pathogens.

Methods

Fungal isolates and molecular identification

Pure fungal isolates were obtained by placing symptomatic strawberry leaves and fruits (surface-sterilized with detergent and 70% ethanol) on PDA medium and incubating them at 24°C in darkness for 7 days. After initial screening, fungi with macroscopic and microscopic characteristics matching the description of *Pilidium lythri* (Palm, 1991; Karimi *et al.*, 2019) were identified through amplification and Sanger sequencing of the Internal Transcribed Spacer (ITS) regions of fungal rDNA, with ITS1 and ITS4 primers (White *et al.*, 1990), as previously described (Frąc *et al.*, 2015). The obtained sequences were deposited in GenBank under accession numbers: PQ632564.1, PQ632565.1, PQ632566.1, PQ632567.1. These sequences were then used to construct phylogenetic tree with other sequences available in Genbank database from the *Pilidium* genus. Initial tree for the heuristic search was obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood

95 value. The tree was drawn to scale, with branch lengths measured in the number of substitutions per
96 site. This analysis involved 57 nucleotide sequences and there were a total of 372 positions in the final
97 dataset. Evolutionary analyses were conducted in MEGA X (Kumar *et al.*, 2018). To fulfil Koch's
98 postulates, a spore suspension of 1.6 x 10^4 spores/mL was prepared, and surface-sterilized strawberry
99 leaves were briefly submerged in the solution. The leaves were then sealed in ziplock bags and
100 incubated at 24°C in darkness for 7 days. Sterile deionized water was used as the untreated control.

101 Metabolic profile arrays

102 Each cell of Phenotype Microarray 21-25 BIOLOG plates (Biolog, Hayward, CA, USA) was inoculated
103 with the fungal mycelium as recommended by the manufacturer and described before (Panek *et al.*,
104 2016). For each plate type, 3 *Pilidium lythri* strains in triplicates and, two temperatures (24 and 30°C)
105 and 4 concentrations were incubated for 96 hours in the darkness. Microplate reader (Biolog, Hayward,
106 CA, USA) was used to determine the optical density at 490 nm (to establish catabolic activity of fungal
107 strains) at the beginning of the experiment and every 24 hours. Obtained data was analysed
108 statistically and visualised in RStudio, using tidyverse (Wickham *et al.*, 2019) and lme4 (Bates *et al.*,
109 2015) packages. First, values obtained at 0 h for each read was divided from read values at 96 h to
110 establish growth of respiration rates, and the data was rescaled from 0 to 1. To establish which factors
111 had an influence on fungal activity, we used generalized linear mixed-effects model (GLLM) with
112 Gamma family, controlling for the plate and plate, and to establish that inhibit its respiration rates, we
113 controlled for plate, strain, temperature and concentration.

114 Pot experiment and isolation of microbial DNA

115 Strawberry plants, obtained from the nursery, were grown in pots containing sieved soil from a
116 certified organic strawberry plantation. After 20 days of initial growth of strawberry plants at 28°C and
117 70% humidity, half of the pots were inoculated with *P. lythri* spores using 10 mL of a $2 \cdot 10^4$ spores mL^{-1}
118 solution per plant. Sterile, deionized water was used as a negative control. Samples were collected
119 from two pots in triplicates (6 samples per niche per time point) for healthy and pathogen-inoculated
120 samples in two timepoints: at the time of inoculation and again after 9 days of incubation. Bulk soil
121 was transferred into sterile 1.5 mL Eppendorf tubes and frozen at -20°C. The rhizosphere was collected
122 as described by Lundberg et al. (Lundberg *et al.*, 2012). Roots, flushed free of rhizosphere soil, were
123 also frozen at -20°C prior to DNA isolation which was conducted with the QIAQUBE instrument (Qiagen,
124 Hilden, Germany) and GeneMATRIX Soil DNA Purification Kit (EURx, Gdańsk, Poland) after
125 homogenization of the samples in the FastPrep-24 instrument (MP Biomedicals) at 4 m s^{-1} for 15 s.
126 DNA isolates were then frozen in -20°C until library construction and sequencing.

127 ## Metataxonomic sequencing, bioinformatics and statistical analysis

128 For the metataxonomic sequencing, microbial DNA ITS2 (Schoch *et al.*, 2012) and 16Sv3 regions
129 (Klindworth *et al.*, 2013) were amplified, indexed and sequenced on Illumina MiSeq instrument
130 (2x300). Raw data was deposited at SRA database under accession numbers: PRJNA1213298 for 16S
131 and PRJNA1213307 for ITS. Raw data was then passed through the custom QIIME2 (Bolyen *et al.*, 2019)
132 pipelines as reported before (Siegieda *et al.*, 2023, 2024). In brief, after primer trimming with Cutadapt
133 (Martin, 2011) and ITSxpress (Rivers *et al.*, 2018), denoising, chimera removal, merging and Amplicon
134 Sequence Variant (ASV) calling was made. The taxonomy was assigned based on UNITE v9 dynamic
135 release (Nilsson *et al.*, 2019) and Silva 138 (Pruesse *et al.*, 2007) databases. The results were then
136 analysed with R (v. 4.0.3), using libraries: phyloseq (McMurdie & Holmes, 2013), tidyverse (Wickham
137 *et al.*, 2019), microeco (Liu *et al.*, 2021), microbiomeMarker (Cao *et al.*, 2022), NetCoMi (Peschel *et al.*,
138 2021). First, for ITS data, only ASVs identified to Fungi and Stramenopiles; and for 16S - Bacteria and
139 Archaea. Then, ITS data was repeatedly rarefied at 6,000 and 16S at 11,000 reads using mirlyn package
140 (McMurdie & Holmes, 2014; Cameron *et al.*, 2021). We employed Effective Number of Species (ENS)
141 (Jost, 2006) on ASV level to evaluate the richness of microbiomes in each niche. We then used Linear
142 Mixed-Effect Models with controlling for the plant to compare differences between control and
143 infected samples. We used supervised machine learning model to evaluate the fungal biomarkers of
144 each niche for healthy and infected samples with Supervised Machine Method (SVM), using Total Sum
145 Scaling normalization method. SourceTracker was used for tracking migration between niches (Knights
146 *et al.*, 2011). RCbray (Bray-Curtis dissimilarity based Raup-Crick metric) and βNTI (β Nearest Taxon)
147 were used to evaluate the ecological processes that dominated the bacterial assemblies (Stegen *et al.*,
148 2013; Liu *et al.*, 2017). When βNTI fell between -2 and 2, microbial assembly was dominated by
149 stochastic mechanisms, and below -2 and higher that 2 - by deterministic. Microbial networks were
150 constructed with 200 most frequent taxa and SPIEC-EASI (Sparse Inverse Covariance Estimation for
151 Ecological Association Inference) method (Kurtz *et al.*, 2015), using glasso (Graphical Least Absolute
152 Shrinkage and Selection Operator) approach. The dataset was resampled 30 times and 20 penalty
153 levels with 1e-4 as the smallest ratio, taking the average of symmetric edges. Fast greedy clustering
154 method was used for network analysis.

155 # Results

156 ## Fungal isolates

157 Fungal ITS sequences, obtained from symptomatic strawberry leaves (G320/23, G321/23, G322/23 and
158 G323/23) showed 100% similarity to those of *Pilidium lythri* already present in the GenBank database
159 and phylogenetic tree revealed clustering of the sequences with other representatives of *Pilidium lythri*

and *Pilidium concavum*. The strains collected after 7 days in 23°C in the darkness on Potato Dextrose Agar were flat and granulose, cinnamon in colour (Karimi *et al.*, 2016; Marin-Felix *et al.*, 2017). The presence of hyaline conidia with smooth walls and slightly pointed tips were confirmed under the microscope. After the inoculation of the surface-sterilized strawberry leaves with the spore suspension and incubation in 24°C in darkness for 7 days, 100% of the leaves showed brown necrotic lesions. Leaves submerged in water did not show symptoms of the disease. Growth of the cinnamon-colour fungal strains were observed after incubation (24°C for 7 days) of infested leaves on the Petri dishes with the PDA medium, fulfilling he Koch's postulate (Figure 1).

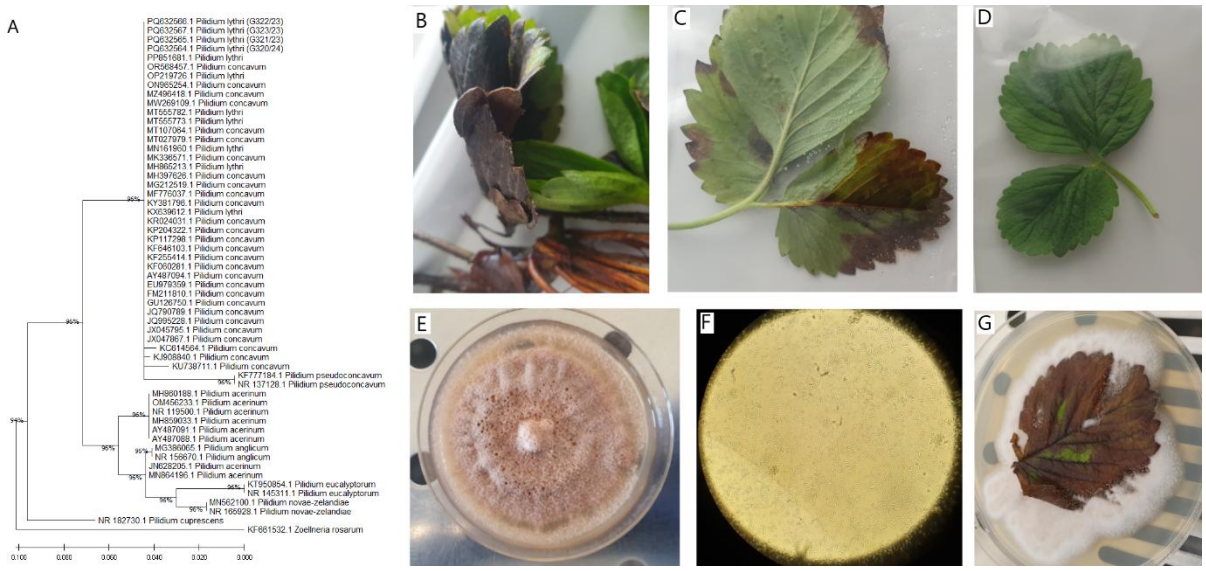

Figure 1. A) Phylogenetic tree of the evolutionary history of *P. lythri* was inferred by using the Maximum Likelihood method and Tamura-Nei model (Tamura & Nei, 1993) and the e tree with the highest log likelihood (-1144.51) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. B,C) Symptoms of the *Pilidium lythri* infection on the strawberry leaves and in the D) control leaf. E) Growth of *P. lythri* after 7 days of growth in 24 °C on PDA medium, F) conidia of *P. lythri* (40x), and G) growth on PDA medium with infected leaf.

Chemical sensitivity

To evaluate *Pilidium lythri*'s sensitivity to various chemicals, BIOLOG PM 21-25 plates were we incubated with the spore and mycelium solution of three fungal strains at 24°C and 30°C for 96 hours (in triplicates, thus 9 replications for each substrate in both temperatures). The plates are coated with 4 different concentrations of the chemical substances. The precise concentrations of the substances used for microarrays are confidential and protected as proprietary information by the manufacturer, thus, for the purposes of this article, we refer to them as concentration levels 1, 2, 3, and 4 to maintain clarity. An evaluation of the model revealed that, as expected, the temperature growth was positively correlated with fungal respiration growth, also substance concentration growth had negative correlation with the fungal respiration. We observed no effect of replicate; and different response of

one of strains - G322/23 was characterised with higher respiration rates compared to other 2 (Supplementary Figure 1). We also evaluated substances that negatively impacted respiration rate growth (Figure 2). Top inhibitors included thiourea, dodecyltrimethyl ammonium bromide, tetrazolium violet, alexidine and fluorodeoxyuridine.

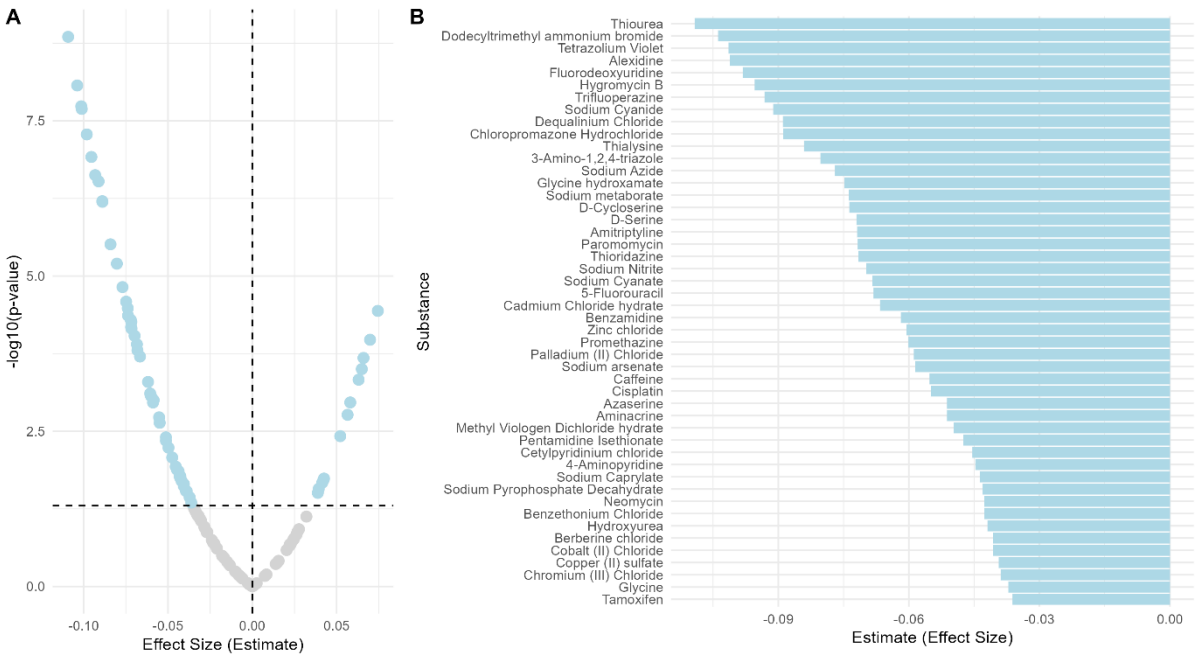

Figure 2. BIOLOG PM21-25 results of the substances that differently influence growth of *Pilidium lythri.* A) Volcano plot visualizing biological and statistical effect for different substances. Gray circles represent no significant importance, light blue - significant results. Dashed vertical lines represent p.value of 0.05 and estimate of -log10(0.05). B) Bar plot representing estimate for each substance significantly inhibiting pathogens respiration rate in tested conditions.

Fungal and bacterial community changes after *P. lythri* inoculation of strawberry

A total of 72 samples from soil (24), rhizosphere (24), and roots (24) were amplified and sequenced. Rarefaction curves plateaued for all samples, indicating sufficient sequencing depth. To evaluate the presence of *Pilidium lythri* in infected samples, we employed a predictive approach to identify 15 most important bacterial and fungal taxa that differentiate healthy and infected strawberry compartments. The analysis revealed that the *Pilidium* fungi belonged to the most important taxa that differentiate between healthy and infected plants and soil (Figure 3). This, along with the visible symptoms of the disease, confirmed that the infection was successful and the pathogen migrated from the soil to rhizosphere and roots.

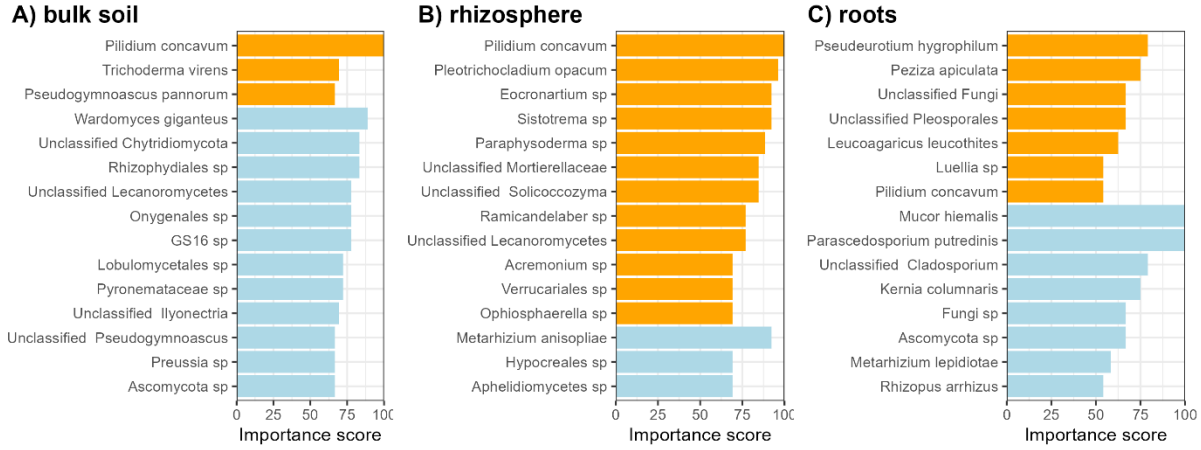

Figure 3. Top 15 fungal taxa found more abundant in (orange) infected and in (blue) healthy soils and plants identified based on Supervised Vector Machine machine learning approach.

After evaluating that infection was successful, statistical analysis was conducted to compare the alpha diversity of bacterial and fungal communities between healthy and inoculated (infected) niches. No significant differences in richness were observed between healthy and infected niches at both sampling times ($p > 0.05$) for bacteria and fungi (Figure 4). Notably, bacterial communities showed a significant increase richness across all three compartments at the second sampling time, whereas fungal community richness remained stable across time points. Additionally, both bacterial and fungal communities exhibited a gradient of decreasing diversity from soil to rhizosphere and root at second sampling.

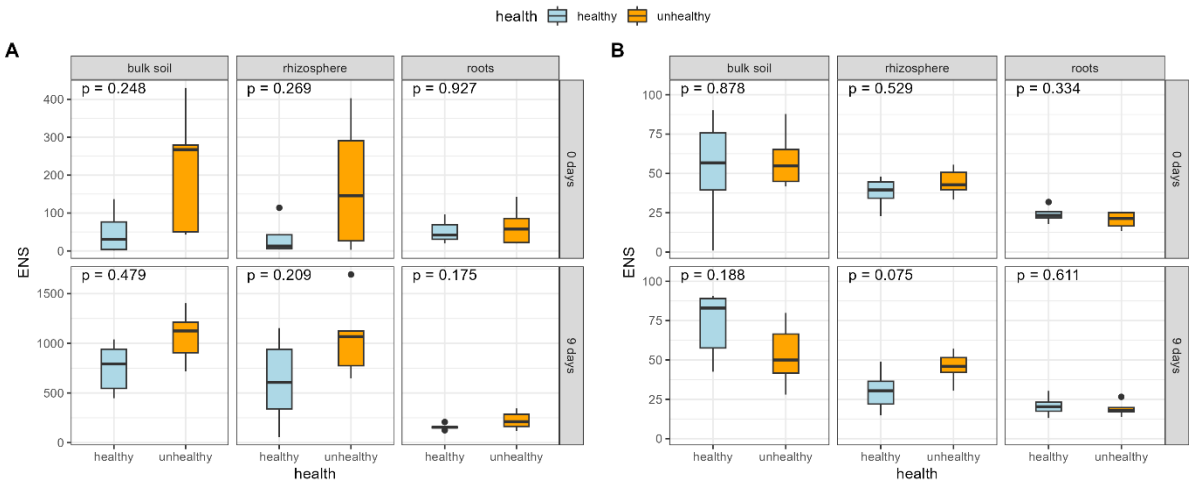

Figure 4. Comparison of alpha diversity (in Effective Number of Species) of bacterial (A) and fungal (B) communities of strawberry niches immediately after *P. lythri* inoculation (0 days) and after 9 days of incubation in 25°C and ~70% of humidity (two). LMER model was used for comparison of non-independent samples. Light blue color represents samples without pathogen inoculation, and orange - with inoculation.

To elucidate the influence of *Pilidium lythri* on microbial migration between the niches, we used samples from sampling at 0 days as a source, and samples from sampling at 9 days as sinks. In bulk soil, only bacterial community revealed significantly higher share of microorganisms in bulk soil and a lower portion from unknown source in infected samples (Figure 5A). Furthermore, we observed migration of bacteria from bulk soil to rhizosphere for both bacteria and fungi in infected communities, but also from rhizosphere to rhizosphere for bacteria in infected communities. Finally, analysis revealed increased migration from rhizosphere to roots in infected samples in bacterial community.

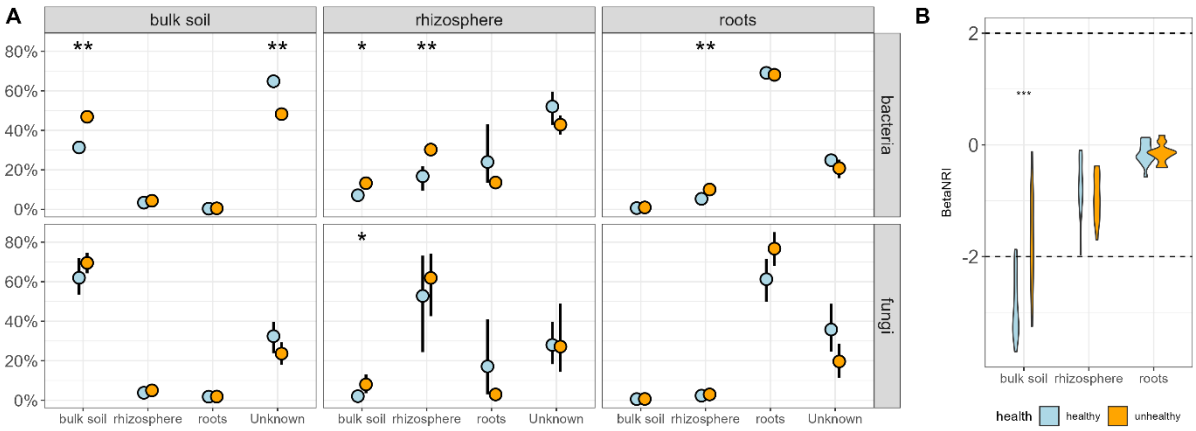

Figure 5. Source tracking analysis (A) of bacterial and fungal microbiomes between sampling one (source) and two (sink). Comparison between healthy (light blue, left) and contaminated (light orange, right) samples. Values are shown as mean standard error from bootstrap. (B) BetaNRI idenx analysis of bacterial community in second sampling. Significant differences on both panels between healthy and contaminated samples are shown with asterisk.

We also evaluated the mechanisms involved in bacterial community assembly by calculating the betaNRI index for bulk soil, rhizosphere, and root samples collected during the second sampling period (Figure 4B). The results revealed a dominance of stochastic processes in both healthy and infected rhizosphere and root samples of strawberry (betaNRI values between -2 and 2). Furthermore, we observed differences in community assembly processes between healthy and infected bulk soil, with a shift from deterministic processes (characterized by lower turnover) in healthy bulk soil to a dominance of stochastic processes in infected soil.

Finally, to evaluate the influence of *Pilidium lythri* on the structure of fungal networks, we compared the network structure in the rhizosphere niche between control and infected samples during the second sampling (Figure 6A). Both networks showed similar characteristics in terms of the number of components (1), clustering coefficient (0.995, 0.999), modularity (-0.007, -0.008), edge density (0.994, 0.999) and natural connectivity (0.375, 0.310), average dissimilarity and average path length (0.6, 0.6). On the other hand, the control rhizosphere exhibited a higher percentage of positive edges (52.308% vs. 43.344%) but lower vertex and edge connectivity (58, 71).

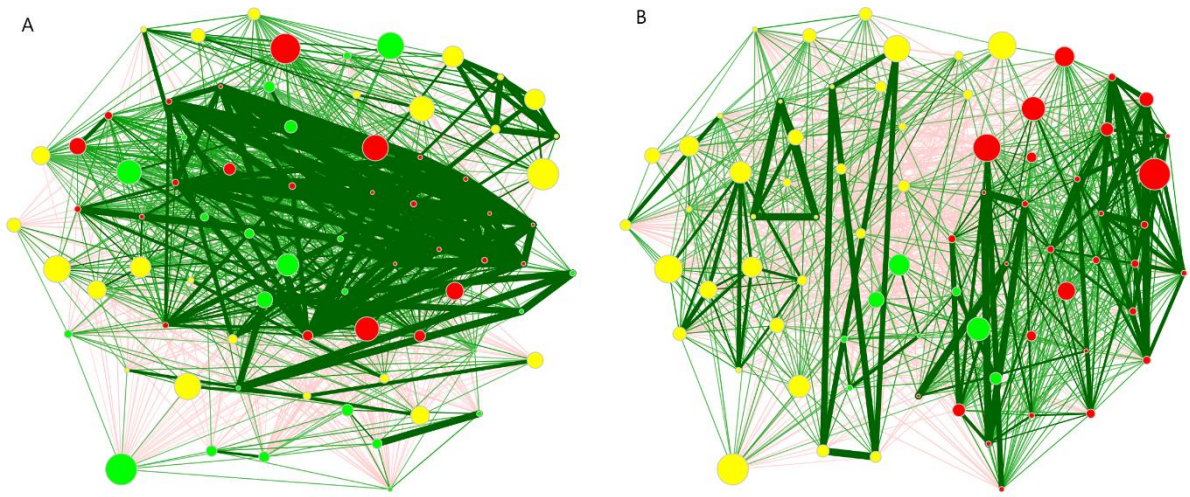

Figure 6. Fungal network analysis in A) control and B) infected rhizosphere samples of strawberry. Each node represents individual fungal ASV, and node size is proportional to number of reads. Node colours represent the same clusters. Green lines represent positive interactions and red- negative. The position of the same nodes is the same in both networks.

Notably, changes were observed in centrality of networks (Jaccard Index). Degree, betweenness and hub taxa measures showed no overlap in most connected, keystone and key hub taxa, significant shift was observed in closeness of most central nodes (Table 1).

Table 1. Centrality changes between networks of rhizosphere samples in control and *Pilidium* contaminated samples. Asterisk shows statistically significant change.

	Degree	Betweenness	Closeness	Eigenvector	Hub taxa
Jaccard Index	0	0	0.071	0.209	0
p-value (P≤Jacc)	1	0.296	0.027*	0.055	1

Discussion

To close the knowledge gap regarding emerging fungal pathogens and theirs influence on microbiomes of plants we used newly isolated strains of *Pilidium lythri*. We identified 3 of them, that were obtained from symptomatic strawberry plants in Polish nursery, by sequencing of the ITS region. We then evaluated the impact of temperature growth on inhibition of fungal development with 112 different chemical substances in 4 concentrations. We then evaluated the impact of this phytopathogen on the structure, assembly processes and association networks of bacterial and fungal metacommunities in soil and plant niches (bulk soil, rhizosphere, roots) of strawberry.

In vitro evaluation of the impact of various chemical substances on the growth and development of the tested phytopathogen at two temperatures (24 and 30 °C) using BIOLOG microarrays raised serious concerns, as *Pilidium lythri* demonstrated overall higher respiration rates at higher temperatures. Global warming has indeed been widely reported to contribute to the spread of fungal phytopathogens

266 to new areas (Ward *et al.*, 2007; Bebber *et al.*, 2013; Chaloner *et al.*, 2021). Among the wide variety of
267 chemical substances tested, some of them presented inhibition mechanism on pathogens respiration
268 rates. Top 5 analysed substances likely disrupted electron chain, destabilized cell membranes, and
269 inhibited DNA/RNA synthesis (Waldorf & Polak, 1983; Mamouei *et al.*, 2018; Ansari *et al.*, 2024; Yang
270 *et al.*, 2025).
271 While the dissemination of fungal pathogens is widely reported (Bebber *et al.*, 2013), still little is known
272 about the effect of such invasive microorganisms on the microbial structure of the plant holobiont.
273 Recent studies have reported significant differences in the α-diversity of microbiome structure
274 between healthy and infected soil and plant niches (Lefcheck *et al.*, 2015; Delgado-Baquerizo *et al.*,
275 2016; Bakker *et al.*, 2018). In the present study, we compared the diversity of bacterial and fungal
276 metacommunities in healthy and *Pilidium lythri*-infected bulk soil, rhizosphere, and roots at two time
277 points: directly after pathogen inoculation and 9 days post-inoculation. The results revealed an
278 evidence of increased α-diversity in the bacterial community 9 days after inoculation in both healthy
279 and inoculated niches, suggesting that bacterial community diversity are less influenced by *Pilidium*
280 *lythri* inoculation and more are responsive to environmental factors. To test this, we analyzed the
281 mechanisms involved in bacterial microbiome assembly by calculating the βNRI index, treating the first
282 sampling as the source of microorganisms and the second sampling as the sink. This index measures
283 how the differences in species composition between the two samplings differ from what would be
284 expected by the null model of random assembly. The results indicated that composition of bacterial
285 communities in healthy and diseased rhizosphere and roots samples was shaped by stochastic
286 processes (i.e. random colonization, dispersal, and ecological drift). Interestingly, we observed a shift
287 from deterministic processes in healthy bulk soil, to stochastic processes in *Pilidium lythri*-infected
288 samples, indicating a disruption in microbial turnover. Infected soil showed random processes that
289 could have been triggered by disturbances in plant biology caused by the infection, described by the
290 Anna Karenina Principle (AKP) (Arnault *et al.*, 2023). The scenario described here can be explained by
291 the loss of plant control over microbiome assembly, resulting in a temporal AKP effect (Cheng *et al.*,
292 2013, 2019). Over time, this could lead to the recovery of the plant's control over microbial assembly
293 or the introduction of new deterministic processes to control bacterial assembly (Gao *et al.*, 2021).
294 Analysis of microbial migration suggests increased migration of bacteria from the soil in diseased
295 samples, as well as increased migration of bacteria from the rhizosphere to the roots in infected plant
296 samples. This inconsistency between migration (suggesting plant filtering) and microbial assembly
297 (suggesting random processes) could be caused by the smaller scale of deterministic processes being
298 masked by larger-scale microbial community assembly. Furthermore, these two mechanisms
299 represent different time scales, where βNRI focuses on broader, longer-term assembly processes
300 (Arnault *et al.*, 2023). Importantly, migration of microorganisms can be caused by random dispersal

301 (stochastic processes) but may appear deterministic as migration increases. Thus, these two processes
302 are not necessarily contradictory.
303 In the fungal microbiome, we observed observed increased migration of fungi from bulk soil to the
304 rhizosphere, highlighting the importance of this niche in fungal microbiome dynamics and the role of
305 plant in microbial recruitment under pathogen stress (Bais *et al.*, 2006; Berendsen *et al.*, 2012). To
306 further asses the influence of the pathogen in microbial metacommunity, we analyzed the fungal
307 networks in the rhizosphere. A number of methods for construction and analysis of co-occurrence
308 networks had been developed, and SPIEC-EASI is characterized with important advantages, such as
309 accounting for compositional type of data and mitigation of non-independence of samples. Also, an
310 implementation of glasso approach allowed for the analysis in high-dimensional setting with more
311 variables than samples (Kurtz *et al.*, 2015). Thus, we implemented these methods for the analysis in
312 current study. Comparison of healthy and infected samples revealed shift in microbial fungal networks
313 under *Pilidium lythri* influence. The fungal networks in infected rhizospheres exhibited lower
314 percentages of positive edges caused lower robustness of networks, suggesting restructuring of fungal
315 community in response to pathogen presence. These findings underline decrease in natural
316 connectivity percentage of positive edges, that suggest lowered positive interactions between fungal
317 taxa. While the presence of the pathogen did not significantly disrupt fungal networks, it did decrease
318 network connectivity, which indicates a shift toward increased competition and antagonism within the
319 fungal microbiome. This suggests adaptation of the fungal meta-community, making it more resistant
320 to further infections and environmental stressors (Hernandez *et al.*, 2021).
321 Concluding, in this study, we isolated and molecularly identified isolates of spreading fungal
322 phytopathogen from symptomatic strawberry plants - *Pilidium lythri*. We observed pathogen's low
323 sensitivity against various chemical substances in 30°C compared with 24°C, highlighting dangers of its
324 spread due to climate change. We also noted significant variability in inhibition caused by chemical
325 substances between strains. We used *P. lythri* to evaluate ecological impacts of spreading fungal
326 phytopathogen on microorganism metacommunities in bulk soil, rhizosphere and roots of strawberry
327 with high-throughput marker sequencing. We revealed that *Pilidium lythri* infection had a major effect
328 on both the structure and dynamics of the plant associated microbiome. Its ability to shift bacterial
329 microbial assembly mechanism from deterministic to stochastic in the bulk soil, underscores its
330 capacity to destabilize plant-associated microbiomes. Additionally, we observed that *P. lythri* shifted
331 the fungal microbiome dynamics in the strawberry rhizosphere towards reducing mutualistic
332 connections, while maintaining overall network stability. This underlined adaptive reorganization of
333 fungal community, potentially increasing resilience to secondary infections and other (a)biotic
334 stresses.

These findings underline the critical role of emerging fungal pathogens like *Pilidium lythri* in destabilizing plant-microbiome interactions. This is significantly important in the context of global warming, which accelerates spread and impact of such pathogens.

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Competing interests

The authors have declared that no competing interests exist.

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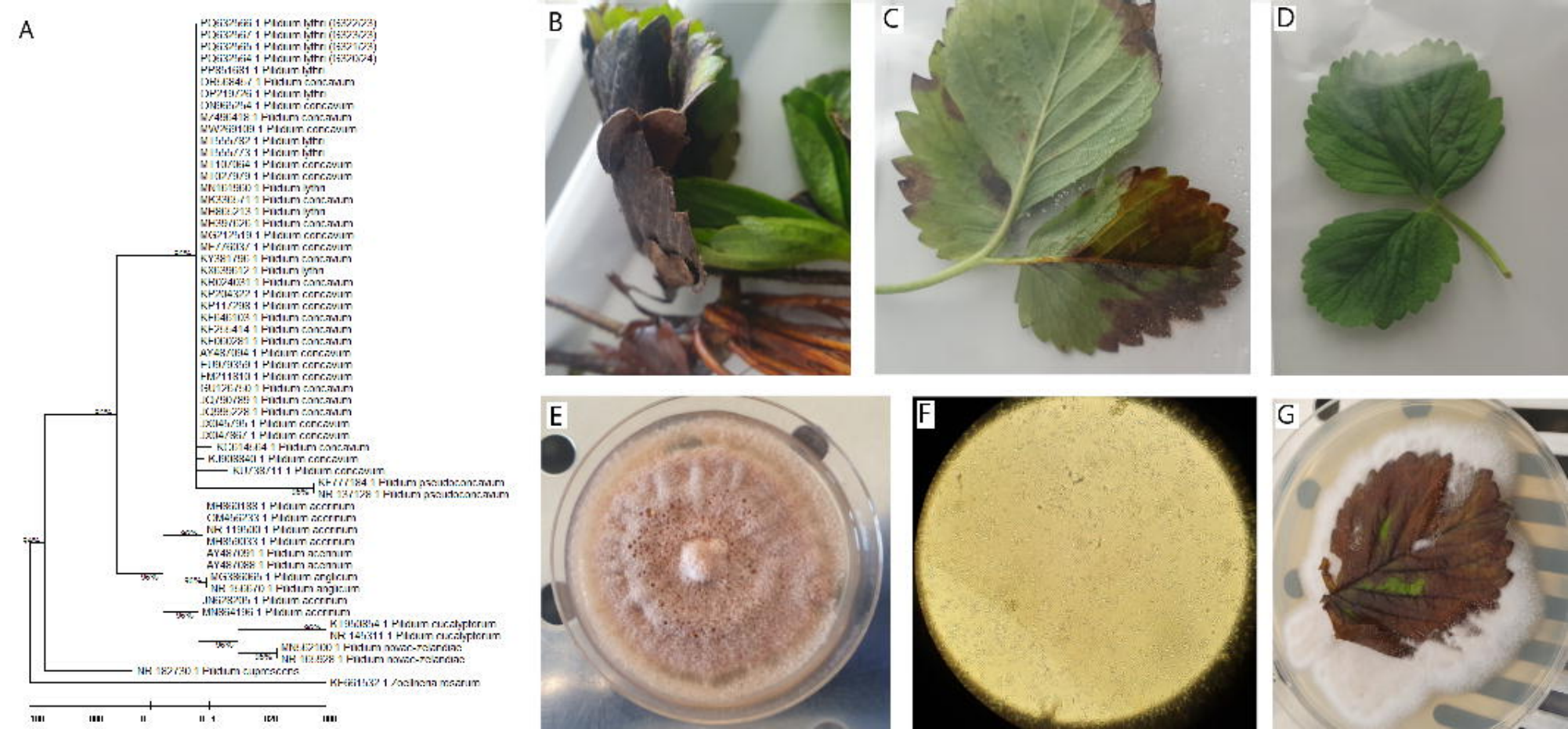
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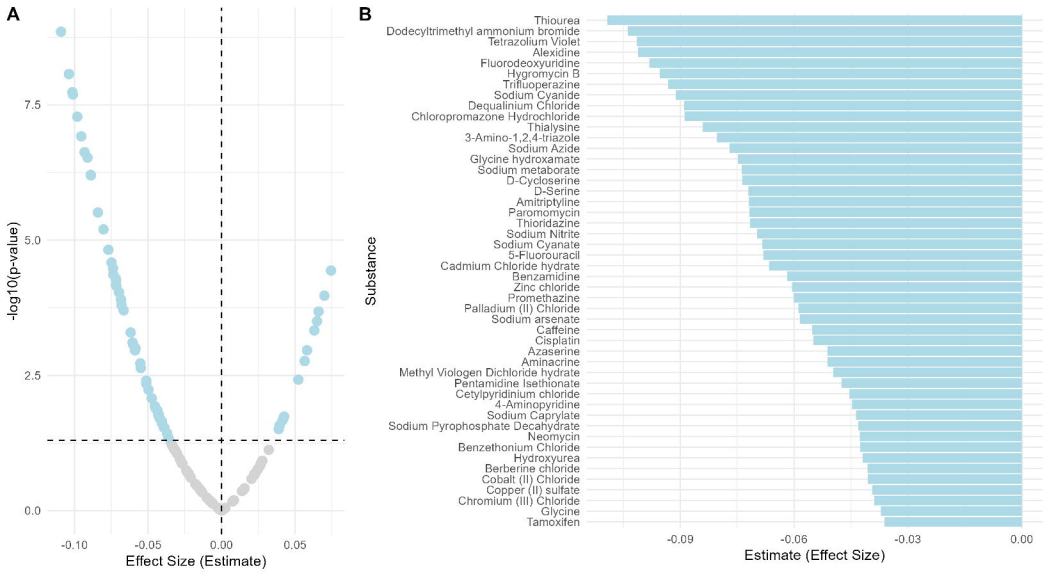
A
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7.5
5.0
2.5
0.0
-0.10
-0.05
0.00
0.05
Effect Size (Estimate)
B
Substance
Thiourea
Dodecyltrimethyl ammonium bromide
Tetrazolium Violet
Alexidine
Fluorodeoxyuridine
Hygromycin B
Trifluoperazine
Sodium Cyanide
Dequalinium Chloride
Chloropromazone Hydrochloride
Thialysine
3-Amino-1,2,4-triazole
Sodium Azide
Glycine hydroxamate
Sodium metaborate
D-Cycloserine
D-Serine
Amitriptyline
Paromomycin
Thioridazine
Sodium Nitrite
Sodium Cyanate
5-Fluorouracil
Cadmium Chloride hydrate
Benzamidine
Zinc chloride
Promethazine
Palladium (II) Chloride
Sodium arsenate
Caffeine
Cisplatin
Azaserine
Aminacrine
Methyl Viologen Dichloride hydrate
Pentamidine Isethionate
Cetylpyridinium chloride
4-Aminopyridine
Sodium Caprylate
Sodium Pyrophosphate Decahydrate
Neomycin
Benzethonium Chloride
Hydroxyurea
Berberine chloride
Cobalt (II) Chloride
Copper (II) sulfate
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Glycine
Tamoxifen
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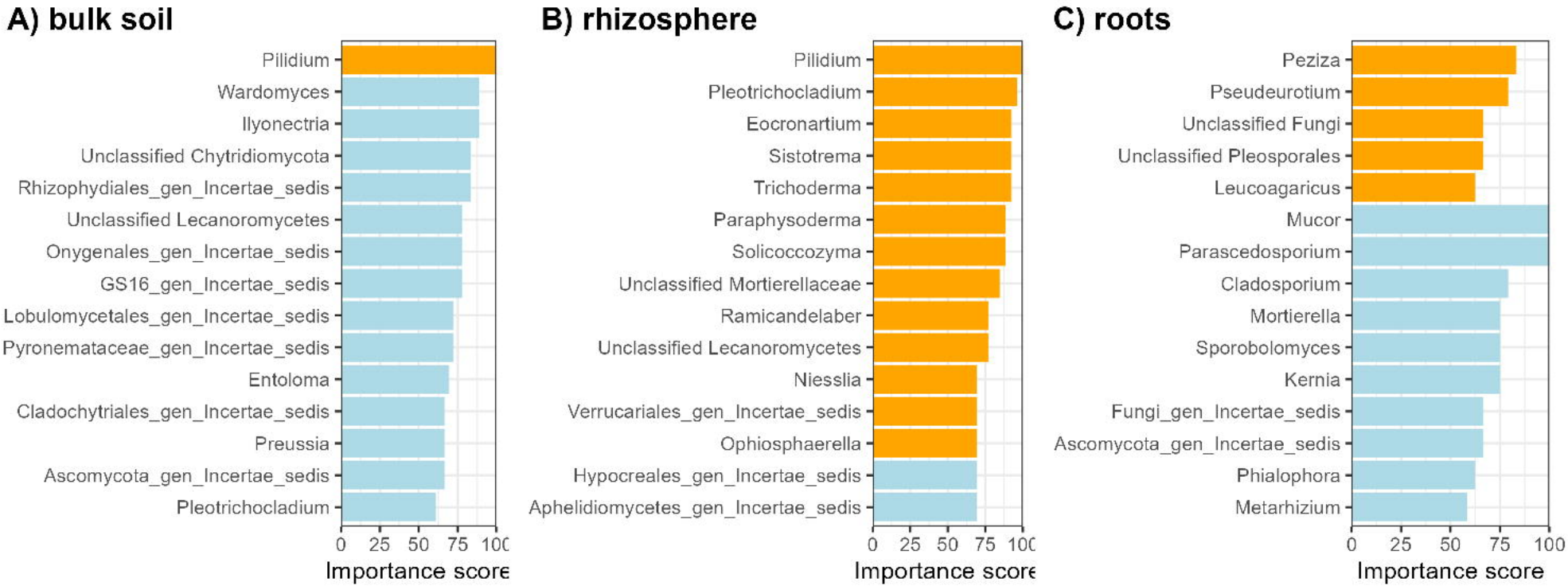
-0.06
-0.03
0.00
Estimate (Effect Size)


A) bulk soil
Pilidium
Wardomyces
Ilyonectria
Unclassified Chytridiomycota
Rhizophydiales_gen_Incertae_sedis
Unclassified Lecanoromycetes
Onygenales_gen_Incertae_sedis
GS16_gen_Incertae_sedis
Lobulomycetales_gen_Incertae_sedis
Pyronemataceae_gen_Incertae_sedis
Entoloma
Cladochytriales_gen_Incertae_sedis
Preussia
Ascomycota_gen_Incertae_sedis
Pleotrichocladium
0 25 50 75 100
Importance score
B) rhizosphere
Pilidium
Pleotrichocladium
Eocronartium
Sistotrema
Trichoderma
Paraphysoderma
Solicoccozyma
Unclassified Mortierellaceae
Ramicandelaber
Unclassified Lecanoromycetes
Niesslia
Verrucariales_gen_Incertae_sedis
Ophiosphaerella
Hypocreales_gen_Incertae_sedis
Aphelidiomycetes_gen_Incertae_sedis
0 25 50 75 100
Importance score
C) roots
Peziza
Pseudeurotium
Unclassified Fungi
Unclassified Pleosporales
Leucoagaricus
Mucor
Parascedosporium
Cladosporium
Mortierella
Sporobolomyces
Kernia
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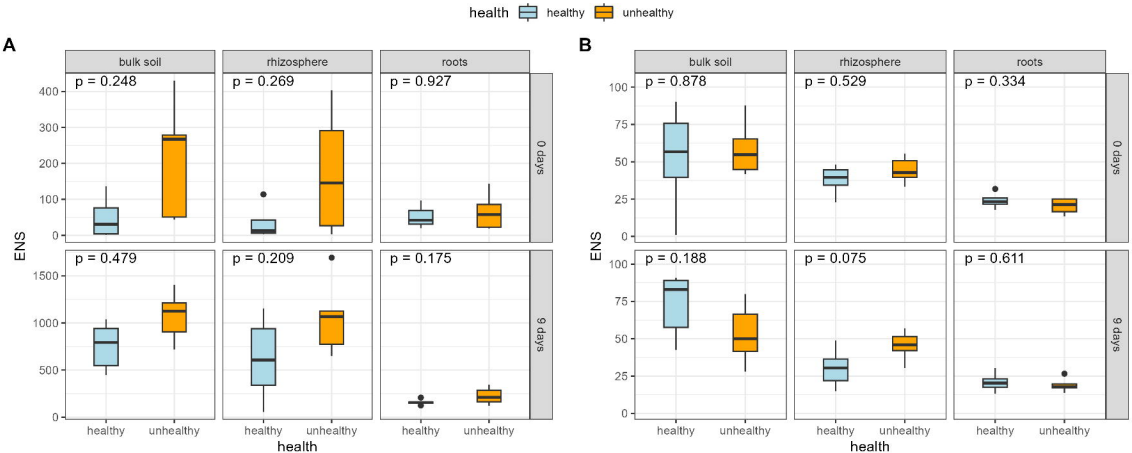
Importance score


health
healthy
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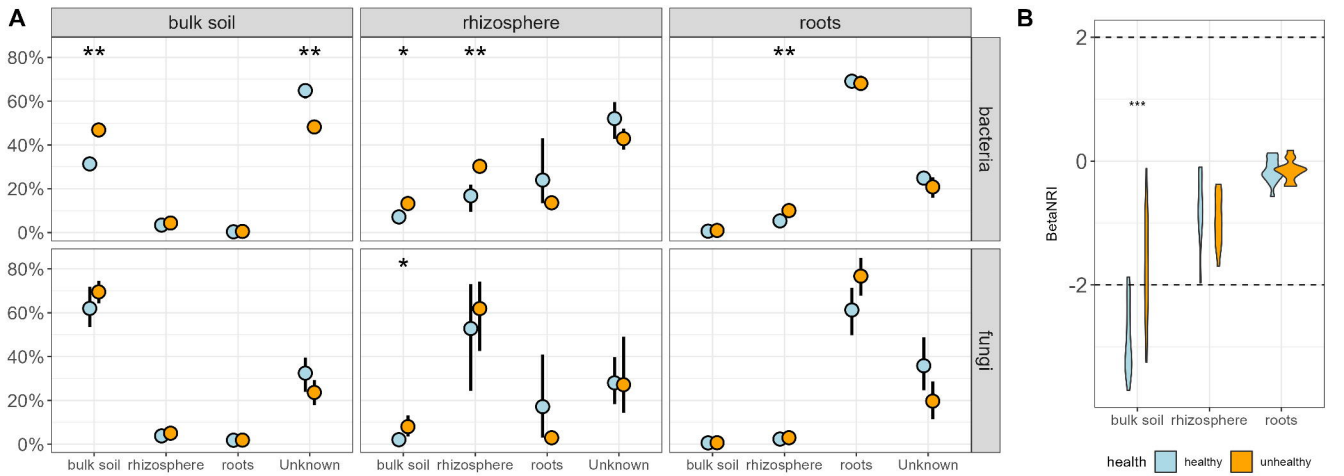
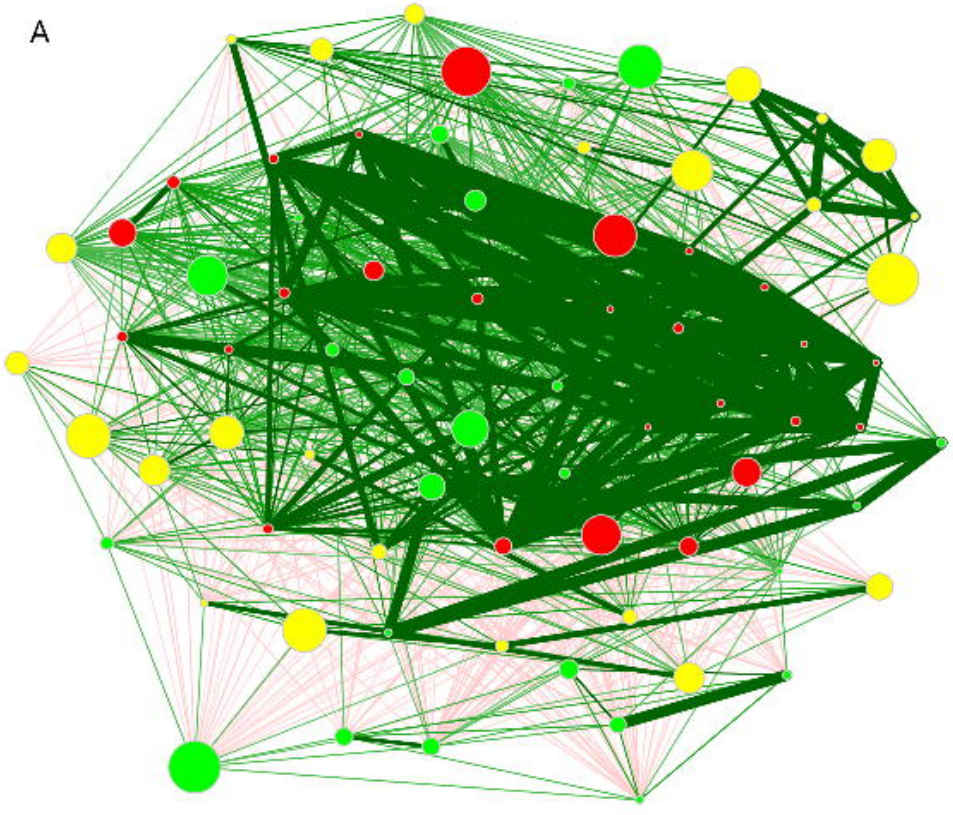
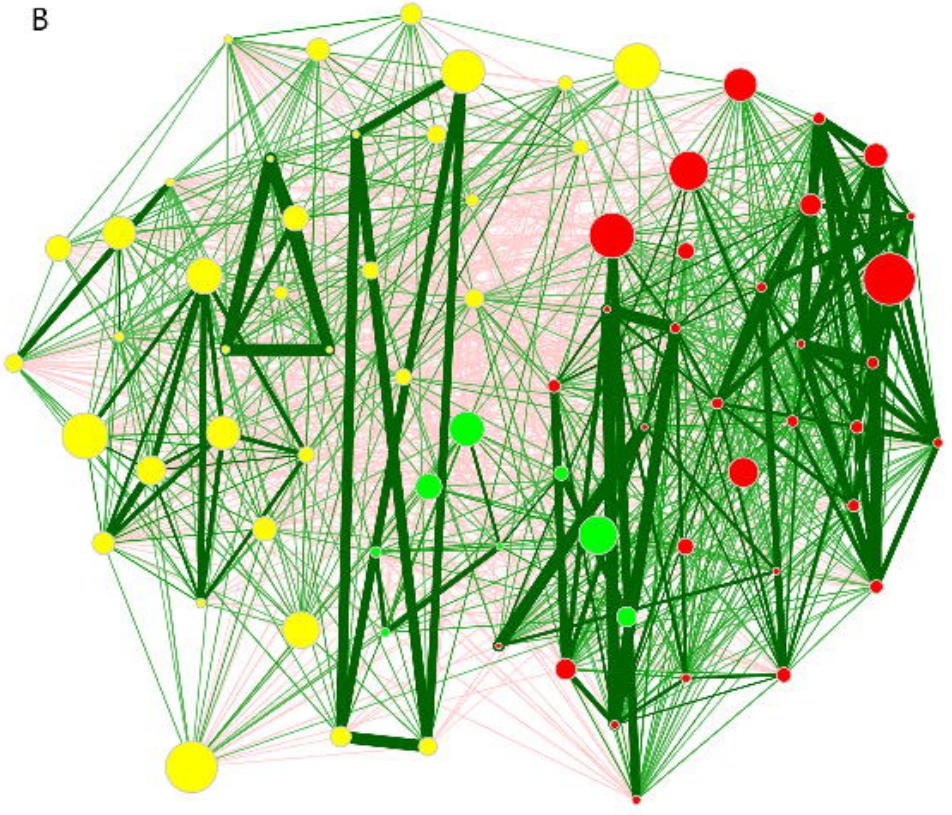
A
bulk soil
rhizosphere
roots
0 days
9 days
p = 0.248
p = 0.269
p = 0.927
p = 0.479
p = 0.209
p = 0.175
ENS
health
B
p = 0.878
p = 0.529
p = 0.334
p = 0.188
p = 0.075
p = 0.611


A
bulk soil
rhizosphere
roots
bacteria
fungi
80%
60%
40%
20%
0%
**
*
bulk soil
rhizosphere
roots
Unknown
B
2
0
-2
***
BetaNRI
health
healthy
unhealthy


A


B