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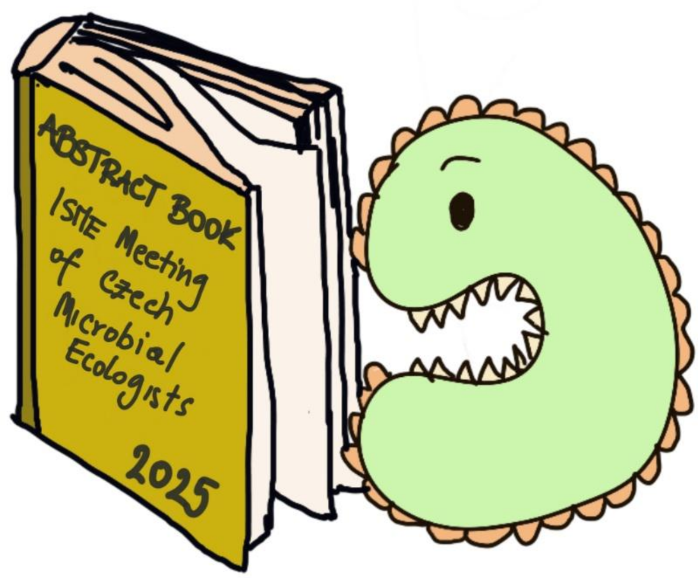
ABSTRACT BOOK



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FUNDAMENTAL NICHES OF ARBUSCULAR MYCORRHIZAL FUNGI ALONG A TEMPERATURE GRADIENT

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Arbuscular mycorrhizal fungi (AMF) are key plant symbionts that enhance plant growth, stress tolerance, and nutrient uptake while contributing to soil health and ecosystem stability. They form associations with most vascular plants and play a vital role in maintaining ecosystem productivity under changing environmental conditions.

Current estimates of their global distributions mostly rely on realized niches, which can be strongly influenced and shifted by biotic interactions. However, as climate change can alter these interactions, predictions based solely on realized niches may misrepresent the true environmental potential of AMF. In this study, we experimentally estimated the fundamental niches of 8 species of AMF across a temperature gradient using controlled growth chambers. Across six temperature and two host plant species, we quantified root colonization and we estimated fungal abundance by using a novel combination of PCR and ddPCR. We found that AMF taxa identified as cold- or warm-preferring based on the GlobalAMFungi database showed similar preferences under experimental conditions, indicating a strong overlap between their realized and fundamental niches. This overlap indicates that the temperature preferences of AMF are probably primarily determined by their own physiological limits rather than biotic interaction.

Key words: arbuscular mycorrhizal fungi, climate change, ecological niche

Funding: The research reported herein was supported by grant from GAČR 22-06936S Towards understanding community assembly in arbuscular mycorrhizal fungi: from structural traits to fundamental and realized niches and GA UK 413622 Response of arbuscular mycorrhizal fungi on global changes: lesson from realized vs. fundamental niches.

THE IMPACT OF PLANT ROOT EXUDATES ON THE TRANSCRIPTOME OF AROMATIC-COMPOUND-DEGRADING *RHODOCOCCUS* STRAINS

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Plant root exudates comprise a diverse mixture of metabolites that shape interactions between plants and soil microorganisms. Beyond serving as carbon and energy sources, these compounds can modulate microbial gene expression, including those of genes whose activity may, in turn, influence plant fitness. Bacterial soil isolates *Rhodococcus* sp. WAY2 and *Rhodococcus opacus* C1 possess genomic clusters for the degradation of aromatic pollutants such as biphenyl, naphthalene, and ethylbenzene, yet their transcriptional activation strongly depends on environmental cues. Our aim was therefore to examine how plant root exudates affect the bacterial transcriptome, with a specific focus on genes responsible for the degradation of aromatic compounds.
Using RNA-Seq, we examined the transcriptomic responses of both strains when exposed to the exudates of perennial ryegrass (*Lolium perenne* L.), a ubiquitous grass species. We detected significant shifts in the expression profiles of genes involved in sugar and amino acid metabolism, along with shifts in the membrane transport genes expression. Notable changes in expression were observed in gene clusters *nah*, *bph*, and *etb*, responsible for aromatic pollutant degradation. These results suggest that root exudates can modulate bacterial capacity for xenobiotic degradation, providing insight into ecological interactions that may be leveraged to enhance bioremediation processes.

Key Words: plant root exudates, aromatic compound degradation, *Rhodococcus* spp.

Funding: The Czech Science Foundation acknowledges financial support under grant no. 20-00291S and the Ministry of Education, Youth and Sports of the Czech Republic grant Talking Microbes – understanding microbial interactions within One Health framework (CZ. 02.01.01/00/22_008/0004597).

RESPONSE OF SOIL MICROBIAL COMMUNITIES AND MICROBE-MEDIATED ECOSYSTEM PROCESSES TO FOREST HARVESTING: THE ROLE OF FINE-SCALE ENVIRONMENTAL HETEROGENEITY

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Forest disturbances profoundly alter soil ecosystems by reshaping microbial communities that mediate nutrient cycling and carbon sequestration. Despite decades of research, the mechanisms driving fungal community assembly following forest gap formation remain poorly understood, particularly in sensitive karst environments. Understanding whether fungal communities are structured by measurable environmental gradients or by fine-scale heterogeneity and stochastic processes is critical for predicting ecosystem responses to future disturbances. Fungal community structure across GAPS transects spanning three distinct localities within high Karst plateaus in Slovenia was examined across three slope positions (upper, mid, lower). Soil samples from 108 plots were characterized using molecular approaches to assess fungal richness, diversity, and beta diversity in relation to soil properties (pH, moisture, carbon, nitrogen, phosphorus, fungal, NLFA, bacterial and total PLFA).

Here it is shown that fungal communities exhibit high within-group variability that is not significantly structured by measured environmental variables or transect position. Environmental variable fitting revealed no significant associations between soil properties and fungal community composition (all $p > 0.05$, $r^2 < 0.02$). However, clustering analysis identified distinct fungal community types corresponding to the three sampled localities (PERMANOVA: $F = 5.014$, $p = 0.001$, $R^2 = 0.087$), with Cluster 1 displaying the highest richness (1,186 species) and Shannon diversity (6.59), while diversity decreased systematically in other clusters. Three-way ANOVA detected only marginal, non-significant trends for slope position × transect interactions in Shannon diversity ($p = 0.135$) and evenness ($p = 0.131$).

These findings suggest that fungal community assembly is driven by unmeasured fine-scale environmental factors, biotic interactions, or stochastic processes rather than broad-scale soil gradients or spatial position. This mechanistic insight highlights the complexity of belowground communities in karst forests and emphasizes the need for high-resolution environmental characterization to predict ecosystem functioning across disturbance gradients.

Key Words: forest gaps, fungal communities, community assembly, beta diversity, karst ecosystems, transect analysis

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HUMAN LAND USE EFFECTS ON GLOBAL PATTERNS OF FUNGAL RICHNESS

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Soil fungal communities exert a large influence on agroecosystem functioning and are in turn themselves significantly altered by land use. However, large-scale assessments of land-use impacts on their diversity remain limited. We use GlobalFungi to analyze fungal richness and community composition across European grasslands and croplands, disentangling land-use type and intensity from climatic and edaphic factors by combining the dataset with the European LAMASUS database. We identify a hump-shaped relationship between grazing intensity and overall fungal richness, with higher diversity at intermediate management levels. Using hierarchical modeling of species communities (HMSC), we find significant effects of land use on fungal community composition, especially for rare species. Fungal functional groups exhibit divergent responses: pathogen richness significantly increases in croplands, while arbuscular mycorrhizal fungi decline with intensification. These trends are consistent across Europe's sampling network, which includes gradients of management practices.
To assess global relevance, we combine our data with the Human Footprint Index (HFI), which describes anthropogenic influence on terrestrial biomes worldwide. Similarly to the European results, this preliminary analysis reveals divergent responses for different functional groups.
Our findings highlight the vulnerability of key functional guilds to land-use changes and underscore the need for management strategies that balance productivity with soil biodiversity conservation.

Key Words: fungi, macroecology, biogeography, land use

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GUT MICROBIAL DIVERSITY AND COMMUNITY STRUCTURE ACROSS THE DIPLOPODA (MILLIPEDES)

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Millipedes (Diplopoda) are key terrestrial detritivores that contribute to nutrient cycling and organic matter decomposition. Despite their ecological relevance, they remain neglected compared to other detritivores such as termites and earthworms. Millipedes possess compartmentalized digestive systems with distinct physicochemical conditions, which may act as ecological filters shaping microbial communities.

Here, we present a comparative analysis of gut bacterial communities along two intestinal compartments (midgut, hindgut) plus the excrements across 13 Diplopoda species collected from diverse habitats and diets. Additionally, we included the feed samples (leaves, wood, and litter) to enable preliminary host–environment comparisons.

Amplicon data (16S rRNA, ITS and 18S rRNA gene amplicon sequencing) were processed using a custom R-based pipeline This pipeline utilized DADA2 for quality filtering, error correction, and ASV inference, followed decontamination (decontam), multiple normalization strategies, diversity and differential analyses (phyloseq framework). Alpha diversity varied markedly across host species, with *E. pulchripes* exhibiting the highest Inverse Simpson diversity and *P. complanatus* the lowest, while the remaining species formed intermediate groups. Differential abundance testing (ANCOM-BC2) revealed taxa enriched in different comparisons (i.e gut compartments), whose ecological roles remain to be explored.

Variance partitioning revealed that host species identity alone explained 25% of the variation in community composition. While average body size appeared as a strong predictor in ordination models ($R^2 = 0.88$, $p = 0.001$), its contribution overlapped entirely with species identity, suggesting that this association exists because species differ from one another in both size and microbiota and not because size, by itself, is an ecological force shaping the microbiota independently. Additional explanatory variables (e.g., pH and redox potential) are currently being evaluated.

Overall, these preliminary results suggest that the millipede gut microbiota exhibits clear compartment-specific structuring, largely shaped by host species identity rather than continuous host traits. A substantial portion of community variation remains unexplained, highlighting the potential influence of other microenvironmental factors such as pH and redox gradients.

Key Words: Diplopoda, gut microbiota, community composition, millipede, amplicon sequencing

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ROOT-ASSOCIATED FUNGAL COMMUNITIES EN FACE OF RECENT CLIMATE CHANGE IN LADAKH

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Mountain ecosystems are warming rapidly, yet the responses of root-associated fungi that underpin plant performance remain poorly resolved. We resampled the roots of 26 plant species at 15 locations across three mountain ranges in Ladakh and Himachal Pradesh, India, from 3,880 to 4,750 m above sea level, in 2015 and again in 2022. Using ITS2 metabarcoding linked with soil properties, climatic factors, and plant identity, we asked which factors structure these fungal communities, whether assembly is mainly deterministic, and if fungal communities show early signals of community shifts in response to climate change. Mountain range, soil properties, and host plant family best explained community variation. Variance partitioning attributed about 45% of the variation to these factors, with unique fractions for soil properties 12.6%, plant family 6.3%, mountain range 5.3%, and climate 2.2%. A βNGI-framework showed that deterministic processes dominated assembly in 89.5% of pairwise comparisons, split between variable selection (43.4%) and homogeneous selection (46.1%), with only 10.5% consistent with stochasticity. Although alpha diversity and overall network topology of fungal communities changed little between years, networks showed pronounced rewiring, including major hub turnover and taxon-specific shifts in centrality. Stress-tolerant and mycorrhiza-associated genera increased in network influence in 2022 in several contexts, while moisture-dependent zoosporic fungi halved in prevalence between sampling years and moss-associated fungi showed negative climate associations, consistent with observed winter drying and snow loss. Together with modest but coherent seasonal warming and precipitation changes, these results indicate stable membership with adaptive reorganization under selection rather than ecological drift. Deterministic filtering by geo-edaphic context and host identity, coupled with flexible interaction networks, may confer resilience to plant fungal partnerships in one of Earth's fastest-warming cold deserts.

Key Words: Rhizosphere, fungi, high-altitude, climate change, soil, Himalaya

Funding: The research reported herein was supported by the Czech Science Foundation 21-04987S; and the Czech Academy of Sciences [project number: RVO 67985939].

ABUNDANCE AND DIVERSITY OF NITRIFIERS IN THE GUT-COMPARTMENT OF MILLIPEDES

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Nitrifiers are globally spread in diverse habitats, where they are classically considered to be aerobes, responsible for aerobic transformation of reactive nitrogen, an ecosystem function linked to nutrient cycling and global change. However increasingly, they are detected in low oxygen and anoxic systems like the gut of millipedes challenging this current understanding. This study investigates the abundance and diversity of nitrifiers in gut compartments of thirteen different in-house reared species of millipedes. Using meta-barcoded 16S rRNA gene sequencing of DNA extracted from different gut compartments, we consistently detected various nitrifiers of the *Nitrosospira, Nitrosomonas,* and *Nitrobacter* genera. Among the ammonia-oxidising community archaea of the *Candidatus* Nitrosocosmicus strain dominated in the midgut while the bacterial genus *Nitrosomonas* dominated in both hindgut and foregut. These diversity patterns of varied nitrifiers between midgut, foregut, and hindgut compartments possibly reflect niche preferences regarding the availability of oxygen and substrate. Through direct redox measurements of selected hindguts we revealed very reduced conditions of -199 mV to -230 mV, demonstrating the absence of standing concentrations of oxygen and other oxidised molecules. Collectively, our data indicate that nitrifiers may survive and could be stable community members in this microanoxic/anoxic environment, but more detailed research is needed to unveil their adaptations and metabolic activities.

Keywords: nitrifiers, millipede, gene sequencing, gut compartments

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ENDEMIC MICROBIAL SPECIES IN LAKE MALAWI REVEALED BY A GLOBAL FRESHWATER METAGENOMIC SURVEY

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Despite its ecological and cultural prominence as one of the African Great Lakes, as well as being an ancient lake with well-recognized endemic fish populations, Lake Malawi remains comparatively understudied in terms of its microbial diversity. Owing to its considerable age, size, depth, and elevated temperatures, Lake Malawi may harbor microbial species that are both abundant and endemic only to this system. To better understand the microbial communities within Lake Malawi, we conducted a comparative metagenomic analysis of twelve hybrid metagenomes (sequenced with Oxford Nanopore and Illumina technologies) collected along a depth-resolved N-S transect from Lake Malawi against a global dataset of around 400 freshwater metagenomes from other African Great Lakes (Tanganyika, Victoria, Kivu), smaller continental lakes across Africa, and lakes from tropical, temperate, and arctic environments spanning five continents.
Our first objective is to identify taxa, resolved to the species level, that are both abundant and uniquely associated with Lake Malawi. The second objective is to evaluate how non-biotic factors such as geographic proximity and climatic conditions influence microbial community recruitment across the global dataset. We hypothesize that Lake Malawi shares several metagenome-assembled genomes (MAGs) with other African Great Lakes, due to their proximity and similar abiotic conditions. Tropical lakes on other continents may share the same microbial genera and a few ubiquitous species with Lake Malawi, while we expect to find fewer similarities to lakes from other climatic regions. Through this comparative framework, we aim to characterize the distinct biotic signatures of Lake Malawi and gain insight into how biogeography and environmental gradients shape microbial community structure in freshwater ecosystems.

Key Words: Lake Malawi, metagenome, MAGs, freshwater microbes

Funding: The research reported herein was supported by the GACR grants 25-15813S (Mechanisms of growth and survival in freshwater oligotrophs (MEGASO), 2025-2027, PI: M.M. Salcher) and 22-03662S (Phylogeography and ecogenomics of ‘*Ca*. Fonsibacter’ (SAR11-IIIb), 2022-2025, PI: M.M. Salcher).

EFFECT OF WILDFIRE ON SOIL MICROBIAL COMMUNITIES IN ARCTIC TUNDRA

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Soils in circumpolar regions contain vast stocks of organic carbon that were protected for millennia from microbial decomposition by low temperatures. Yet this is changing as due to climate change. Rising air temperatures and extended growing seasons together with more frequent summer heatwaves is intensifying wildfire activities across Arctic tundra (Hu et al., 2015). While fire impacts on vegetation and soil chemistry have been increasingly studied, effects on soil microbial communities, which underpin carbon sequestration and nutrient cycling, remain less understood. The aim of this study was to describe the effect wildfires have on the abundance, diversity and function of soil bacteria and fungi in Arctic tundra. To my knowledge, this is the first study examining the effect of wildfires on soil microbial community in Arctic tundra in longer term. It was shown that fungal community is more strongly affected by wildfires than bacterial community. Fire had no significant effect on total prokaryote nor fungal abundance. Taxonomic composition of microbial community was mainly shaped mainly by sampling site and soil layer, rather than soil chemistry. This is in contrast with studies investigating factors shaping bacterial community composition at other sites (Delgado-Baquerizo & Eldridge, 2019; Ganzert et al., 2014; Tripathi et al., 2019). However, relative abundance of several taxa, especially fungi, varied with fire intensity and interacted with vegetation cover and nitrate levels. These results are a good starting point for investigation what roles do soil microbial community play in Arctic tundra recovery after wildfires and how fires may influence nutrient cycles in this biome.

Key Words: tundra, wildfires, soil microbiome, climate change

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SURVIVAL OF AEROBIC METHANOTROPHS UNDER OXYGEN-DEPLETED CONDITIONS: INSIGHTS FROM *METHYLOBACTER*

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The globally distributed genus *Methylobacter*, an aerobic methanotroph from the *Gammaproteobacteria*, plays a crucial role in mitigating methane emissions from diverse ecosystems, including freshwater and marine habitats, wetlands, soils, sediments, groundwater, and landfills. In some of these ecosystems, this genus may be responsible for oxidising most of the methane produced *in situ*, therefore significantly lowering methane emissions. In recent years, numerous reports have been published on *Methylobacter* spp. presence and often activity in oxygen-depleted environments were made; however, it remains uncertain what kind of metabolism they use and how much they contribute to methane oxidation under these conditions. Here, we first used comparative genomics with publicly available 44 high-quality MAGs and genomes covering the known diversity in the genus to generate hypotheses regarding alternative metabolisms that could allow for activity of *Methylobacter* spp. under oxygen-limited and -depleted conditions. Through this *in silico* analysis, we identified a range of possible anaerobic dissimilatory metabolisms, with hydrogen metabolism standing out, featuring one to four different hydrogenases in each species. Hydrogen is an abundant resource produced in oxygen-depleted conditions, and methanotrophs from other classes are known to be able to metabolise it. In a follow-up pure culture experiment, we showed that under oxygen-depletion (0.6%-0%), *Methylobacter tundripaludum* SV96 can co-oxidise hydrogen with methane, likely providing additional energy. To our surprise, we did not detect differentially expressed genes between *M. tundripaludum* amended with methane or with methane and hydrogen and genes encoding for a type 1d [NiFe]-hydrogenase were expressed in all the tested oxygen-depleted conditions. Our work provides a first glimpse into the previously overlooked metabolism of gammaproteobacterial aerobic methanotrophs during oxygen-depleted conditions.

Key Words: metabolic versatility, aerobic methanotrophy, oxygen-depleted conditions, hydrogen metabolism

Funding: The research reported herein was supported by the grant 21-17322 M from GA ČR

CLIMATE AND SOIL CHEMISTRY SHAPE GLOBAL GRADIENTS IN SOIL BACTERIAL DIVERSITY

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Biodiversity patterns in bacteria present an enigma. Even though bacteria represent significant portion of biodiversity in terms of kingdoms, phyla and families, how their diversity is distributed in space remains unclear due to a lack of global data, resulting in conflicting hypotheses. Here, we leverage a newly compiled, comprehensive global database for bacteria: GlobalBacteria. Based on amplicon sequencing, the database covers 12,000 samples covering all major world's continents, biomes and climates. We find that bacteria show the classic latitudinal gradient in alpha, beta, and gamma diversity, such that their diversity peaks in the tropics. This pattern is consistent with the results for most organisms, but diverges from it in a compelling aspect, namely bacterial diversity peaks outside the tropical rainforests and, instead, reaches its maximum in the tropical savannahs. Beta diversity, which captures changes in species composition, also increases toward the equator but, surprisingly, seems to be driven by environmental pH rather than by physical distance between the bacterial communities. Finally, gamma diversity varies across continents, reaching its highest value in Africa. These results were robust to the choice of sampling corrections, modelling approach, and geographic scale. Altogether, our findings confirm that tropics serve as the global hotspot of biodiversity, which seems to apply across a wide variety of taxa, including bacteria, which provide key ecosystem functions and services.

Key Words: soil bacteria, latitudinal diversity gradient, global diversity maps, bacterial biogeography

Funding: The research reported herein was supported by Czech Science Foundation (25-15251S).

FROM DEPTHS TO LAB: DETECTION, ISOLATION AND CULTIVATION OF NOVEL *PATESCIBACTERIOTA* LINEAGES

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Candidate Phyla Radiation (CPR) or *Patescibacteriota* is a recently identified bacterial phylum with a peculiar lifestyle. Their reduced genome, metabolic repertoire and cell sizes, restrict them to an epiparasitic or episymbiotic lifestyle. Recently, environmental microbiologists uncovered their presence across natural microbial communities, including thermokarst lakes, hypersaline soda lake sediments and groundwaters, where they can make up to 20-70% of the total bacterial community. While metagenomic analyses do serve this purpose well, only a few have been isolated into culture, such as members of the *Saccharimonadia class*, therefore, studying their ecological role remains a challenge.

In order to characterize the prokaryotic community and obtain stable cultures from diverse CPR lineages, we sampled six lakes from Central Europe: the subalpine Lake Maggiore (Italy), the alpine lakes Cadagno (Switzerland) and Hallstättersee (Austria), and the acidic lakes Plešné and Čertovo (Czechia) during 2024-2025. Metagenomic sequencing was conducted on these time-series, resulting in >7000 medium and high-quality metagenome assembled genomes, out of which 676 belong to *Patescibacteriota*. Isolation efforts entail a dilution-to-extinction (DTE) cultivation in 96-well plates containing three different artificial lake water media. Currently, 24480 wells have been screened using flow cytometry for potential CPR presence, and 2567 stable co-cultures and mixed cultures were prepared and maintained. So far, the presence of *Patescibacteriota* has been confirmed in 105 of them. Importantly, a pure and stable co-culture of '*Candidatus* Boromeibacter lacustris' and its host *Undibacterium* sp. has been obtained from the hypolimnion (200 m depth) of Lake Maggiore.

'*Ca.* B. lacustris' has a genome size of 1.4 Mbp containing 1428 genes, including 519 that encode for hypothetical proteins (~36%), lacking genes coding for the citric acid cycle, lipid biosynthesis, and carbohydrate metabolism, most amino acids and cofactors. Lipid analysis through gas chromatography of an axenic *Undibacterium* sp. culture and its coculture with '*Ca.* B. lacustris' reveals a shift in lipid production from energy storage palmitic-oleic-linoleic and trilaurin-dipalmitin-olein triglycerides to phospholipids (two types of phosphatidylethanolamines), diacylglycerols and long-chain unsaturated and saturated triglycerides under CPR infection, hinting at metabolic stress. Overall, this work highlights the importance of further isolation efforts into CPRs, shedding light on the ecological importance of CPRs in controlling microbial communities.

Key Words: *Patescibacteriota*, lipids, lakes, isolation, coculture.

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GLOBAL ECOLOGY OF HUMAN-OPPORTUNISTIC FUNGAL PATHOGENS

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Fungi are among the most diverse eukaryotes on Earth and include numerous environmental species capable of causing severe opportunistic infections in humans. Despite their growing public-health importance, the environmental niches and global distribution of these pathogens remain poorly understood. Here we analyse 87,540 environmental samples spanning all continents, major terrestrial biomes and land-use types to map the global ecology of human-opportunistic fungal pathogens. We uncover strong global asymmetries in pathogen occurrence, with markedly higher prevalence in Africa and Asia than in Europe or North America. *Aspergillus* was detected in 40% of all African samples, while Asia emerged as a hotspot of overall pathogen diversity. Climatic factors (temperature and precipitation) explained broad-scale variation in pathogen richness but did not account for the exceptionally high diversity observed in Asia. Instead, diversity hotspots were concentrated in croplands, where crop type—most notably maize—strongly influenced pathogen concentration. These findings reveal previously unrecognised links between agricultural practices, environmental conditions and the proliferation of opportunistic fungal pathogens, providing an ecological basis for assessing population-level risk and highlighting the need for more sustainable land-use strategies to mitigate emerging fungal threats.

Key Words: Opportunistic fungal pathogens, Environmental reservoir, Global distribution, Croplands, Climate

Funding: The research reported herein was supported by the grant Holistic management practices, modelling and monitoring for European forest soils – HoliSoils (EU Horizon 2020 Grant Agreement No 101000289)

NOVEL BACTERIAL PHYLA FOUND IN JÁCHYMOV THERMAL SPRINGS

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Groundwater ecosystems are reservoirs of phylogenetic novelty but are often poorly explored due to their inaccessibility. Most of these novel prokaryotic lineages are yet impossible to culture and only way to study them is through culture-independent methods like metagenomics. The radioactive thermal springs in Jáchymov have been used to treat musculoskeletal diseases for over 100 years, yet their microbial composition remains undescribed. Metagenomic sequencing of two Jáchymov thermal springs revealed two previously undescribed phyla: one represented by two metagenome-assembled genomes (MAGs) from the Běhounek spring and another represented by a single MAG from the Curie spring. Metabolic predictions suggest that both phyla encode the Wood–Ljungdahl pathway, which is likely to operate in the reductive direction for carbon fixation. Notably, the Běhounek MAGs possess a hybrid carbon monoxide dehydrogenase/acetyl-CoA synthase complex composed of bacterial and archaeal subunits. These organisms appear to generate energy through hydrogen oxidation coupled to a simple electron transport chain. In contrast, the phylum represented by the Curie MAG may be capable of ferric iron reduction via extracellular electron transfer. These findings highlight the Jáchymov springs as a source of evolutionarily distinct prokaryotes.

Key Words: metagenomics, groundwater, phylogenetic novelty

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CHARACTERIZATION OF ENDOGENOUS VIRUSES FROM NEWLY ISOLATED FRESHWATER OOMYCETES

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Viruses are a major part of freshwater microbial communities, having a central role in biogeochemical cycles and food webs. However, research regarding viral diversity and their interactions with certain groups of freshwater protists is still scarce, as is the case for oomycetes. Different viruses can have diverse effects on the microbial population dynamics, especially during bloom periods. For example, polinton-like viruses (PLVs) may defend their protist hosts against giant viruses (GVs), which are associated with the spring bloom decline and collapse. PLVs are small double-stranded DNA viruses (14–40 kb) with a dual lifestyle: they can exist both integrated within the genomes of their eukaryotic hosts, and as free capsid-bound particles which are particularly abundant in aquatic ecosystems.
During the April 2025 spring bloom, oomycetes were isolated from the freshwater Římov Reservoir (Czech Republic), from epilimnetic biomass and detrital leaves. These isolates were then exposed to 0.2–0.8 µm viral fractions from the same location. High-quality genomes for eight oomycete strains were obtained by combining high-molecular-weight DNA isolation and long-read sequencing. Their endogenous viral elements were identified based on hallmark genes, GC-content shifts, and terminal repeats (TRs). Only four isolated oomycetes contained PLVs, with the most abundant being found in *Phytophthora gonapodyides* (n=50), followed by *Pythium sp.* (n=17), and were scarce in the two distinct *Phytopythium litorale* strains (n=2). Gene annotations revealed that these PLVs can integrate into hosts`s genomes by employing either retrovirus-type integrases or tyrosine recombinases. Further oomycete infection experiments are expected to advance our understanding of protist-virus interactions.

Key Words: polinton-like viruses, oomycetes, freshwater, aquatic ecosystems

Funding: The research reported herein was supported by the grant project GACR 25-15920S: Polinton-like Viruses: Guardians or Predators of Protists; (duration 2025-2027).

PLANT-BACTERIA SYSTEM WITH PGP ACTIVITY FOR ENHANCED DEGRADATION OF TOLUENE AND XYLENE

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Biodegradation is a common strategy for the efficient removal of aromatic pollutants, including toluene and xylene. To enhance this process, we assembled a plant-bacteria system consisting of a genetically modified tobacco plant (*Nicotiana tabacum*) transformed with a gene encoding an aromatic ring cleavage enzyme (*bphC*) and hydrocarbon-degrading bacteria exhibiting plant growth promoting (PGP) activity. Soil contaminated by petrochemical production provided the source of the bacteria. The bacterial samples were identified and dereplicated by MALDI-TOF MS or 16S rRNA sequencing to create a set of non-recurring isolates. These isolates were then tested for two traits: their degradation activity using toluene or xylene as the sole source of carbon and their PGP activity: nitrogen fixation, phosphate solubilization, ACC deaminase biosynthesis and IAA production using biochemical tests based on Nfb, NBRIB, Dworkin-Foster and TSB media, respectively. Degradation rate of the best-performing bacterial isolate, *Pseudomonas alloputida* ER57, was then measured using gas chromatography. A single culture of this isolate achieved 67% xylene removal after 12 hours and 98.8% toluene removal after 24 hours. The effect of the complete plant-bacteria system on the overall biodegradation rate is currently being evaluated in semi-field conditions using the original contaminated soil, combining the tobacco plant and *P. alloputida* ER57.

Key Words: Biodegradation, Plant growth promotion, Toluene, Xylene, Dereplication, *Pseudomonas alloputida*

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CULTIVATION AND GENOMIC CHARACTERIZATION OF NOVEL METHANOGENS FROM ARID DESERT BIOCRUST

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Methanogens are strictly anaerobic archaea capable of energy conservation by methane production, yet their presence in oxic and arid environments challenges existing paradigms. In this study, we enriched and genomically characterized seven methanogenic cultures from desert biocrusts, affiliated with the genera *Methanobacterium*, *Methanosarcina*, and *Methanocella*. Six of these new cultures represent new species. Nonetheless, phylogenomic analyses revealed close genetic relationships with organisms from anoxic environments, indicating the absence of an evolutionary distinction. Comparative genomics exposed diverse though non-unique repertories of antioxidant (e.g. catalase, superoxide dismutase and desulfoferrodoxin), and desiccation-resistance genes (including genes for maintaining osmotic pressure and repair of cell wall and membrane), with *Methanobacterium* spp possessing the lowest gene abundance and diversity for oxygen and desiccation tolerance. Nevertheless, the occurrence of a Class I methanogen such as *Methanobacterium* in arid soils challenges the notion that members of this class are less oxygen tolerant than Class II. Pangenome analysis further uncovered unique genes enriched in membrane-associated functions and potentially non-functional stress-related genes. Via a global metagenomic survey we find that methanogens are underdetected in dryland soils, likely due to sequencing depth limitations. Our findings highlight previously overlooked methanogen diversity and ecological plasticity in oxic and desiccated habitats, and emphasize the need for further studies to elucidate their survival strategies.

Key Words: archaea, methanogens, ROS, desiccation, comparative genomics

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ACCLIMATIZATION-ENABLED ANAEROBIC OXIDATION OF METHANE BY *CA*. METHANOPEREDENS AFTER PROLONGED ACID STRESS

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Members of the archaeal genus *Candidatus* Methanoperedens are able to oxidise methane under anoxic conditions while utilising available electron acceptors such as nitrate. The most advanced laboratory enrichment cultures of *Ca.* Methanoperedens perform methane oxidation at near-neutral pH. However, amplicon data from acidic peatlands show the presence of Methanoperedens also in acidic conditions of pH 4-5. Here we investigated whether an enrichment culture of *Ca.* Methanoperedens Vercelli Strain 1 is able to adapt to a stepwise decrease in pH in a cultivation bioreactor. Bioreactor batch activity assays utilising 13C-CH4 in the headspace were used to compare methane oxidation rates at individual pH checkpoints on the gradient from 7.25 to <6. The enrichment culture adapted to pH decrease, as evidenced by sustained oxidation activity. The side community in the bioreactor decreased in abundance, while the relative proportion of *Ca.* Methanoperedens cells to the side community remained stable. Analyses of archaeal membrane-spanning lipids revealed that acidic conditions did not alter the composition of the cell membrane. Energy harvested from oxidation may not be invested in substantial growth of *Ca.* Methanoperedens. We hypothesize that cells of *Ca.* Methanoperedens remain metabolically active at sub-optimal pH values, allocating the harvested energy to maintenance rather than growth. Such observation can be used to predict the ability of important anaerobic methane oxidisers to colonize distinct environmental niches including acidic peatlands.

Key Words: anaerobic oxidation of methane, acidification, ANME

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PERSISTENCE OF *ACINETOBACTER* SPECIES IN MANURED FIELDS

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Cattle manure, commonly used as soil fertilizer, is known to harbor antibiotic residues and antibiotic resistant bacteria. *Acinetobacter* spp., including pathogenic *Acinetobacter baumannii* occur in cattle manure. Moreover, previous soil microcosm studies have shown that *Acinetobacter* species and their associated antibiotic resistance genes can disseminate from manure to soil. However, it remains unclear whether acinetobacters can also persist in agricultural fields following manure application.
In this study, we monitored *Acinetobacter* abundance and species composition on farmers' fields over three months after manure application. In two Czech conventional dairy farms, we collected manure (fresh or composted) and bulk soil from five plots per field before manuring and at 5-6 time points afterward. In addition, rhizosphere soil was sampled at Farm 1 at three months post-manuring. The average *Acinetobacter* abundance in fresh manure (Farm 1) was ~1E+10 16S copies/g dry weight (6% of total bacteria), whereas in composted manure (Farm 2) it was only ~7E+07 16S copies/g dry weight (0.07%). Before manuring, acinetobacters were below the detection limit (~1E+06 copies/g dry weight) in both fields. Following manure application, *Acinetobacter* abundance fluctuated over time. At Farm 1, acinetobacters were detectable over three months, peaking at ~1E+08 16S copies/g dry weight after four weeks. In contrast, at Farm 2, abundance declined below the detection limit by that time. Therefore, *Acinetobacter* species composition in field soils was analyzed only at Farm 1 using *rpoB* metabarcoding. Within the first two weeks (t3), the *Acinetobacter* community showed high spatial heterogeneity, which became more homogeneous after 4-8 weeks (t4–5). At three months (t6), when crops (a mixture of wheat and legumes) were growing, spatial heterogeneity increased again. After three months, the dominating *Acinetobacter* species in the field was either *Acinetobacter pseudolwoffii* or *Acinetobacter terrae*. Additionally, *Acinetobacter guillouiae* and potentially soil-borne *Acinetobacter calcoaceticus* were prevalent in several rhizosphere samples. Importantly, *A. baumannii* was not transferred to any of the manured fields.
This study shows that acinetobacters can persist in the field for at least three months following manure application and are also able to colonize the rhizosphere of cultivated plants. These findings highlight the need for further investigation of their contribution to the spread of antibiotic resistance in agricultural fields.

Key Words: *Acinetobacter*, cattle farm, manure, qPCR, *rpoB* metabarcoding

Funding: The research herein was supported by the Charles University Grant Agency (grant number 160125; Transmission of antibiotic resistance genes from manure to agricultural field via *Acinetobacter* plasmids) and the Czech Science Foundation (grant number 22-05373S; Cattle excrements and manure as a reservoir of acinetobacters representing risk to human health, 2022-2024).

HIERARCHICAL MODE OF EVOLUTION IN FRESHWATER SAR11 DRIVEN BY SPECIES DISPERSAL AND LAKE HISTORY

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Microorganisms dominate aquatic ecosystems, yet the evolutionary forces shaping their biogeography remain poorly understood. Freshwater habitats, acting as “isolated islands", may generate distinct evolutionary patterns in contrast to globally connected marine systems. Here, we investigated the globally abundant freshwater SAR11 genus *Fontibacterium* using a comparative population genomics framework. We analysed genome-wide single nucleotide variants (SNVs) from three species with contrasting dispersal capacities: an African endemic (*F. africanum*), a temperate specialist (*F. temperatum*), and a cosmopolitan species (*F. commune*). All species exhibited significant lake-specific population structure, but the scale of differentiation was constrained by their dispersal potential, ranging from complete genetic isolation between ancient lakes in the endemic species to transcontinental cohesion in the cosmopolitan one. Notably, the cosmopolitan species showed near-complete genetic cohesion between antipodal populations (Japan and Brazil) suggesting stochastic long-distance dispersal without geographic barriers. Furthermore, population genetic signatures varied among lake systems, especially elevated nucleotide diversity and pN/pS ratios in ancient African lakes suggesting relaxed purifying selection; a pattern that may reflect their unique evolutionary history. These findings support a hierarchical model where a species' dispersal capacity sets the broad-scale potential for gene flow, while local lake history determines the fine-scale genetic structure of its populations.

Key Words: SAR11-IIIb, *Fontibacterium*, biogeography, population genomics, freshwaters

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IMPROTANCE OF FOREST SOIL BIODIVERSITY MONITORING

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Soil microorganisms are key regulators of forest biogeochemical processes, including nutrient cycling, organic matter decomposition, and greenhouse gas (GHG) exchange. However, belowground biodiversity remains insufficiently monitored at larger spatial scales, particularly in current times of increasing natural disturbances intensity driven by ongoing climate change, combined with the impacts of traditional forest management practices. Understanding how microbial communities respond is essential for predicting ecosystem resilience.

Here, we provide an overview of soil microbial biodiversity responses observed within the HoliSoils project (H2020; https://holisoils.eu/), which aims to study microbial and biogeochemical dynamics across 23 forest sites in 8 European countries, including boreal, temperate and Mediterranean forests. Standardized sampling protocols and harmonized analytical workflows enable cross-site comparison of microbial composition (16S rRNA and ITS2 amplicon sequencing), microbial biomass, extracellular enzyme activity, soil respiration and soil chemistry (C, N, pH, available P). At selected sites, metagenomics and multi-year sampling permit assessment of functional potential and temporal development following disturbance.

Across sites, high-intensity disturbance consistently reduced the relative abundance of symbiotic fungal groups and microbial biomass, often with increasing effects over time. Bacterial community composition was relatively stable but showed an indication of a functional response to extreme management practices, as in the case of clearcutting. Post-disturbance management practices, such as the addition of wood residues, seem to restore some functionality, as evidenced by enzyme activities returning to levels comparable to control conditions.
Root exclusion via trenching resulted in consistent loss of microbial biomass accompanied by shifts in community composition, with a higher relative abundance of saprotrophic fungi compared to ectomycorrhizal fungi. Interestingly, the fungal-to-bacterial biomass ratio emerged as one of the strongest predictors of soil CO_2 fluxes, pointing at a need to include microbial biomass ratio both to modelling and future biodiversity monitoring.

Our findings demonstrate that coordinated, long-term monitoring of microbial diversity, biomass and function is essential for detecting early ecosystem change and predicting biogeochemical consequences in disturbed forest soils.

Key Words: soil microbial biodiversity, biomass ratio, forest disturbance

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NOTES