Pilidium lythri: A Climate-Driven Disruptor of Plant-Associated Microbiomes and Ecological Stability

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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. After assessing high adaptability of emerging *Pilidium lythri* to growing temperatures, we analysed its impact on microbiome structure of soil (bulk soil, rhizosphere) and strawberry plant (roots) niches. For this, we employed high-throughput marker sequencing and various statistical models. Our results 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. Fungal microbiome diversity increased locally in infected rhizospheres, while microbial migration patterns highlighted shifts in community interactions. Network analysis underscored *P. lythri*'s role in destabilizing fungal community connectivity, enhancing antagonistic interactions, and occupying a central ecological niche. 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 (Baldrian, 2016; Berg, 2009; Gupta et al., 2021; Marco et al., 2022; Michl et al., 2023; Trivedi et al., 2020). These finely tuned interactions are widely studied and hold great potential for improving agricultural productivity in a sustainable manner (Frąc et al., 2022; Trivedi et al., 2020; Vishwakarma et al., 2020).

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; X. Liu et al., 2019). Despite this urgent challenges, limited attention has been given to understand how these emerging pathogens alter and interact with native 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 and Payak 1965), Iran (Arzanlou et al., 2013; Sayami et al., 2013) and China (Duan et al., 2010; Zhang et al., 2008). Notably, its occurrence had been recently identified in several temperate climate countries, including Belgium (Lopes et al., 2010), Croatia (Milicević et al., 2014), Spain (Aguín et al., 2018), France (Caesar et al., 2012; Cardin et al., 2009) 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 (Ayoubi et al., 2016; Geng et al., 2012; Lopes et al., 2010), 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 (Bruckart et al., 2013; Caesar et al., 2012; Cardin et al., 2009; Garfinkel & Chastagner, 2019; Zhang et al., 2008). 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 (Ayoubi et al., 2016; Karimi et al., 2016; Lopes et al., 2010). 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 ideal model organism to explore relationships between emerging fungal pathogens, theirs host plants and native 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, *Pilidium lythri* was used as a model organism. We isolated pure fungal strains 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 at 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* (Karimi et al., 2019; Palm, 1991) 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

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

Metabolic profile arrays

Each cell of Phenotype Microarray 21-25 BIOLOG plates (Biolog, Hayward, CA, USA) was inoculated with the fungal mycelium as recommended by the manufacturer and described before (Panek et al., 2016). For each plate type, 3 *Pilidium lythri* strains in triplicates and in two temperatures (24 and 30°C) were incubated for 96 hours in the darkness. Microplate reader (Biolog, Hayward, CA, USA) was used to determine the optical density at 490 nm at the beginning and every 24 hours. Obtained data was analysed statistically and visualised in RStudio, using tidyverse package (Wickham et al., 2019). First, values obtained at 0h for each replicate was divided from read values at 96h, and values <0 were assigned as 0. To compare differences in fungal growth between different temperatures for each concentration we performed Wilcoxon test with False Discovery Rate method to correct for multiple comparisons. Next, to differentiate growth of different strains, we used Kruskal-Wallis method with *post-hoc* pairwise comparison with Wilcoxon test.

Pot experiment and isolation of microbial DNA

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

125 ## Metataxonomic sequencing, bioinformatics and statistical analysis

126 For the metataxonomic sequencing, microbial DNA ITS2 (Schoch et al., 2012) and 16Sv3 regions
127 (Klindworth et al., 2013) were amplified, indexed and sequenced on Illumina MiSeq instrument
128 (2x300), and raw data was then passed through the custom QIIME2 (Bolyen et al., 2019) pipelines as
129 reported before (Siegieda et al., 2023, 2024). In brief, after primer trimming with Cutadapt (Martin,
130 2011) and ITSxpress (Rivers et al., 2018), denoising, chimera removal, merging and Amplicon Sequence
131 Variant (ASV) calling was made. The taxonomy was assigned based on UNITE v9 dynamic release
132 (Nilsson et al., 2019) and Silva 138 (Pruesse et al., 2007) databases. The results were then analysed
133 with R (v. 4.0.3), using libraries: phyloseq (McMurdie & Holmes, 2013), tidyverse (Wickham et al.,
134 2019), microeco (C. Liu et al., 2021), microbiomeMarker (Cao et al., 2022), NetCoMi (Peschel et al.,
135 2021). First, for ITS data, only ASVs identified to Fungi and Stramenopiles; and for 16S - Bacteria and
136 Archaea. Then, ITS data was repeatedly rarefied at 6,000 and 16S at 11,000 reads using mirlyn package
137 (Cameron et al., 2021; McMurdie & Holmes, 2014). We employed Effective Number of Species (ENS)
138 (Jost, 2006) on ASV level to evaluate the richness of microbiomes in each niche. We used supervised
139 machine learning model to evaluate the fungal biomarkers of each niche for healthy and infected
140 samples with Supervised Machine Method (SVM), using Total Sum Scaling normalization method.
141 SourceTracker was used for tracking migration between niches (Knights et al., 2011). RCbray (Bray-
142 Curtis dissimilarity based Raup-Crick metric) and βNTI (β Nearest Taxon) were used to evaluate the
143 ecological processes that dominated the bacterial assemblies (C. Liu et al., 2017; Stegen et al., 2013).
144 When βNTI fell between -2 and 2, microbial assembly was dominated by stochastic mechanisms, and
145 below -2 and higher that 2 - by deterministic. Microbial networks were constructed with 1,000 most
146 frequent taxa (using Pearson correlation measure) and fast greedy clustering method.

147 # Results

148 ## Fungal isolates

149 Fungal ITS sequences, obtained from symptomatic strawberry leaves (G320/23, G321/23, G322/23 and
150 G323/23) showed 100% similarity to those of *Pilidium lythri* already present in the GenBank database
151 and phylogenetic tree revealed clustering of the sequences with other representatives of *Pilidium*
152 *lythri*. The strains collected after 7 days in 23°C in the darkness on Potato Dextrose Agar were flat and
153 granulose, cinnamon in colour (Karimi et al., 2016; Marin-Felix et al., 2017). The presence of hyaline
154 conidia with smooth walls and slightly pointed tips were confirmed under the microscope. After the
155 inoculation of the surface-sterilized strawberry leaves with the spore suspension and incubation in
156 24°C in darkness for 7 days, 100% of the leaves showed brown necrotic lesions. Leaves submerged in
157 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).

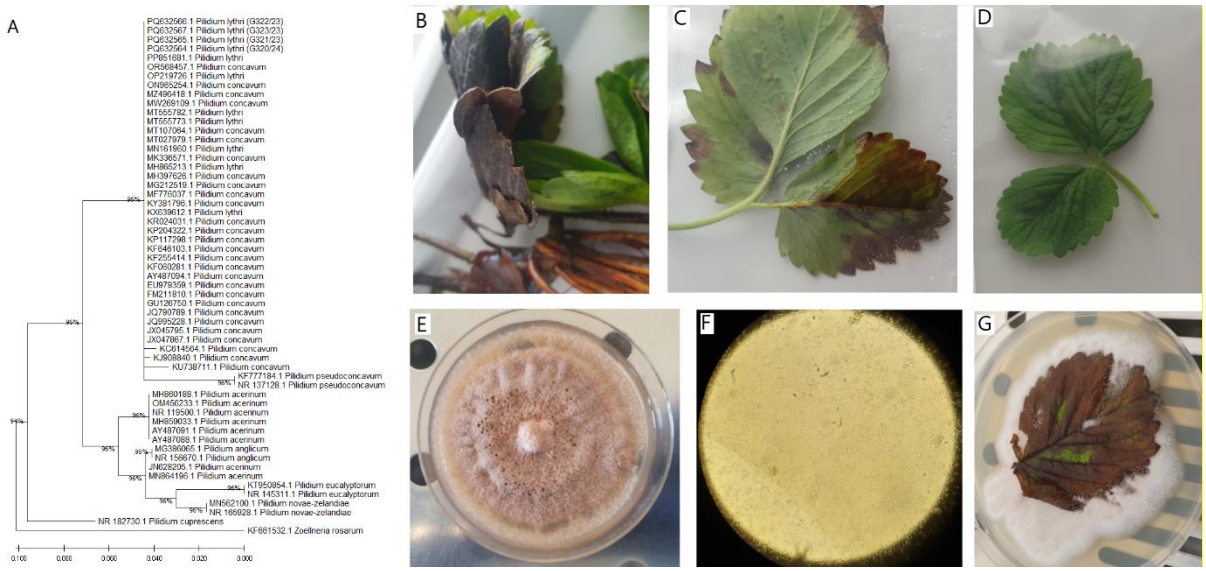


Figure 1. A) Phylogenetic tree of the evolutionary history of *P. lythri* was inferred by using the Maximum Likelihood method and Tamura-Nei model (Tamura & Nei, 1993) and the e tree with the highest log likelihood (-1144.51) is shown. The percentage of trees in which the associated taxa clustered together 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 of three strains at 24°C and 30°C for 96 hours. These 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. For the purposes of this article, we refer to them as concentration levels 1, 2, 3, and 4 to maintain clarity. The substances were grouped in 8 groups, based on their structure and function: antibiotics, antimicrobials, chelators, drugs (used in medicine), membrane function compounds, nitrogen compounds, salts and others (Panek et al., 2016). Overall, strains exhibited lower sensitivity to chemical compounds at higher temperature (30 °C) in concentration 3 and 4 (p-values < 0.01, Supplementary Figure 1). We also observed variation between strains in higher temperature (Supplementary Figure 2), where the strain G321/23 was characterized with the highest sensitivity, and G322/23 the lowest. Increase in substance concentration showed decrease in median fungal growth in both temperatures (excluding zeros) (Supplementary Table 1). Alarmingly, only Niaproof (in level 4 concentration) effectively inhibited growth off all 3 strains at both temperatures (Figure 2, Supplementary Figure 3, Supplementary Figure 4, Supplementary Figure 5).

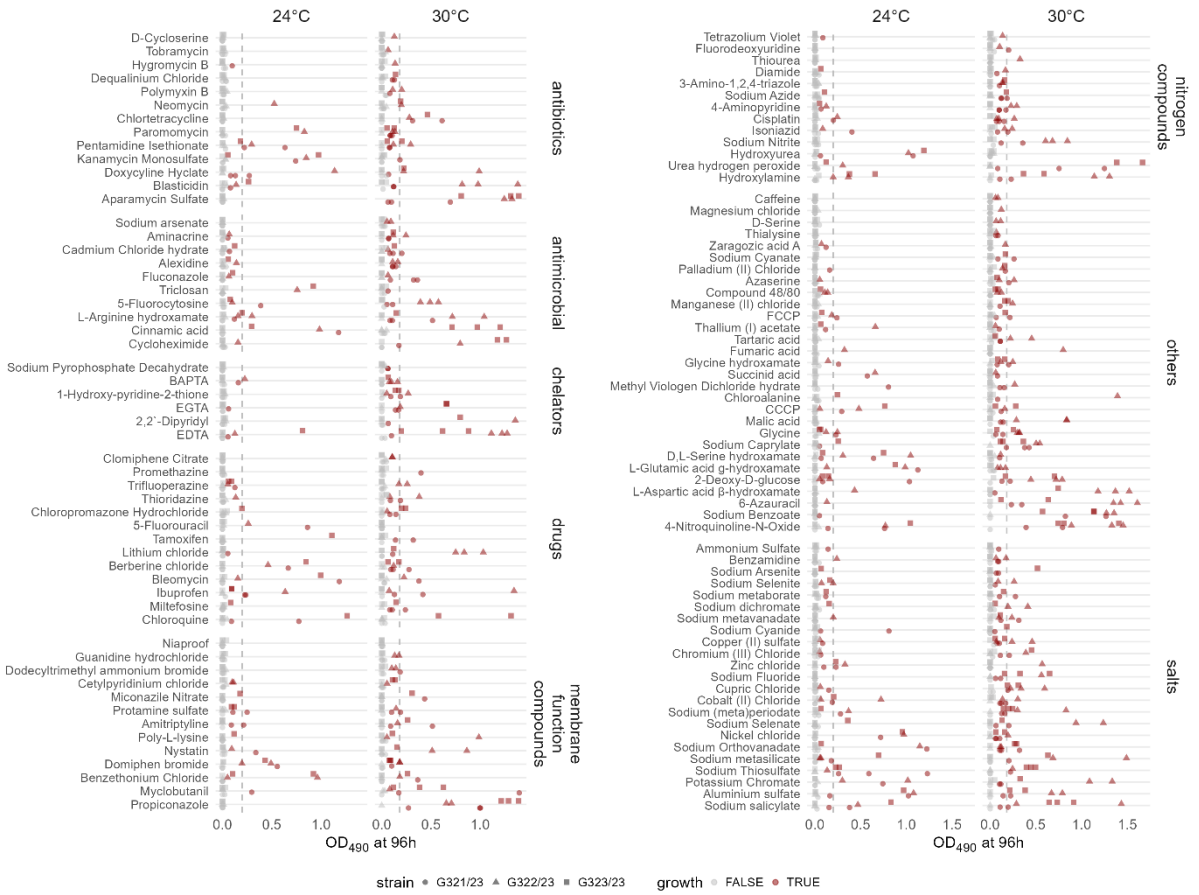


Figure 2. BIOLOG PM21-25 results of the cells with highest concentration of chemical substances (concentration level 4). Shapes represent different strains of *Pilidium lythri.* Gray symbols represent no growth, red - growth of the strain after 96 hours. Dashed vertical grey line represents average response for each chemical substrate in each temperature (excluding zeros).

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

A total of 72 samples from soil (2x12), rhizosphere (2x12), and roots (2x12) 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.

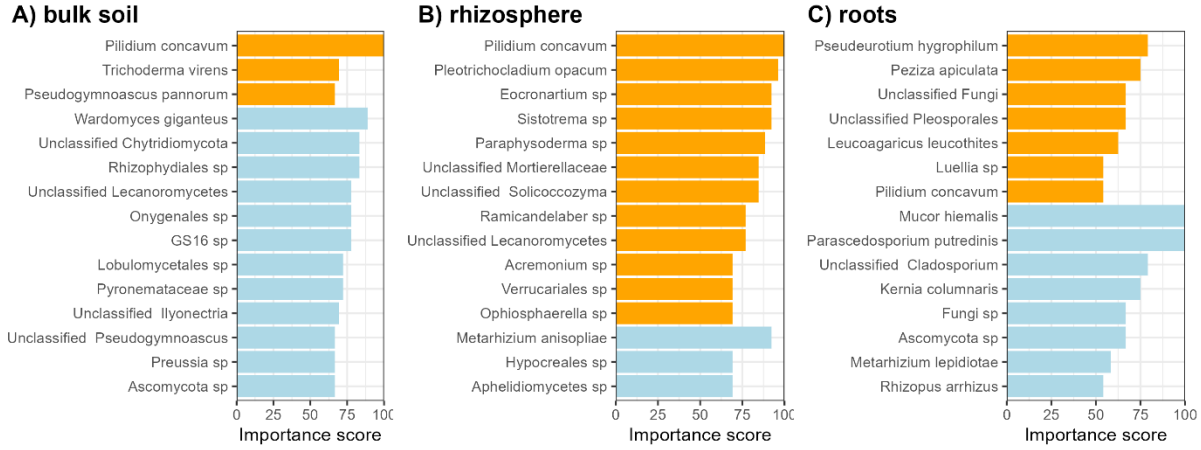


Figure 3. Top 15 fungal taxa found more abundant in (orange) infected and in (blue) healthy soils and plants identified based on Supervised Vector Machine machine learning approach.

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 ($p > 0.05$) (Figure 4). Only fungal community in the infected rhizosphere at the second sampling displayed higher richness ($p < 0.05$). Notably, bacterial communities showed a significant increase richness across all three compartments at the second sampling time ($p < 0.05$), whereas fungal community richness remained stable across time points ($p > 0.05$). Additionally, both bacterial and fungal communities exhibited a gradient of decreasing diversity from soil to rhizosphere and root at second sampling.

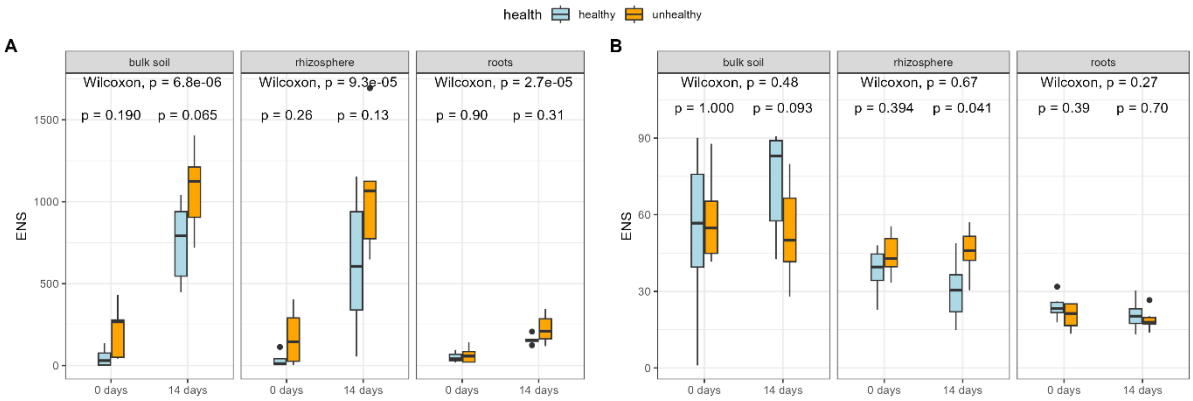
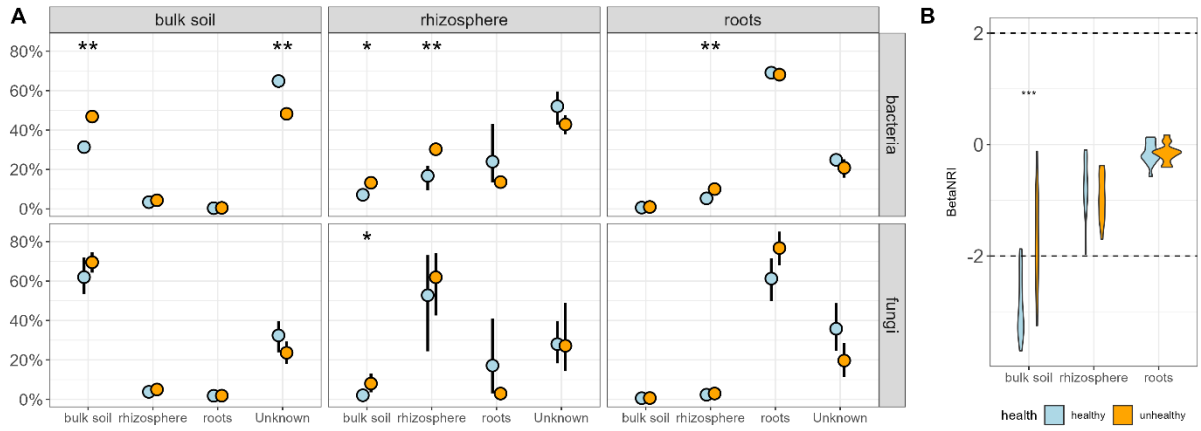


Figure 4. Comparison of alpha diversity (in Effective Number of Species) of bacterial (A) and fungal (B) communities of strawberry niches immediately after *P. lythri* inoculation (one) and after 9 days of incubation in 25°C and ~70% of humidity (two). Higher result represent full model comparison, and lower - pairwise comparisons within each sampling. Light blue color represents samples without pathogen inoculation, and orange - with inoculation.

214 To elucidate the influence of *Pilidium lythri* on microbial migration between the niches, we used
215 samples from sampling at 0 days as a source, and samples from sampling at 9 days as sinks, and did
216 this both fungal and bacterial communities. In bulk soil, only bacterial community revealed significantly
217 higher share of microorganisms in bulk soil and a lower portion from unknown source in infected
218 samples (Figure 4A). Furthermore, we observed migration of bacteria from bulk soil to rhizosphere for
219 both bacteria and fungi in infected communities, but also from rhizosphere to rhizosphere for bacteria
220 in infected communities. Finally, analysis revealed increased migration from rhizosphere to roots in
221 infected samples in bacterial community.

222 

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

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

234 Finally, to evaluate the influence of *Pilidium lythri* on the structure of fungal networks, we compared
235 the network structure in the rhizosphere niche between control and infected samples during the
236 second sampling (Figure 6A). Both networks showed similar characteristics in terms of the number of
237 components (1), natural connectivity (0.339, 0.302), and vertex and edge connectivity (72, 69). On the
238 other hand, the control rhizosphere exhibited a higher percentage of positive edges (50.15% vs.
239 46.55%) but lower average dissimilarity and average path length (0.644 vs. 0.670). Notably, the ASV

identified as *Pilidium* demonstrated higher values of betweenness (0 vs. 3) and closeness centrality (1170 vs. 154) in the infected samples.

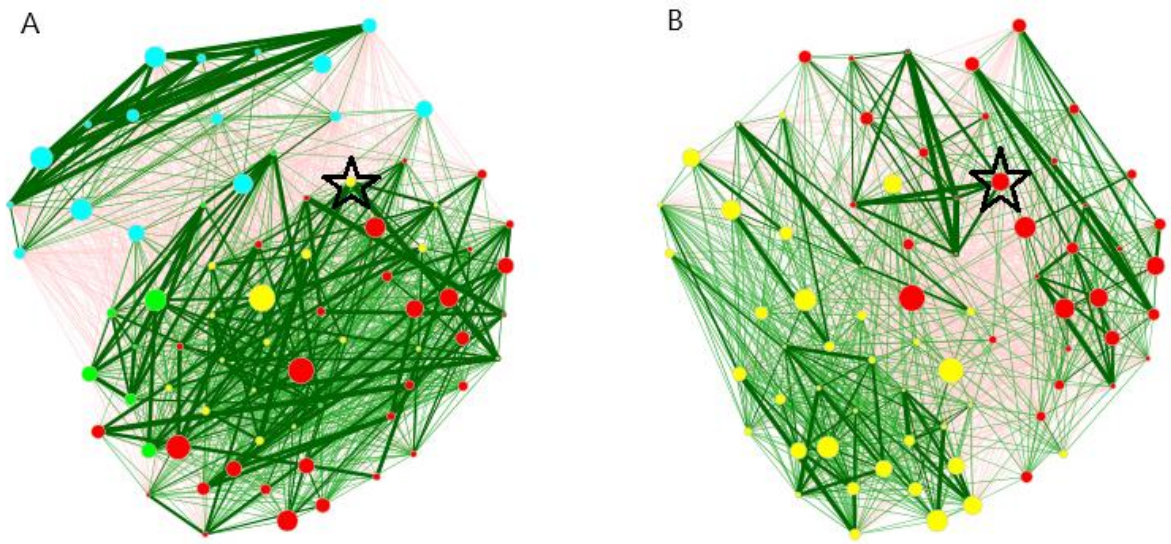


Figure 6. Fungal network analysis in A) control and B) infected rhizosphere samples of strawberry. Each node represents individual fungal ASV, and node size is proportional to number of reads. Node colours represent the same clusters. Green lines represent positive interactions and red- negative. The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

Discussion

To close the knowledge gap regarding emerging fungal pathogens and theirs influence on native plants we used newly isolated *Pilidium lythri* as a model organism. We identified 3 strains obtained from symptomatic strawberry plants in Polish nursery by sequencing of the ITS region and evaluated the impact of temperature growth on inhibition of fungal development with 112 different chemical substances. We then evaluated the impact of this phytopathogen on the structure 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 using BIOLOG microarrays raised serious concerns, as *Pilidium lythri* demonstrated lower susceptibility to chemicals at higher temperatures, with most of the tested compounds showing limited or no inhibitory effects on fungal growth. Notably, increased temperature allowed the pathogen to grow even in the presence of these substances, highlighting the high potential for environmental adaptability of this fungus. Global warming has indeed been widely reported to contribute to the spread of fungal phytopathogens to new areas (Bebber et al., 2013; Chaloner et al., 2021; Ward et al., 2007). Among the wide variety of chemical substances tested, only Niaproof showed inhibition at both temperatures, and could be further tested to evaluate their antifungal effects. Since Niaproof (tetradecyl sodium sulfate) is an anionic surface-active agent, its

antifungal mechanism may involve disrupting the fungal cell membrane, thus preventing the pathogen from surviving in the environment.

As the dissemination of fungal pathogens, is widely reported (Bebber et al., 2013), still little is known about the effect of such invasive microorganisms on the native microbial structure of the plant holobiont. Recent studies have reported significant differences in the α-diversity of microbiome structure between healthy and infected soil and plant niches (Bakker et al., 2018; Delgado-Baquerizo et al., 2016; Lefcheck et al., 2015). In the present study, we compared the diversity of bacterial and fungal metacommunities in healthy and *Pilidium lythri*-infected bulk soil, rhizosphere, and roots at two time points: directly after pathogen inoculation and 9 days post-inoculation. The results revealed strong evidence of increased α-diversity in the bacterial community 9 days after inoculation in both healthy and inoculated niches, suggesting that bacterial communities are less influenced by *Pilidium lythri* inoculation and more responsive to environmental factors. To test this, we analyzed the mechanisms involved in bacterial microbiome assembly by calculating the βNRI index, treating the first sampling as the source of microorganisms and the second sampling as the sinks. This index measures how the differences in species composition between the two samplings differ from what would be expected by the null model of random assembly. The results indicated that composition of bacterial communities in healthy and diseased rhizosphere and roots samples was shaped by stochastic processes (i.e. random colonization, dispersal, and ecological drift). Interestingly, we observed a shift from deterministic processes in healthy bulk soil, to stochastic processes in *Pilidium lythri*-infected samples, indicating a disruption in microbial turnover. Infected soil showed random processes that could have been triggered by disturbances in plant biology caused by the infection, described by the Anna Karenina Principle (AKP)(Arnault et al., 2023). The scenario described here can be explained by the loss of plant control over microbiome assembly, resulting in a temporal AKP effect (C. Cheng et al., 2013; Y. T. Cheng et al., 2019). Over time, this could lead to the recovery of the plant's control over microbial assembly or the introduction of new deterministic processes to control bacterial assembly (Gao et al., 2021). Analysis of microbial migration suggests increased migration of bacteria from the soil in diseased samples, as well as increased migration of bacteria from the rhizosphere to the roots in infected plant samples. This inconsistency between migration (suggesting plant filtering) and microbial assembly (suggesting random processes) could be caused by the smaller scale of deterministic processes being masked by larger-scale microbial community assembly. Furthermore, these two mechanisms represent different time scales, where βNRI focuses on broader, longer-term assembly processes (Arnault et al., 2023). Importantly, migration of microorganisms can be caused by random dispersal (stochastic processes) but may appear deterministic as migration increases. Thus, these two processes are not necessarily contradictory.

296 In the fungal microbiome, we observed a localized increase in α-diversity in the infected rhizosphere
297 at the second sampling, which contrasts with recent results from strawberry fields that showed no
298 differences in microbiome composition based on health status (Siegieda et al., 2023). We also observed
299 increased migration of fungi from bulk soil to the rhizosphere, highlighting the importance of this niche
300 in fungal microbiome dynamics and the role of plant in microbial recruitment under pathogen stress
301 (Bais et al., 2006; Berendsen et al., 2012). To further asses the influence of the pathogen in microbial
302 metacommunity, we analyzed the fungal networks in the rhizosphere. Comparison of healthy and
303 infected samples revealed the important role *Pilidium lythri* plays in rhizosphere networks and overall
304 microbiome assembly. The fungal networks in infected and control samples were similar, yet infected
305 rhizospheres exhibited lower percentages of positive edges and longer average path lengths. This
306 suggests that the presence of the pathogen does not significantly disrupt fungal networks but does
307 decrease network connectivity, indicating a shift toward increased competition and antagonism within
308 the fungal microbiome. This could destabilize the fungal meta-community, making it more vulnerable
309 to further infections and environmental stressors (Hernandez et al., 2021). Furthermore, we noted the
310 central role of *Pilidium lythri*, as it exhibited higher betweenness and closeness centrality in the
311 infected samples. This suggests that the pathogen plays a pivotal role in reshaping the fungal
312 community balance. Increased betweenness centrality indicates that the pathogen acts as a key
313 connector in the infected rhizosphere, potentially by disrupting connections between beneficial fungal
314 microorganisms or recruiting microbial partners to facilitate its distribution within and between the
315 soil and plant niches.
316 Concluding, in this study, we isolated and molecularly identified pure isolates of spreading fungal
317 phytopathogen from symptomatic strawberry plants - *Pilidium lythri*. We observed pathogen's low
318 sensitivity against various chemical substances in 30°C compared with 24°C, highlighting dangers of its
319 spread due to climate change. We revealed that only Niaproof is a promising substance that could be
320 used to control its spread, but further investigations on its safety in the field conditions and
321 mechanisms of actions are necessary. We used *P. lythri* as a model phytopathogenic microorganism to
322 evaluate ecological impacts of spreading fungal phytopathogens on native microorganism
323 metacommunities in bulk soil, rhizosphere and roots of strawberry with high-throughput marker
324 sequencing. We revealed that *Pilidium lythri* infection had a major effect on both the structure and
325 dynamics of the plant associated microbiome. Its ability to shift bacterial microbial assembly
326 mechanism from deterministic to stochastic in the bulk soil, underscores its capacity to destabilize
327 plant-associated microbiomes. Observed mechanism aligned with Anna Karenina Principle, suggesting
328 loss of control over microbiota assembly in infected soil, which is particularly important for perennial
329 crops, such as strawberry. Additionally, we observed that *P. lythri* plays an important role in fungal
330 network in the strawberry rhizosphere, where it shifted the fungal microbiome dynamics towards

competition and antagonism, amplifying vulnerability of strawberry rhizosphere to secondary infections and other (a)biotic stresses.

These findings underline the critical role of emerging fungal pathogens like *Pilidium lythri* in destabilizing native 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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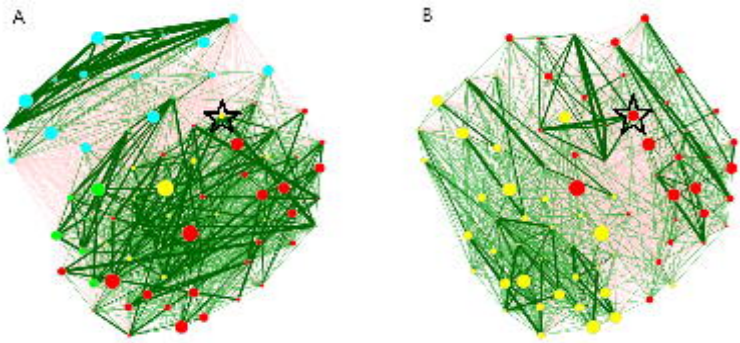
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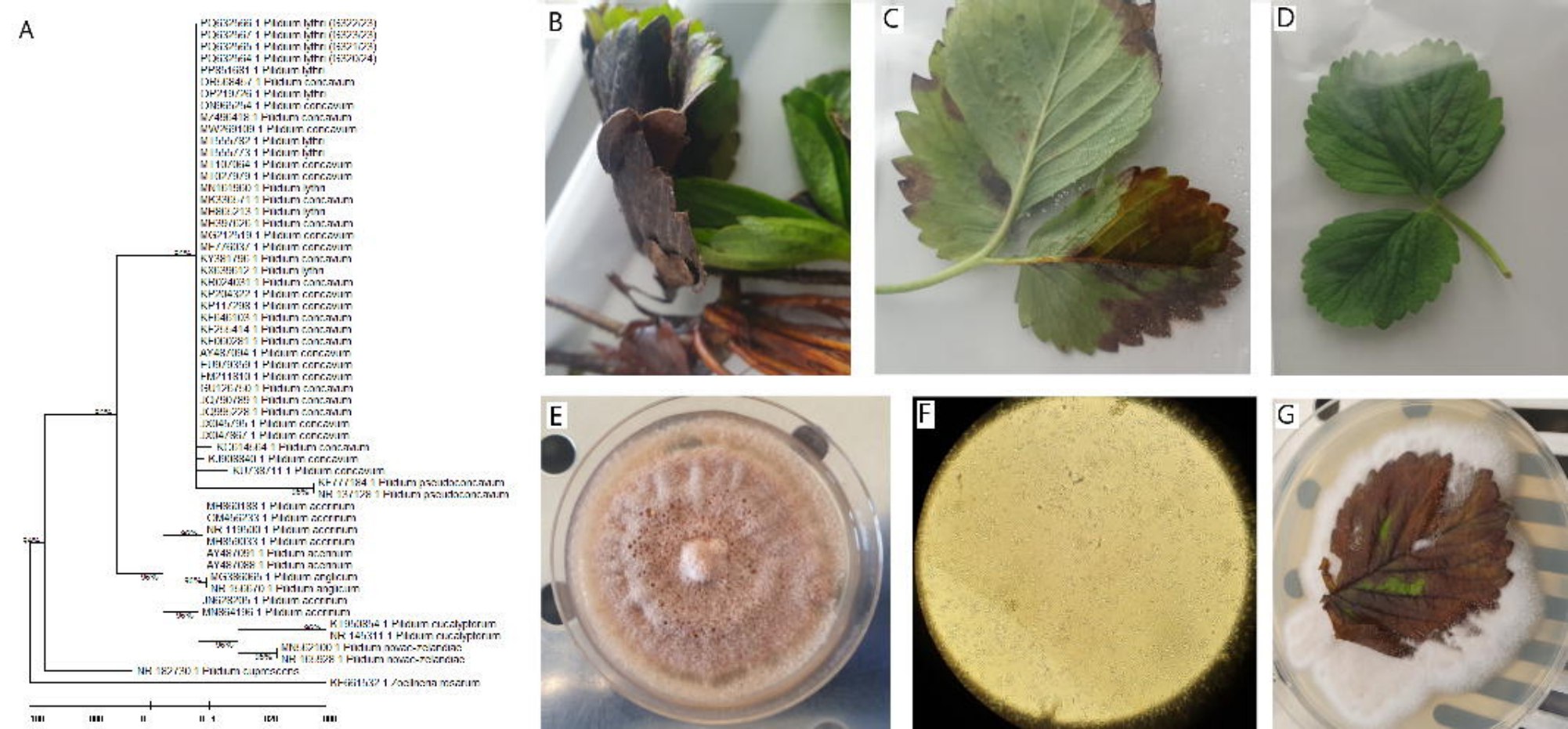
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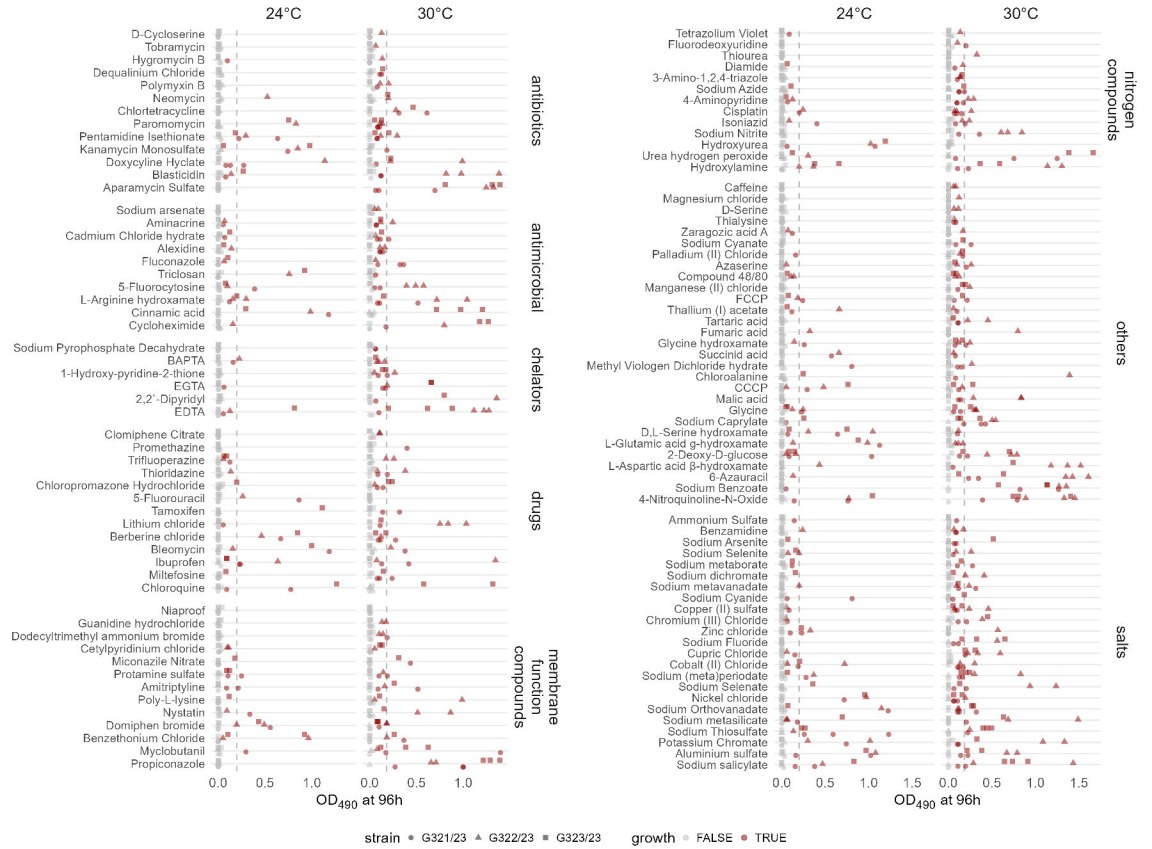
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Doxycycline Hyclate
Blasticidin
Aparamycin Sulfate
antibiotics
Sodium arsenate
Aminacrine
Cadmium Chloride hydrate
Alexidine
Fluconazole
Triclosan
5-Fluorocytosine
L-Arginine hydroxamate
Cinnamic acid
Cycloheximide
antimicrobial
Sodium Pyrophosphate Decahydrate
BAPTA
1-Hydroxy-pyridine-2-thione
EGTA
2,2'-Dipyridyl
EDTA
chelators
Clomiphene Citrate
Promethazine
Trifluoperazine
Thioridazine
Chloropromazone Hydrochloride
5-Fluorouracil
Tamoxifen
Lithium chloride
Berberine chloride
Bleomycin
Ibuprofen
Miltefosine
Chloroquine
drugs
Niaproof
Guanidine hydrochloride
Dodecyltrimethyl ammonium bromide
Cetylpyridinium chloride
Miconazile Nitrate
Protamine sulfate
Amitriptyline
Poly-L-lysine
Nystatin
Domiphen bromide
Benzethonium Chloride
Myclobutanil
Propiconazole
membrane function compounds
0.0
0.5
1.0
1.5
OD490 at 96h
Tetrazolium Violet
Fluorodeoxyuridine
Thiourea
Diamide
3-Amino-1,2,4-triazole
Sodium Azide
4-Aminopyridine
Cisplatin
Isoniazid
Sodium Nitrite
Hydroxyurea
Urea hydrogen peroxide
Hydroxylamine
nitrogen compounds
Caffeine
Magnesium chloride
D-Serine
Thialysine
Zaragozic acid A
Sodium Cyanate
Palladium (II) Chloride
Azaserine
Compound 48/80
Manganese (II) chloride
FCCP
Thallium (I) acetate
Tartaric acid
Fumaric acid
Glycine hydroxamate
Succinid acid
Methyl Viologen Dichloride hydrate
Chloroalanine
CCCP
Malic acid
Glycine
Sodium Caprylate
D,L-Serine hydroxamate
L-Glutamic acid g-hydroxamate
2-Deoxy-D-glucose
L-Aspartic acid β-hydroxamate
6-Azauracil
Sodium Benzoate
4-Nitroquinoline-N-Oxide
others
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Benzamidine
Sodium Arsenite
Sodium Selenite
Sodium metaborate
Sodium dichromate
Sodium metavanadate
Sodium Cyanide
Copper (II) sulfate
Chromium (III) Chloride
Zinc chloride
Sodium Fluoride
Cupric Chloride
Cobalt (II) Chloride
Sodium (meta)periodate
Sodium Selenate
Nickel chloride
Sodium Orthovanadate
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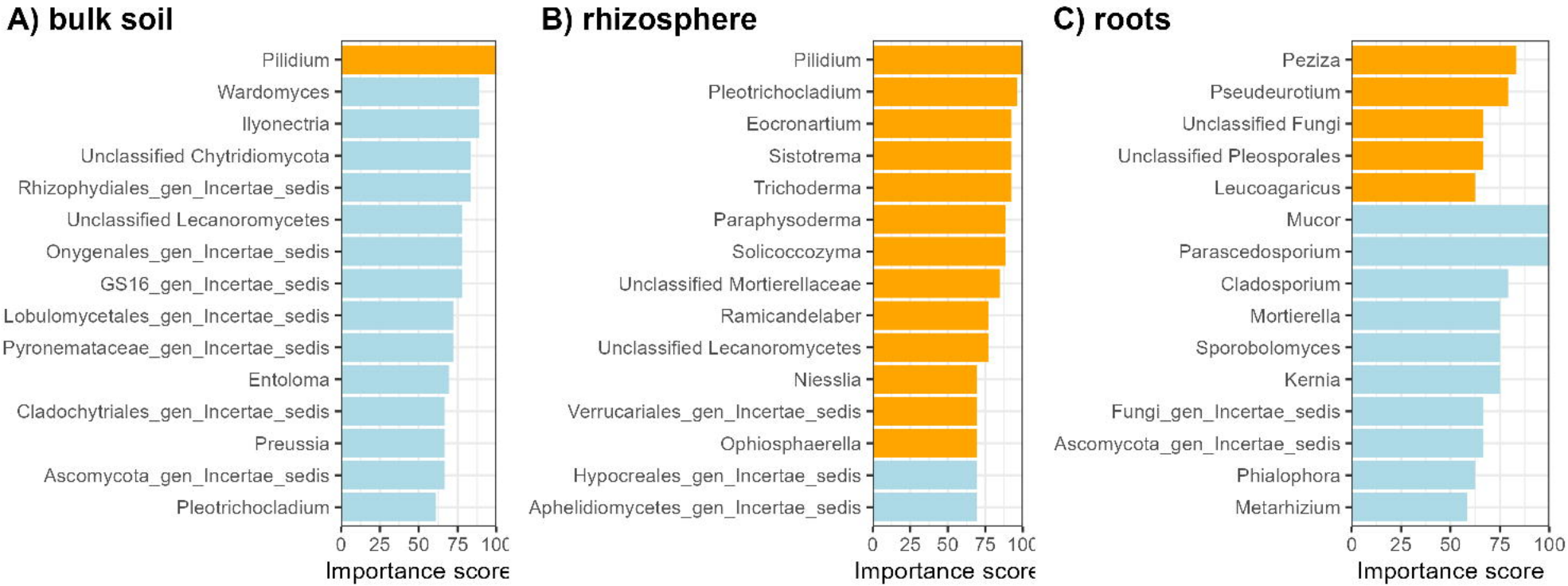
A) bulk soil
Pilidium
Wardomyces
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Unclassified Chytridiomycota
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Unclassified Lecanoromycetes
Onygenales_gen_Incertae_sedis
GS16_gen_Incertae_sedis
Lobulomycetales_gen_Incertae_sedis
Pyronemataceae_gen_Incertae_sedis
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
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Niesslia
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Ophiosphaerella
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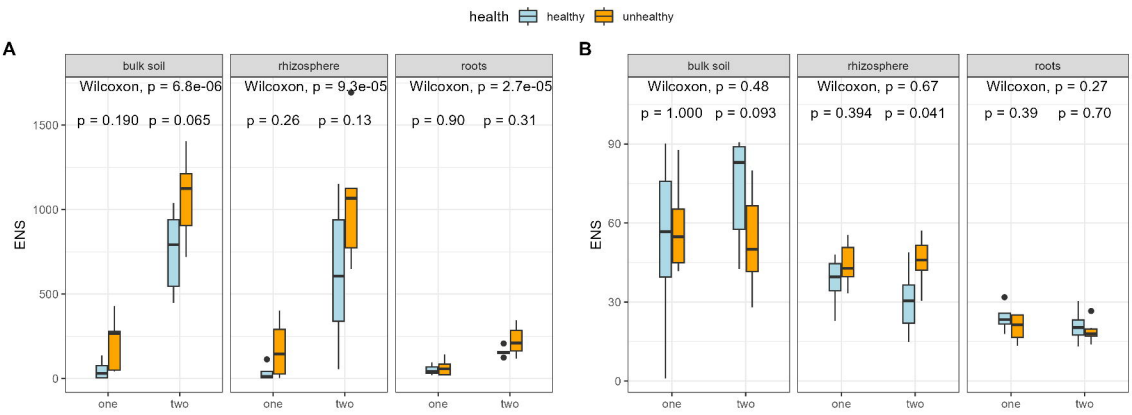
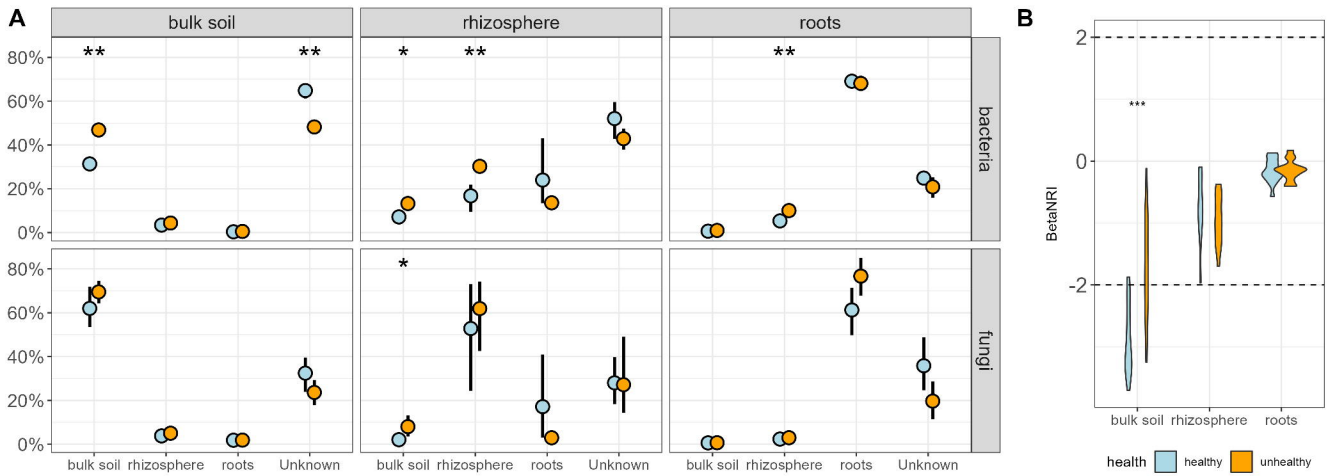
0 25 50 75 100
Importance score
C) roots
Peziza
Pseudeurotium
Unclassified Fungi
Unclassified Pleosporales
Leucoagaricus
Mucor
Parascedosporium
Cladosporium
Mortierella
Sporobolomyces
Kernia
Fungi_gen_Incertae_sedis
Ascomycota_gen_Incertae_sedis
Phialophora
Metarhizium
0 25 50 75 100
Importance score


health
healthy
unhealthy
A
bulk soil
rhizosphere
roots
Wilcoxon, p = 6.8e-06
p = 0.190 p = 0.065
Wilcoxon, p = 9.3e-05
p = 0.26 p = 0.13
Wilcoxon, p = 2.7e-05
p = 0.90 p = 0.31
ENS
1500
1000
500
0
one
two
B
Wilcoxon, p = 0.48
p = 1.000 p = 0.093
Wilcoxon, p = 0.67
p = 0.394 p = 0.041
Wilcoxon, p = 0.27
p = 0.39 p = 0.70
90
60
30
0


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