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RESEARCH ARTICLE

Health or disease – a question of rhizomicrobial ecology? The case of grapevine trunk disease

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Summary

Aims The incidence of the apoplectic breakdown associated with grapevine trunk diseases (GTDs) is promoted by climate change, which has become a challenge for viticulture worldwide. Outbreak of these conditional diseases is expected to depend on the rhizomicrobiome. However, the impact of the rhizomicrobiome on grapevine resilience has remained poorly understood, particularly regarding its ecological aspects. This study explores the link between GTDs, the rhizomicrobiome, and soil chemistry in vineyards along the Upper Rhine.

Methods Using amplicon sequencing for both fungal and prokaryotic communities, we show that around half of the fungal rhizosphere community is endowed with pathotrophic potential, independently of the health status of the plant, including seventeen previously reported GTD-associated taxa, predominantly Black Foot Disease.

Results In contrast to fungi, bacterial diversity is shifted depending on the micronutrients Fe, Cu, Mn, and Zn. Moreover, taxa enriched in the rhizosphere of asymptomatic vines, such as *Pseudophialocephala* and *Collarina* for the mycobiome, and *Caulobacter*, *Kitasatospora*, and *Entotheonellaceae* for the bacteriome, showed correlations with soil properties. The most prominent feature associated with disease outbreaks was drastic changes of microbial co-occurrence networks. These were significantly increased in the fungi, especially for GTDs taxa, such as *Fomitoporia, Stereum, Phaeomoniella*, and *Neofusicoccum*. By contrast, there was a depletion of many bacteria and their microbial interactions under disease outbreak such as *Isoptericola, Caulobacter, Rhodomicrobium* and *Thioprofundum*.

Conclusion Thus, likely microbial interactions and not the mere presence of GTDs taxa explains disease outbreak. This finding opens new strategies for sustainable management of GTDs.

Keywords Climate Change and Pathogens · Co-Occurrence Networks · Grapevine Trunk Diseases · Rhizomicrobiome · *Vitis vinifera*

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Introduction

Grapevine trunk diseases (GTDs) threaten viticulture worldwide, accelerated by ongoing climate change. In France alone, yield losses attributed to GTDs reached 25% in 2016, corresponding to an economic deficit of approximately 5 billion US dollars (https://www.maladie-du-bois-vigne.fr). GTDs comprise a diverse group of vascular and wood-decaying diseases, including Botryosphaeria dieback, Esca syndrome, Eutypa dieback, Diaporthe dieback, and black foot disease, and are associated with a wide range of fungal taxa (Li et al. 2023a; Romanazzi et al. 2009). Multiple genera have been reported as causal agents for each disease complex, such that GTDs cannot be attributed to single pathogens but rather to consortia of wood-colonising fungi with variable epidemiology and aggressiveness, (for review, see Guan et al. 2016; Kenfaoui et al. 2022; Fontaine et al. 2025).

Unlike classical plant diseases, GTDs do not follow Koch's postulates. They are conditional diseases, meaning that meaning that the expression of symptoms is not correlated to pathogen abundance, but rather depend on the condition of the host. For instance, the aggressive Botryosphaeria dieback fungus *Neofusicoccum parvum* switches from an endophytic to a necrotrophic lifestyle when grapevines experience severe drought stress. This transition is linked to the accumulation of the lignin precursor ferulic acid, which acts as a "plant surrender" signal, inducing secretion of fungal virulence factors that trigger programmed cell death in the host (Khattab et al. 2023). In the absence of ferulic acid, the fungus manipulates the homeostasis between defense and growth of the host by secreting an auxin mimic, 4-hydrophenylacetic acid, interfering with specific branches of phytoalexin synthesis (Flubacher et al. 2023).

Conditional pathogenesis is not unique to GTDs. While plant–pathogen interactions are often conceptualised as a battle between host and pathogen, this view represents a reduction of a much more complex ecological reality. In fact, the outcome of this battle depends on numerous environmental factors, including the presence of other microorganisms that can strongly influence infection processes. Resistance of plants to soil-borne diseases has repeatedly been associated with differences in rhizosphere microbiota rather than pathogen load alone (Kwak et al. 2018). As sessile organisms, plants actively shape the microbial communities in their rhizosphere, and numerous studies have shown that beneficial soil microbes enhance plant survival under biotic and abiotic stress (De Vries et al. 2020; Field et al. 2015; Ren et al. 2019). These observations support the concept that plant–rhizomicrobiome interactions are shaped by co-evolutionary dynamics and that the rhizomicrobiome functions as a "second genome" that contributes to plant health and resilience (De Vries et al. 2020; Mendes et al. 2011).

Accordingly, even for the same host genotype and similar soil physicochemical properties, disease outcome can range from complete breakdown to full asymptomatic persistence depending on rhizomicrobiome composition (Wei et al. 2019). Shifts in rhizomicrobiome can therefore serve as early indicators of plant resilience or susceptibility (Gu et al. 2022; Wei et al. 2019). Furthermore, and experimental enrichment of protective microbial consortia has been shown to suppress disease outbreaks in several crop systems (Lee et al. 2021). These findings highlight that the microbial interactions, rather than individual taxa, could be critical determinants of disease outbreak.

In the context of GTDs, however, the role of the rhizomicrobiome has hardly been investigated. Most studies have focused on fungal communities in wood tissues, while belowground microbial ecology remains poorly explored. The few available studies suggested that GTD outbreak were more linked to the incidence of *Fusarium spp.* in the rhizosphere (Li et al. 2023a, b), or the depletion of specific bacterial taxa, e.g., members of bacterial family *Bacillaceae* (Fotios et al. 2021). These observations suggest that GTD outbreak may be driven by broader ecological changes in the rhizomicrobiome rather than by pathogen incidence alone.

The composition and function of soil microbiomes are strongly shaped by physicochemical soil properties. Soil acidification, for instance, reduces the capacity of microbial communities to suppress fungal pathogens, e.g. Fusarium (Li et al. 2023a, b), while micronutrient availability modulates microbial richness, diversity, and function (Dai et al. 2023a, b). Large-scale surveys have identified metallic micronutrients such as iron, manganese, copper, and zinc as major drivers of soil microbiome structure. These elements are essential cofactors for microbial enzymes

involved in respiration, redox reactions, and nutrient cycling, and can directly influence competitive interactions between microbes (Dai et al. 2023a, b). For iron in particular, experimental evidence shows that rhizosphere microbiomes can suppress pathogens under iron limitation through siderophore-mediated competition, whereas this suppression is lost when iron becomes abundant (Gu et al. 2020).

In viticulture, even small differences in soil properties and water management can have pronounced effects on yield and grape quality, a phenomenon traditionally referred to as terroir. Increasing evidence suggests that these effects are at least partly mediated by shifts in soil and plant-associated microbiomes (Gilbert et al. 2014). Taxonomic composition and diversity of vineyard soil microbiomes are influenced by geography, management practices, and soil chemistry (Coller et al. 2019). and soil microbial communities can act as reservoirs for a reservoir for vine-associated microbial flora (Zarraonaindia et al. 2015). However, how soil-driven microbiome patterns intersect with GTD outbreak remains largely unresolved.

The interplay between grapevine rhizomicrobiome, soil properties, and GTD outbreak has not yet been systematically addressed. Filling this gap is critical for developing sustainable strategies to enhance grapevine resilience, as classical breeding approaches are slow and insufficient to cope with rapidly progressing climate-associated challenges. Targeting beneficial rhizomicrobiota promoting grapevine resistance might act as a fast and sustainable approach against GTDs. This study employed a microbial-ecological strategy, probing the rhizomicrobiome from symptomatic and asymptomatic vines coming from the same vineyard, and sampling over a transsect of ten vineyards differing in soil composition. We used this approach to elucidate the roles of rhizomicrobiome structure and soil nutrient dynamics in grapevine resilience to trunk diseases.

The main question of this study is whether the GTD outbreak is driven only by GTD-associated fungi, or whether it could be associated with broader shifts in the rhizomicrobiome and microbial interactions. Specifically, we tested whether (i) GTD-associated fungi show similar levels in both symptomatic and asymptomatic vines, (ii) disease outbreak correlates with the depletion of specific microbial taxa or altered microbial co-occurrence networks, and (iii) soil micronutrient could shape rhizomicrobiome composition and diversity in vineyards. Understanding these interactions between microbes, nutrients, and disease symptoms will help develop sustainable GTD management strategies.

Methodology

Sampling of rhizosphere soil

To identify whether the outbreak of GTDs might be associated with shifts in the composition of rhizosphere microbes, ten vineyards were sampled in August 2022 along the German side of the Upper Rhine representing Northern (Rauenberg), central (Ringsheim), and Southern (Eichstetten, Ihringen) domains within the viticulture region Baden. The majority (eight sites) comprised the commercially important variety Müller-Thurgau, and two sites the traditional variety Silvaner (Suppl.Fig. 1). Rhizosphere soil was collected at 20 cm below the surface from the root-hair zone of plants that either displayed GTD symptoms or were asymptomatic. The soil was immediately transferred to dry ice and remained there during transport, before long-term storage at −80 °C. Each vineyard is represented by six rhizosphere samples, three samples from symptomatic, and three from asymptomatic plants.

Extraction of DNA and amplicon sequencing

Soil DNA was extracted from aliquots of 400 mg soil using the DNeasy PowerSoil Pro Kit (Qiagen, Hilden-Germany) following the instructions of the manufacturer. Phenolic compounds were removed by washing the DNA with 10% v/v of sodium acetate, then, DNA concentration was quantified using the Qubit® 3.0 fluorometer (Thermo Fisher Scientific) with the Qubit™ dsDNA HS Assay Kit, and quality assessed spectrophotometrically (NanoDrop™ 2000/2000c spectrophotometer, Thermo Fisher Scientific). To analyse the taxonomic structure of the microbial community, 10 ng of the purified DNA were used as template to either amplify 16S ribosomal RNA gene of prokaryotes, or the Internal Transcribed Spacer (ITS) of fungi. For the 16S rRNA, V4-V5 region was targeted using, 0.16 µM of the primer set 5'-GTGCCA GCMGCCGCGGTAA-3' and 5'-CCGTCAATTCCT TTGAGTTT-3' ligated with the Illumina adapter. For the ITS, the ITS2 region was addressed with the

same concentrations of primers 5'-GCATCGATG AAGAACGCAGC-3' and 5'-TCCTCCGCTTAT TGATATGC-3'. To increase specificity, the PCR was conducted using touchdown cycling at 52–56 °C. Annealing took place at 55 °C. After PCR, an amplification step was included using 0.04 U/µL of Q5 High-Fidelity DNA Polymerase in presence of Q5 High GC Enhancer (Thermo Fisher). Amplicons were then cleaned up with the DNA Clean & Concentrator Kit (Zymo research, Germany) and subsequently used to prepare amplicon sequencing libraries. Here, 110 ng of cleaned amplicon were selected for a fragment size of 400–600 bp in two steps using 0.4x and 0.7×Agencourt Ampure XP beads (Beckman Coulter). Upon size selection, amplicons were ligated to dual index primers NEBNext® Multiplex Oligos for Illumina® (New England Biolabs, Frankfurt, Germany) following the protocol of the manufacturer, and cleaned afterwards using Ampure XP beads. The prepared libraries were diluted, pooled for equimolarity, and sequenced on a Illumina Novaseq platform to generate 150,000 pair-end reads per sample (2×250 bp) (Novogene, Munich, Germany).

Soil chemical analysis

For every vineyard, 6 soil cores were pooled to form a composite representative sample. Soil samples were then sent for chemical analysis using standards assays on soil type, pH, as well as content of macronutrients and micronutrients (Agricultural Analytical and Research Authority of the State of Rheinland-Pfalz, Speyer) following the rules of the German Fertiliser Regulation (DüV).

Analysis of sequence reads

The obtained paired-end reads from the Illumina sequencer were subjected to quality assessment using the FASTQC tool (Andrews, 2010). Low-quality reads and Illumina adapters were trimmed, and subsequently merged using FASTP (Chen et al., 2018). The merged fastq reads were further denoised to filter out chimeric reads as well as reads shorter than 250 bp using the DADA2 plugin in the QIIME2 pipeline based on the denoise-single method (Bolyen et al. 2019). The samples were then mapped to the corresponding sequences and their frequencies calculated, and the resulting operational taxonomic units (OTUs) were then classified either using the database Silva_99 (Robeson et al. 2021) for 16S reads, or the UNITE database (Abarenkov et al. 2024) for reads of fungal ITS. The fungal OTUs were classified with respect to their trophic mode using the FUNGuild database, which classifies fungal taxa based on their trophic mode (saprotroph, symbiotroph, or pathotroph), their associated hosts, and, in case of pathogens, their preferred colonization target (wood, root or leaf) (Nguyen et al. 2016). Since fungal taxa associated with GTDs are not specified in this or alternative databases, we classified, for the current study, wood-trophic taxa that had been previously reported as GTD causal agents (Li et al. 2023a; Martín et al. 2022) as GTDs community. To visualise the high complexity, heatmaps were constructed based on relative frequencies using the ComplexHeatmap software and the circlize tool, clustering variants of rows and columns variants based on their Euclidean distances (Gu 2022).

Statistical analysis

To identify rhizomicrobiome members correlated with the outbreak of GTDs, we probed for potential differential abundance among the Müller-Thurgau vineyards using the Analysis of Compositions of Microbiomes with Bias Correction (ANCOM-BC) tool implemented in R. This tool has been developed to derive statistically consistent parameters on the base of samples that differ in size (Lin & Peddada 2020). Here, the status of the plant (asymptomatic versus asymptomatic) was set as covariate of interest, while the OTUs classified with respect to their role in GTDs were scored per rhizosphere sample. Significantly shifted OTUs were then plotted on a log-linear scale over the plant status to yield log-fold changes, test statistics, standard errors, P values, adjusted P values, and differential abundance.

Diversity metrics and correlations analyses

To quantify differences in the composition of the rhizomicrobiome in relation to chemical soil profiles and GTD outbreak, we used several parameters. To address α-diversity (the diversity in a given location), we used the Shannon index as overall estimate (Shannon 1948). To account for the fact that rare OTUs might be underrepresented due to sampling bias, we also calculated the Chao1 indices (Chao 1987), and

the Faith Phylogenetic Diversity (Faith_PD) index, a parameter that also considers phylogenetic relationships between the taxa (Faith 1992). All these parameters were calculated using the respective QIIME2 tools. The non-parametrical Kruskal–Wallis test was used to test statistical significance for differences in the a-diversity indices over chemical profile of the soil and the GTD symptomatics.

As alternative approach to assess β-diversity (i.e., differences between different locations), we conducted a Principal Coordinate Analysis to detect commonalities between the sites. For parametrisation, we used here either the Bray–Curtis distance using *vegan* package for R for ecological analyses (Oksanen et al., 2024), or the Weighted-Unifrac distance (implemented in QIIME2). For visualisation, we employed differentially coloured polygons through the ggplot2 plugin of R, and the stat_ellipse command set at a confidence level of 95%. To classify the chemical profiles of the vineyards, a Principal Component Analysis was carried out using the FactoMinor and factoextra packages of R. In addition, Pearson correlations between rhizomicrobiome diversity metrics and soil chemical profile were calculated and plotted using Hmisc and corrplot packages of R (Harrell, 2024; Wei & Simko, 2021).

Construction of Co-occurrence networks

Changes in the rhizomicrobiome dynamics and interactions under GTDs outbreak were studied by calculating co-occurrence networks either for asymptomatic or symptomatic vines with a resolution to the genus level. Correlation networks were assessed and visualized using R packages phyloseq (McMurdie & Holmes, 2013), microbiome (Lahti & Shetty, 2017), Hmisc (Harrell, 2024), igraph (Csardi & Nepusz, 2006), and ggplot2 (Wickham, 2016). Fungal and prokaryotic community networks were constructed at the genus level. After elimination of non-annotated OTUs, pairwise correlations were determined using Spearman's correlation coefficients, filtered based on thresholds of $P < 0.05$ for statistical significance and $|r| > 0.6$ for correlation strength.

Results

Differences in chemical profile are reflected in differences of the rhizomicrobiome

A Principal Component Analysis of soil chemical properties (Suppl.Fig. 2a) revealed several types of chemical profile. Soils of vineyards C, D, I, and J showed similar chemical characteristics. Vineyards G and H were categorized separately, mostly due to their low levels of organic Carbon (C), Nitrogen (N), and Boron (B), whereas vineyard F exhibited the opposite profile, characterized by elevated concentrations of these elements. In addition, vineyards E and B were clustered together with comparably high contents of Mn and K (Suppl.Fig. 2; Suppl.Fig. 3).

Regarding the rhizomicrobiome structure, ITS amplicons showed a total of 8,893 featured fungal operational taxonomic units (OTUs) after the removal of low-quality and chimeric reads). On the phylum level, the taxonomic structure was relatively comparable between the vineyards. The most dominant fungi were the *Ascomycota*, followed by *Basidiomycota*. Furthermore, the phylum *Rozellomycota* was more prevalent in Vineyard F (Suppl.Fig. 2b). Among the twenty most dominant fungal genera, seventeen belonged to the phylum *Ascomycota*. In addition, *Fusarium* was the most abundant across all vineyards, shaping 12–23% of the total fungal community. Based on the most dominant fungi, the Rauenberg vineyards clustered together using Euclidean distance (Fig. 1a), mirroring the pattern observed in the PCA of soil chemical properties (Suppl.Fig. 2a). These two vineyards exhibited the lowest relative abundance of Fusarium, but higher levels of two other pathogenic genera, *Penicillium* and *Dactylonectria*, as well as an elevated presence of the beneficial fungus *Solicoccozyma*, known to promote root growth (Albornoz et al., 2025). The Eichstetten vineyard also showed distinct profiles of other two fungi, *Fusidium* and *Subulicystidium* (Fig. 1a).

The 16S amplicons reads exhibited higher chimeric read rates, with approximately 30% of reads filtered out. Handling such chimeric reads in prokaryotic communities over 60 complex soil samples would limit the outputs and could introduce bias for 16S amplicon profiling. similar artifacts ratios were reported previously in complex communities, e.g. gut microbiome and soil, where PCR amplification across highly diverse templates increased the chimera formation (Reitmeier et al. 2021; Straub et al. 2020). Furthermore, the amplified V3–V4 regions of 16S rRNA gene in our study may contribute to chimera formation, as the conserved region between

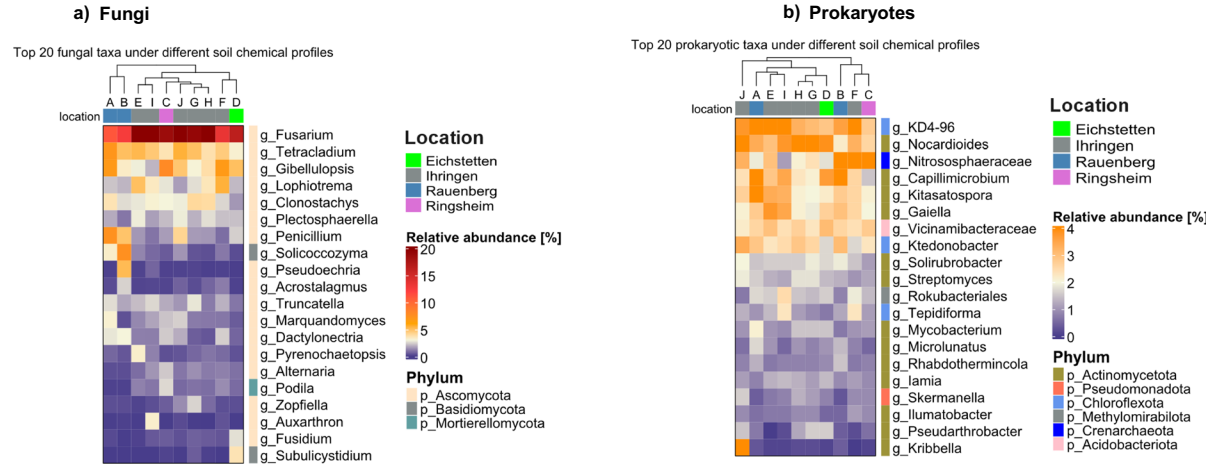


Fig. 1 Taxonomic composition of dominant microbial taxa in vineyards rhizomicrobiome across the Upper Rhine Valley; **a**) Relative abundance of the top 20 fungal taxa based on ITS rDNA sequences. **b**) Relative abundance of the top 20 prokaryotic taxa based on 16S rRNA gene sequences. Every vineyard was represented by six rhizosphere samples. Rows are color-coded according to the taxonomic phylum of each OTU, while columns are color-coded to indicate the geographical origin of each vineyard

V3–V4 regions can increase the frequency of mis-primings during PCR. Following denoising step, the 16S reads represented 84,221 prokaryotic OTUs. Here, the prokaryotic community in the vineyard rhizosphere was significantly enriched, with 10 times more OTUs than observed in the fungal community. At the species level, 1566 Amplicon Sequence Variants (ASVs) of the fungal community were identified, along with 2786 ASVs of prokaryotic origin. Among the prokaryotes, *Actinomycetota* were the dominant bacterial phylum shaping the rhizobacteriome across the vineyards. *Pseudomonadota, Chloroflexota*, and *Acidobacteriota* were next in relative abundance, respectively (Suppl.Fig. 2c). In terms of archaeal communities (overall constituting only a minor fraction), the *Crenarchaeota* were more abundant in vineyards B and F. Additionally, analysis of the twenty most dominant bacterial genera in the vineyard rhizomicrobiome showed that fourteen belonged to the phylum *Actinomycetota*. Despite this, the most dominant genus overall was *KD4-96* from the phylum *Chloroflexota*, followed by *Nocardioides* (Fig. 1b). Notably, the two vineyards from Ihringen (I and F) exhibited distinct profiles characterized by higher abundances of *Tepidiforma* and *Rokubacteriales*, while vineyard (J) in particular showed a pronounced enrichment of *Kribbella*.

Wood-colonising fungi dominate in the vineyard rhizomicrobiome

The defined fungal OTUs were annotated resolving to the genus level using the FUNGuild database. Around half of the fungal taxa were pathotrophs (Fig. 2a). Most of them were opportunistic pathotrophs, otherwise living as saprotrophs or symbiotrophs, suggesting that their function might vary depending on the condition of the host. In contrast, beneficial taxa (non-pathogenic with symbiotic potential) were found to be less prevalent compared to pathogenic taxa, ranging from only 2.9% to 7.4% (Fig. 2a). As alternative approach, we investigated the relative abundance of wood-trophic taxa including those classified as GTDs according to Li et al. (2023a) and Martín et al. (2022). The highest (84–94%) incidence of pathogenic wood-colonising fungi was found in the Southern part of the sampling area, in the vineyards of Ihringen, and Ringsheim,(E, I, And C) whereas the vineyards of Rauenberg (A, and B) in the Northern part harboured less wood pathotrophs, comprising 68–74% of the wood trophic community. By contrast, the Rauenberg vineyards showed the highest abundance of the non-pathogenic saprotrophs (Fig. 2b).

Abundance of GTD-associated fungi depends on vineyards, but not on symptom expression

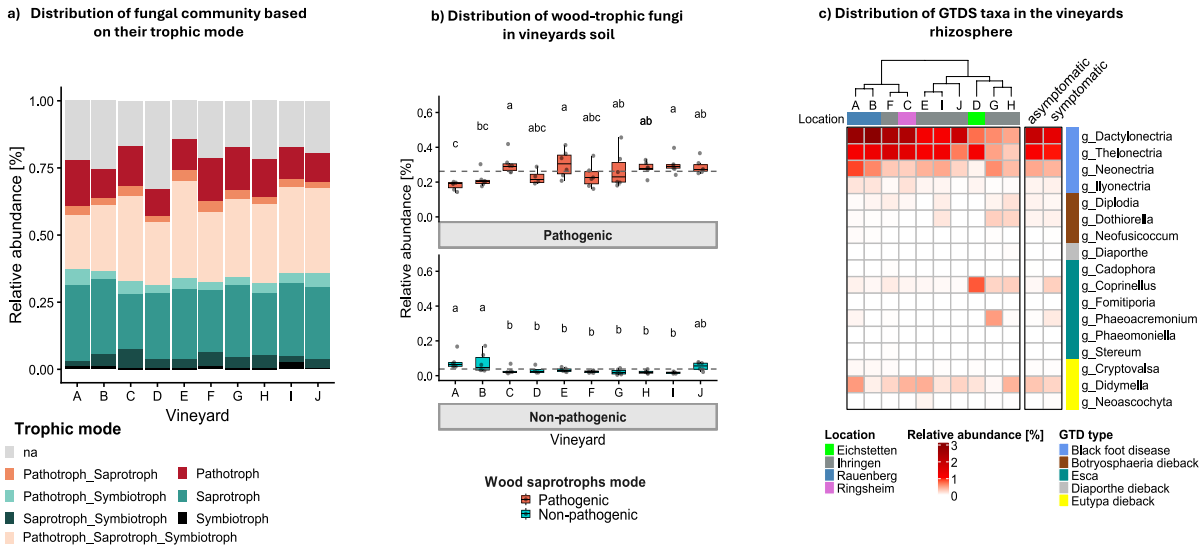


Fig. 2 Fungal community annotation in vineyards rhizomicrobiome across varied geographical and soil chemical profiles. **a**) Annotation of ITS sequences by trophic mode using FUNGuild database. **b**) Relative abundance of wood-trophic OTUs categorized as pathogenic (coral) or non-pathogenic (cyan) to the plants. Significant differences denoted by different letters (Duncan test, $P > 0.05$), while every vineyard was represented by six rhizosphere samples. **c**) Heatmap displaying relative abundance of seventeen GTD-annotated OTUs per vineyard or vine status (asymptomatic vs. symptomatic), irrespective of cultivar or location. GTD taxa are color-coded according to associated GTD type

OTUs representing GTD-associated fungi were identified based on the previously reported GTDs taxa (Li et al. 2023a; Martín et al. 2022). In fact, seventeen taxa could be detected in the vineyard rhizosphere linked with GTDs of different type, including Black Foot Disease, Botryosphaeria Dieback, ESCA, Eutypa Dieback, or Diaporthe Dieback. Here, taxa associated with Black Foot Disease, such as g_*Dactylonectria*, g_*Thelonectria*, and g_*Neonectria* were generally the most prevalent GTD. They were more abundant in the vineyards of Rauenberg (A and B), in the Northern part of the transsect that shared a similar overall profile of GTD-associated fungi, reported by their clustering in terms of Euclidean distance (Fig. 2c). Contrasting with Black Foot Disease, fungi associated with Botryosphaeria Dieback (g_*Diplodia,* g_*Dothiorella*, and g_*Neofusicoccum*) were significantly rarer, and found mainly in vineyards G, H, and I, near Ihringen in the Southern part of the transsect, characterised by *loess* soils and a warm and dry climate. The third disease, Esca, was represented by six OTUs: g_*Coprinellus*, g_*Cadophora*, g_*Phaeoacremonium,* g_*Stereum*, g_*Fomitiporia,* and g_*Phaeomaniella*. Among them, g_*Coprinellus* was the most abundant Esca taxon in all tested vineyards, particularly in vineyards D, G, and H, followed by g_*Phaeoacremonium*, especially in vineyard G. Three OTUs associated with Eutypa Dieback: g_*Cryptovalsa,* g_*Neoascochyta*, as well as g_*Didymella* which was significantly detected in all vineyards. To a low extent, g_*Diaporthe*, associated with Diaporthe Dieback was found, without a particular vineyard preference. To assess whether the outbreak of GTDs is linked with a higher abundance of GTD taxa, their relative abundance in the rhizosphere of symptomatic versus asymptomatic vines was calculated, pooling over all vineyards. Here, *Coprinellus* was the only OTU that showed significant accumulation for symptomatic plants ($P < 0.01$, Kruskal–Wallis test) (Fig. 2c). Thus, with exception of g_*Coprinellus*, we do not see any link between disease outbreak and abundance of GTD-associated fungi.

GTD outbreak correlates with rhizomicrobiome shifts

To test whether GTD outbreak correlated with significant shifts of the rhizomicrobiome, a differential abundance analysis was carried out, using ANCOM-BC. Since the covariate of interest was the health status of the vine, all rhizosphere samples

were pooled into two categories, symptomatic versus asymptomatic vines. Then, for the shifted OTUs at the genus level, the Log Fold Changes (LFC) for symptomatic over asymptomatic vines, and their statistical significance were calculated, regardless of the cultivar or geographical location. This approach revealed significant shifts of both, the fungal (Fig. 3a), and the prokaryotic (Fig. 3b) rhizomicrobiome that were also dependent on the chemical properties of the soil. These shifts are described in the following:

Fungal shifts

In the fungal community, seven OTUs were depleted with symptomatic vines (Fig. 3a). Five of these belong to the *Ascomycota*: *Cistella*, *Pseudophialocephala*, *Populomyces*, *Tetracoccosporium*, and

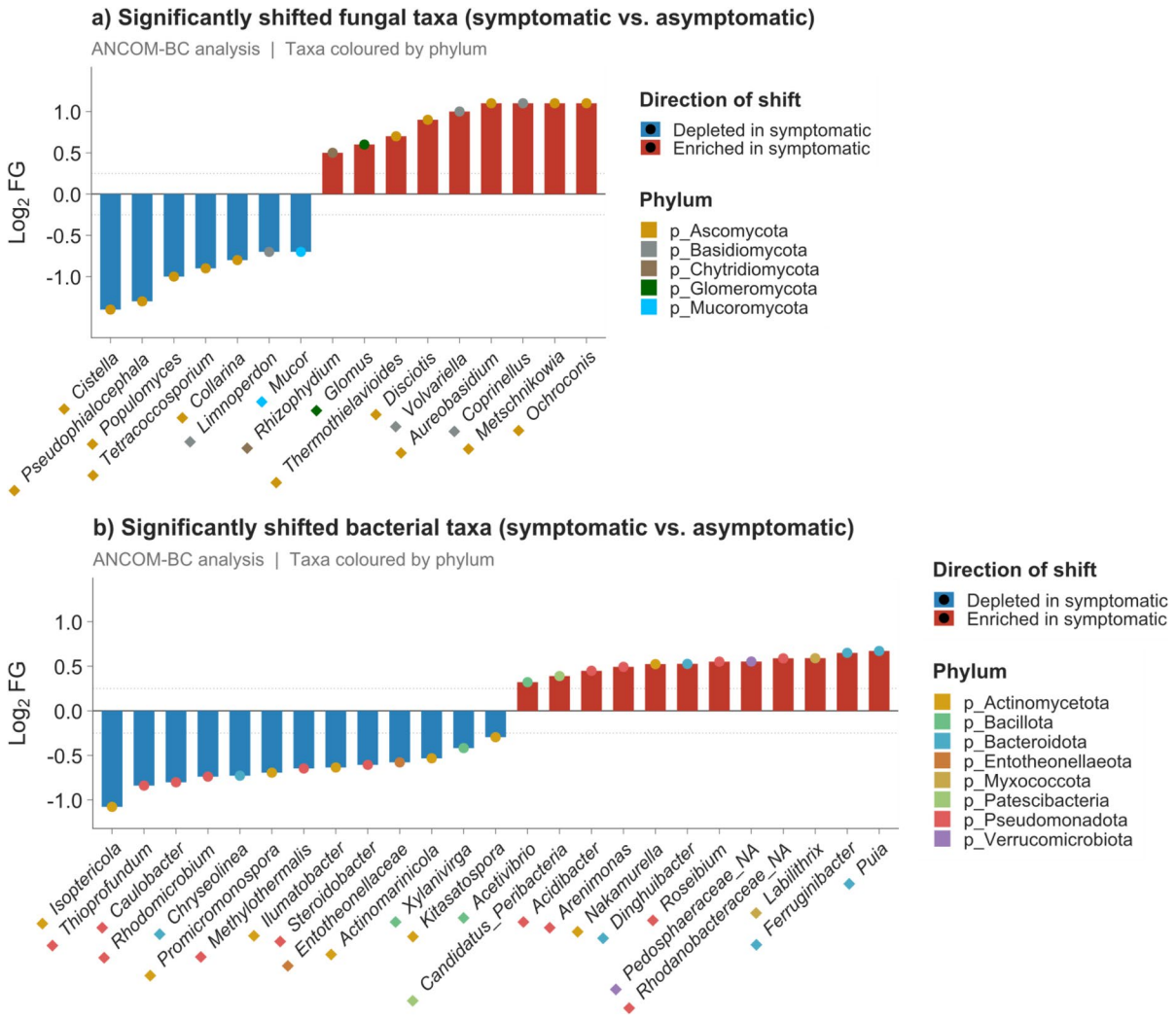


Fig. 3 Differentially abundant microbial taxa in vineyards rhizomicrobiome associated with grapevine trunk disease (GTD) outbreak. **a**) Fungal and **b**) bacterial taxa exhibiting significant abundance differences between symptomatic and asymptomatic vines as determined by ANCOM-BC analysis which implements bias correction for sampling fraction differences between samples and Wald Test for testing coefficients in the linear model. Log-fold change (LFC) values indicate the magnitude of differential abundance; positive LFC values refer to taxa enriched in the rhizosphere of thirty symptomatic vines relative to thirty asymptomatic vines. Taxa labels are color-coded by their respective phyla, highlighting shifts in community composition potentially linked to GTD outbreak

Collarina. Additionally, two genera from different phyla shifted in parallel: *Mucor* from *Mucoromycota*, and *Limnoperdon* from *Basidiomycota*. On the other hand, nine OTUs were enriched under disease outbreak; Among them were g_*Coprinellus*, proposed as driver of Esca, as well as the epiphytic pathogenic fungus, g_*Aureobasidium*. Pearson correlations were then calculated between fungi enriched with healthy vines and individual soil nutrients, adding those fungal phyla that had been found to be generally abundant in the rhizomicrobiome (Fig. 4a). We saw significant associations of specific fungal taxa with specific traits of soil chemistry. For instance, the phylum *Basidiomycota* was positively correlated with the micronutrients Fe, Cu, and Zn, but negatively correlated with $CaCO_3$ and pH (alkalinity). Likewise, the phylum *Rozellomycota* showed positive correlations with Org_C and N, but also with the micronutrient Boron (B). Generally, the taxa that decreased during disease outbreaks, seemed more responsive to soil micronutrients. Here, *Collarina* as the taxon with the largest fluctuations with respect to soil properties exhibited positive correlations with Zn, Cu, Mn, Fe, Mg, and C.N ratio, followed by g_*Pseudophialocephala*, with significant correlations with Zn, Cu, Fe, Mg, and K. Also, for *Cistella* a link with Fe levels was observed.

Prokaryotic shifts

The abundance of many bacterial OTUs dropped significantly in symptomatic vines (Fig. 3b). Among those taxa that correlated with healthy vines, the two phyla *Actinomycetota* and *Pseudomonadota* exhibited five OTUs. The most affected taxa were *Isoptericola*, *Thioprofundum*, *Caulobacter*, *Rhodomicrobium,* as well as *Chryseolinea* from p_*Bacteroidota*. Other phyla had only one depleted OTU. For instance, in p_*Bacillota*

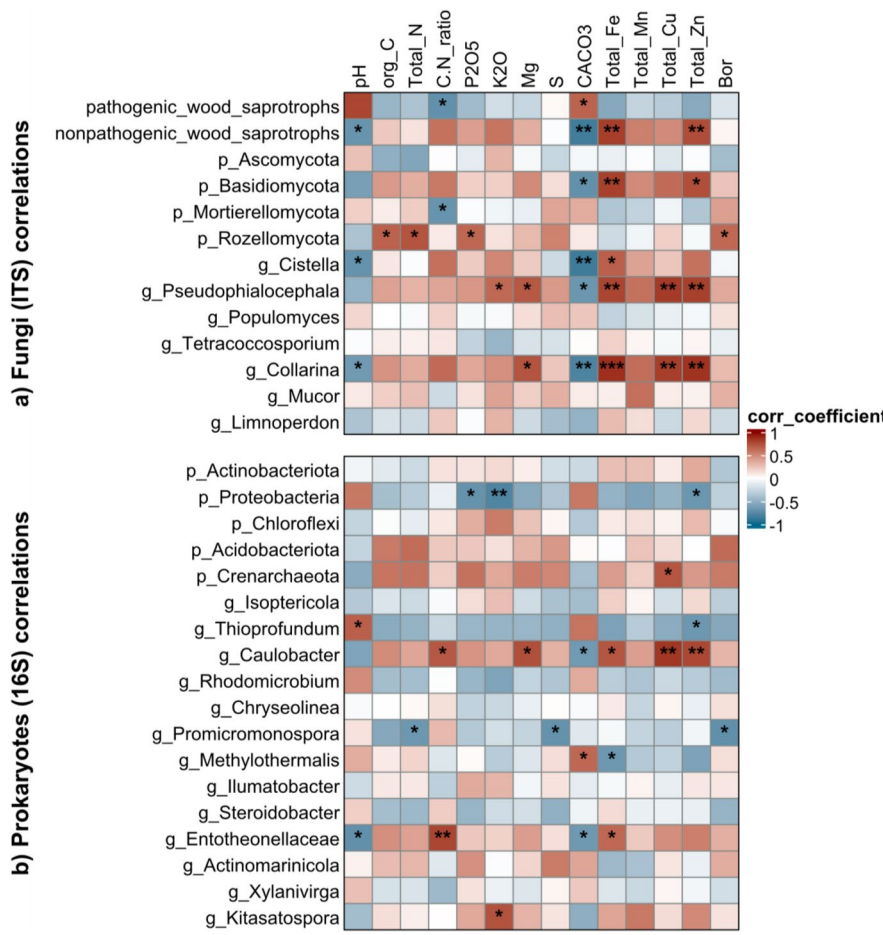
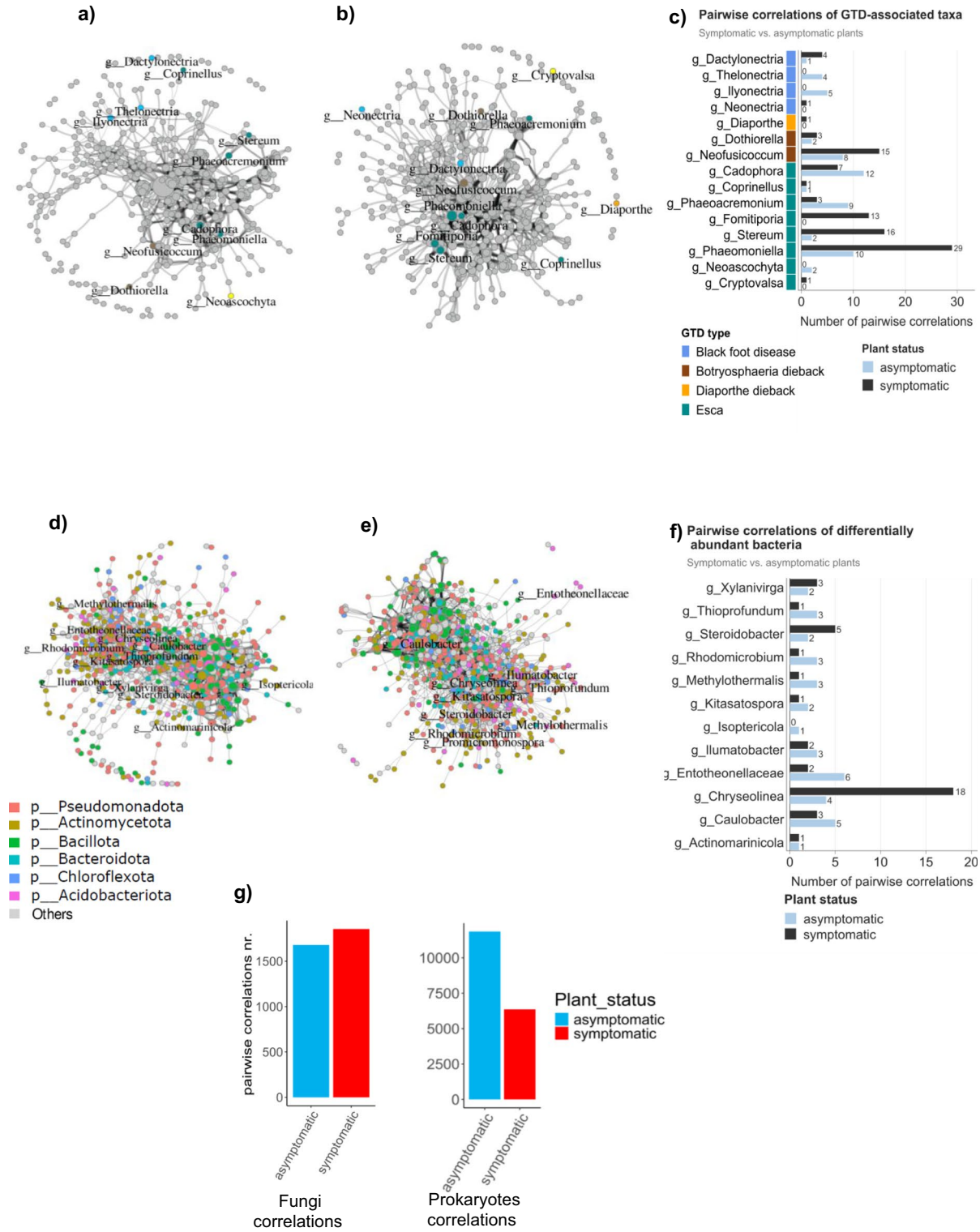


Fig. 4 Correlations between rhizomicrobiome composition and soil physicochemical parameters. **a**) Pearson correlation patterns between wood saprotrophs, dominant fungal phyla, and differentially abundant fungal taxa (between thirty symptomatic and thirty asymptomatic vines) with measured soil parameters. **b**) Pearson correlation patterns of dominant bacterial phyla and taxa negatively associated with symptomatic vines in relation to soil variables. Asterisks (*, **, and ***) indicate significant ANOVA P-values of ≤ 0.05, ≤ 0.01, and ≤ 0.001, respectively


a)
g__Dactylonectria
g__Coprinellus
g__Thelonectria
g__Ilyonectria
g__Stereum
g__Phaeoacremonium
g__Cadophora
g__Phaeomoniella
g__Neofusicoccum
g__Dothiorella
g__Neoascochyta
b)
g__Cryptovalsa
g__Neonectria
g__Dothiorella
g__Phaeoacremonium
g__Dactylonectria
g__Neofusicoccum
g__Phaeomoniella
g__Diaporthe
g__Cadophora
g__Fomitiporia
g__Stereum
g__Coprinellus
c) Pairwise correlations of GTD-associated taxa
Symptomatic vs. asymptomatic plants
g_Dactylonectria
g_Thelonectria
g_Ilyonectria
g_Neonectria
g_Diaporthe
g_Dothiorella
g_Neofusicoccum
g_Cadophora
g_Coprinellus
g_Phaeoacremonium
g_Fomitiporia
g_Stereum
g_Phaeomoniella
g_Neoascochyta
g_Cryptovalsa
Number of pairwise correlations
GTD type
Black foot disease
Botryosphaeria dieback
Diaporthe dieback
Esca
Plant status
asymptomatic
symptomatic
d)
g__Methylothermalis
g__Entotheonellaceae
g__Chryseolinea
g__Rhodomicrobium
g__Caulobacter
g__Thioprofundum
g__Kitasatospora
g__Ilumatobacter
g__Xylanivirga
g__Steroidobacter
g__Isoptericola
g__Actinomarinicola
p__Pseudomonadota
p__Actinomycetota
p__Bacillota
p__Bacteroidota
p__Chloroflexota
p__Acidobacteriota
Others
e)
g__Entotheonellaceae
g__Caulobacter
g__Humatobacter
g__Chryseolinea
g__Thioprofundum
g__Kitasatospora
g__Steroidobacter
g__Methylothermalis
g__Rhodomicrobium
g__Promicromonospora
f) Pairwise correlations of differentially abundant bacteria
Symptomatic vs. asymptomatic plants
g_Xylanivirga
g_Thioprofundum
g_Steroidobacter
g_Rhodomicrobium
g_Methylothermalis
g_Kitasatospora
g_Isoptericola
g_Ilumatobacter
g_Entotheonellaceae
g_Chryseolinea
g_Caulobacter
g_Actinomarinicola
Number of pairwise correlations
Plant status
asymptomatic
symptomatic
g)
pairwise correlations nr.
asymptomatic
symptomatic
Fungi correlations
Prokaryotes correlations
Plant_status
asymptomatic
symptomatic

◂**Fig. 5** Shifts in rhizomicrobiome co-occurrence networks in vineyards in relation to vine health status. Co-occurrence networks were constructed separately for fungal (**a, b**) and prokaryotic (**d, e**) communities associated with asymptomatic (**a, d**) and GTD-affected (**b, e**) vines. Networks were inferred using Spearman correlations, filtered for statistical significance ($p < 0.05$) and correlation strength ($|r| > 0.6$). Each node represents an operational taxonomic unit (OTU), the size of the node represents the number of the positive pairwise correlations, and each edge represents a significant positive correlation between OTUs. In the fungal networks, GTD-associated taxa are color-coded by disease type, while non-GTD taxa are shown in gray. Prokaryotic nodes are color-coded according to the six most dominant bacterial phyla. OTUs labeled in the prokaryotic networks correspond to taxa significantly reduced under GTD outbreak. **c**) Number of significant pairwise correlations for GTD fungal taxa in the rhizosphere of asymptomatic vs. symptomatic vines, with color codes reflecting GTD types. **f**) Number of significant pairwise correlations for negatively shifted bacterial taxa of rhizomicrobiome under GTD outbreak in the rhizosphere of asymptomatic vs. symptomatic vines. **g**) Total number of significant pairwise correlations within fungal and prokaryotic communities in the vineyards rhizosphere based on the health status

only one OTU (*Xylanivirga*) decreased, as well as in p_*Entotheonellaeota* (*Entotheonellaceae*). On the other hand, there were also several rhizobacteriome members which accumulated significantly in symptomatic vines: *Puia*, and *Ferruginibacter*, from p_*Bacteroidota*, were enriched most, but also four OTUs from *Pseudomonadota*, as well as a single OTU from each of the phyla *Verrucomicrobiota,* p_*Patescibacteria* and *Myxococcota* were significantly increased.

We searched for chemical properties of the soil that correlated with these changes of bacterial abundance, but also with dominance of specific prokaryotic phyla independent of disease symptomatics (Fig. 4b). The generally most dominant bacterial phylum, p_*Actinomycetota*, exhibited a negative correlation with only $CaCO_3$ while p_*Pseudomonadota* were negatively correlated with P, K, and Zn. Positive correlations were seen for the p_*Acidobacteriota* with B and for p_*Crenarchaeota* with Cu. Soil properties had also a significant impact on several taxa associated with the asymptomatic phase of GTDs. Specifically, g_*Caulobacter* displayed a positive correlation with C.N ratio, as well as with Mg, Fe, Cu, and Zn, but a negative correlation with $CaCO_3$ (Fig. 4b). Along with *Caulobacter*, also *Entotheonellaceae* became enriched depending on C.N ratio, Fe, and $CaCO_3$, but were depleted in alkaline pH.

Rhizomicrobial co-occurrence networks shift depending on GTD symptoms

To assess whether the interactions and relationships among different rhizomicrobiome taxa are influenced by the health status of the vine, co-occurrence networks were inferred for both, the fungal (Fig. 5a,b) and the prokaryotic (Fig. 5d,e) microbiome in symptomatic versus asymptomatic vines. Here, the shift of the co-occurrence networks responded qualitatively different in fungi versus prokaryotes. While 1680 significant correlations were detected among the fungal taxa in healthy vines, there was an increase to 1856 significant correlations under GTD outbreak (Fig. 5g). A salient component of this increase was the doubling for correlations of GTD-associated taxa with other fungal taxa upon host transition to the symptomatic phase (Suppl_table1**;** Fig. 5c). Here, most GTD taxa sharply changed their correlation profiles. For instance, *Fomitiporia* displayed thirteen significant correlations under disease outbreak, but none in asymptomatic plants. Likewise, correlations of *Stereum* were amplified eightfold. Furthermore, this fungus extended its associations with other GTD taxa, such as *Phaeomoniella* and *Fomitiporia*, as well as with other six wood-saprotrophic and pathogenic taxa (Suppl_table1). Likewise, *Phaeomoniella*, in symptomatic vines, displayed 29 correlations, not only with *Fomitiporia*, but also with eleven other pathogenic taxa, contrasting with only 10 correlations in healthy vines. The aggressive genus, *Neofusicoccum,* responsible for Botryosphaeria dieback, entertained 15 different correlations during GTD outbreak, compared to only 8 in the asymptomatic phase. For Diaporthe dieback linked with *Diaporthe*, a significant correlation, with *Paurocotylis*, was only seen in symptomatic vines (Suppl_table1). Another case, where the pathogenic partner of a GTD fungus was swapped by another pathogenic partner, is represented by *Coprinellus*, which was more prevalent in symptomatic vines (Fig. 3a). This fungus correlates with *Keissleriella* in asymptomatic vines, but switches to a significant correlation with *Tulasnella* under the conditions of a GTD outbreak.

Contrasting with fungi, the connectivity for the 1167 prokaryotic genera, was drastically decreased from 11,856 significant correlations in the healthy vines to 6361 significant correlations under GTDs outbreak (Fig. 5g**;** Suppl_table2). Here, taxa

associated with the asymptomatic phase showed different interaction profiles with other soil prokaryotes. For instance, *Entotheonellaceae* constituted a core node with six strong pairwise correlations in the rhizosphere of healthy vines, but upon GTD outbreak turned into a peripheral node with only two correlations. Also, *Isoptericola* and lost their correlations and even disappeared from the co-occurrence network, while *Thioprofundum, Kitasatospora, Rhodomirobium, Methylothermalis, Illumatobacter, and Caulobacter* robustly lost their positive correlations with other bacteria in symptomatic plants (Fig. 5f**;** Suppl_table2). Only few taxa showed an inverse pattern: *Steroidobacter and Chryseolinea* established more positive correlations only in symptomatic vines.

Bacterial diversity depends mainly on soil, fungal biodiversity also on geography

As markers for ecosystem robustness, we determined a panel of diversity metrics for the rhizomicrobiome over the different vineyards with their differences in soil parameters and geographical location, either in asymptomatic plants or under disease outbreak. Bacterial and fungal diversity metrics of the grapevine rhizomicrobiome were assessed across multiple vineyards differing in soil properties, geographic location, and disease status. Overall, alpha- and beta-diversity measures revealed no consistent shifts associated with GTDs outbreak (Suppl.Fig. 5). Only vineyard-specific effects were observed: bacterial Shannon diversity differed significantly in vineyards E and G, while fungal beta diversity (Bray–Curtis) differed between asymptomatic and symptomatic vines in vineyards D and F. Across vineyards, alpha-diversity metrics (Shannon and Chao1) remained largely stable and showed no significant correlations with macro- or micronutrient levels, suggesting that metrics relying solely on taxon richness and evenness were relatively insensitive to environmental variation **(**Fig. 6**;** (Suppl. Fig. 6).

In contrast, diversity measures incorporating phylogenetic relationships revealed pronounced patterns. Faith's phylogenetic diversity (PD) was the most variable alpha-diversity metric and showed strong associations with soil chemistry, particularly in prokaryotic communities, where PD correlated positively with Fe, Cu, Zn, and Mg (Fig. 6d). Similarly, beta-diversity metrics incorporating taxonomic distance substantially increased. Here, weighted UniFrac captured 30% of the variance compared to only 4% using Bray–Curtis for prokaryotes. Fungal beta diversity differed significantly by geographic location, with vineyards in the northern sites (Rauenberg and Ringsheim) clearly separated from those in the southern site (Eichstetten). This spatial structuring was largely independent of soil properties, with $CaCO_3$ being the only soil parameter showing a notable association (Fig. 6a, b). In contrast, prokaryotic community composition was more tightly linked to soil properties, with Fe, Zn, Mg, and Cu consistently explaining both phylogenetic and non-phylogenetic beta-diversity patterns (Fig. 6; Suppl.Fig. 6). Together, these results indicate that bacterial diversity is primarily shaped by soil chemistry, whereas fungal community structure is more strongly influenced by geographic context, and that phylogenetic metrics are essential to uncover these relationships.

Discussion

This study explored the relationship between the outbreak of grapevine trunk diseases (GTDs) and rhizomicrobiome composition and dynamics across ten vineyards sampled along a north–south transect in the Upper Rhine Valley. By comparing symptomatic and asymptomatic grapevines within vineyards, The transition from the latent to the necrotrophic phase of the GTD-associated fungi was accompanied by taxonomic shifts in both the fungal and the prokaryotic rhizomicrobiomes. Using multiple diversity metrics and correlation network analyses, we detected strong associations between prokaryotic community composition and soil chemical properties, whereas fungal taxa were primarily structured by host status and geographic context. Together, these findings raise three central questions that structure the following discussion: **(i)** why do prokaryotic communities primarily respond to soil chemistry, whereas fungal communities do not show comparable abundance shifts? **(ii)** what mechanisms may underlie the association of specific prokaryotic taxa with grapevine health? and **(iii)** what do altered fungal correlation networks imply for our understanding of GTD pathogenesis?

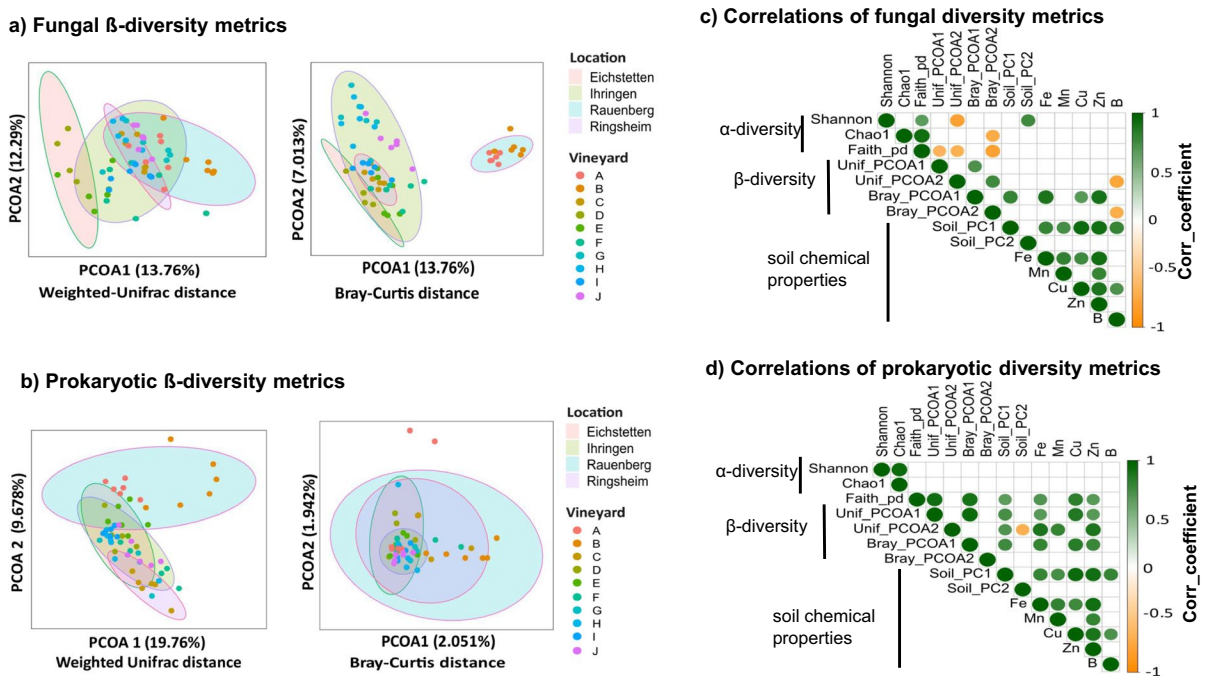


Fig. 6 Changes in beta-diversity metrics of the fungal (**a**) and prokaryotic (**b**) communities under different geographical locations and soil chemical properties: Principle coordinate analysis (PCOA1, PCOA2) based on either Weighted-Unifrac distance or Bray–Curtis distance for the fungal and prokaryotic communities of different vineyards soils repsectively. β-diversity metrics per geographical location (Eichstetten, Ihringen, Ringsheim, Walldorf) were separated into different colored polygons. To statically evaluate the significance of distant polygons "β-diversity metrics per location", the stat_ellipse function from the ggplot package was set to a level of 0.95. Pearson correlations of the diversity metrics for the fungal **(c)** and prokaryotic **(d)** communities were calculated in relation to the changes in soil chemical paramters, such as the principal component analysis of soil chemical properties (PC1, PC2) and the micronutrients concentrations. The colorcode of the correlation plots represents the value of the correlation coefficient with significance level = 0.05; green color refers to significantly positive correlation coefficient; orange color represents significantly negative correlation, while the blank color represents the insignificant values of correlation coefficient

Soil ecology: fungi respond by metabolic state, bacteria by proliferation (H1, H3)

The rhizomicrobiome plays a central role in maintaining plant health and resilience against environmental challenges (Berendsen et al. 2012; Gu et al. 2022). Accordingly, understanding how soil ecology shapes microbial community composition and function is essential for sustainable viticulture. Two salient features emerge from our analysis.

For the fungal community, host status rather than soil properties appears to define disease outbreak. The dominant fungal phyla in the vineyard rhizosphere were *Ascomycota* and *Basidiomycota* (Suppl. Figure 1), consistent with previous vineyard studies across cultivars and, different geographical (Bao et al. 2022; Berlanas et al. 2019; Coller et al. 2019; Lailheugue et al. 2024). In addition, no significant differences in the incidence of symbiotrophic or pathotrophic fungal taxa were observed among vineyards (Fig. 2a). Approximately 50% of detected fungal taxa were multi-trophic with pathotrophic potential, supporting a scenario in which disease outbreak is not defined by increased pathogen abundance but rather by changes in fungal behaviour. Such conditional pathogenesis has been demonstrated for *Neofusicoccum parvum*, where transitions to necrotrophy are triggered by host-derived metabolic signals (Khattab et al., 2023; Flubacher et al. 2023). Given that most fungal secondary metabolite gene clusters remain silent unless activated by specific environmental cues (Brakhage & Schroekh, 2011), it is plausible that differences in soil ecology are reflected in altered fungal metabolic states rather than in abundance shifts.

In contrast, the prokaryotic community was strongly structured by geography and soil chemistry. *Actinomycetota* dominated the bacterial rhizomicrobiome, followed by *Pseudomonadota, Chloroflexota*, and *Acidobacteriota* (Suppl. Figure 2c), a pattern consistent with environmental filtering. Comparable phylum-level dominance has been reported across vineyards in Europe and China, although with strong regional variation (Bao et al. 2022; Berlanas et al. 2019; Coller et al. 2019; Lailheugue et al. 2024; Marasco et al. 2018). Such comparisons between different studies have to be taken with care, because timing of sampling might also affect the rhizomicrobiome structure (Berlanas et al. 2019). However, for the data from the current study, seasonal differences can be excluded because the samples were collected in August.

Our data indicate that differences in bacterial abundance patterns primarily derive from soil properties rather than disease status. This is supported by correlations between *Pseudomonadota* and Zn, K, and P, as well as between *Acidobacteriota* and B and nitrogen content (Fig. 4b). More generally, strong associations between phylogenetic diversity metrics (Faith_PD, UniFrac) and micronutrients such as Fe, Cu, Mn, and Zn suggest that micronutrient availability is a key driver of bacterial community assembly (Fig. 6c). These elements play central roles in microbial metabolism, respiration, redox reactions, and nutrient cycling (Dubinsky et al. 2010; Whalen et al. 2018; Dai et al. 2023a). Furthermore, the abundance of genes regulating P metabolism in the soil was strongly correlated with Mn (Shepherd & Oliverio 2024), supporting a model in which bacteria respond to environmental change primarily through shifts in proliferation and community composition.

In contrast to fungi, which can respond to environmental cues by activating silent metabolic modules, bacteria rely on restructuring metabolically cohesive consortia (Pascual-García et al. 2020). In shorthand, fungal communities respond to soil ecology predominantly through metabolic plasticity, whereas bacterial communities respond through changes in abundance and composition.

GTD outbreak is associated with loss of beneficial bacterial taxa and reduced microbial network connectivity.

Under disease outbreak, we observed pronounced shifts in bacterial community composition, reflected most clearly in co-occurrence networks. The strong reduction in positive correlations among network nodes indicates a loss of connectivity and functional stability in the bacterial rhizomicrobiome under disease outbreak (Fig. 5g). Consistent depletion of taxa such as *Isoptericola*, *Thioprofundum*, *Caulobacter*, *Rhodomicrobium*, and *Chryseolinea* suggests their association with stable, health-related rhizomicrobiome structures (Fig. 3b). The disappearance of positive interactions involving taxa such as *Isoptericola* further supports the interpretation that disease outbreak reflects a breakdown of beneficial microbial interactions rather than the emergence of new dominant taxa (Fig. 5e; Suppl. Table 2). Possible reasons might be increased competition for nutrients, reduced functionality, but also the rise of outbreak-associated bacterial communities.

Given that approximately half of the fungal rhizomicrobiome exhibits pathotrophic potential (Fig. 2a), depletion or destabilisation of bacterial networks may facilitate the transition of conditional fungal pathogens toward pathogenicity. While the underlying mechanisms remain unresolved in grapevine, studies in other crops show that root exudates from healthy plants promote rhizomicrobiomes that enhance nutrient cycling and plant immunity (Chen et al. 2024; Du et al. 2024; Wilhelm et al. 2023). Under severe stress conditions preceding GTD outbreak (Khattab et al., 2023), altered root exudation patterns may disrupt established microbial networks, leading to loss of beneficial taxa and promotion of pathogenic lifestyles.

These findings suggest that stabilising health-associated bacterial networks could represent a strategy for sustainable GTD management. Micronutrient supplementation may contribute to this goal, as micronutrients were strongly correlated with phylogenetic diversity metrics and with higher abundance of health-associated bacteria (Fig. 4**;** Fig. 6). Although causality cannot be inferred from correlative data, the identified bacterial taxa represent promising candidates for functional testing. Indeed, co-inoculation with *Actinomycetota* and *Bacillota* has previously been shown to mitigate GTD progression (Cobos et al. 2022), either through direct antagonism or via activation of host defence responses (Álvarez-Pérez et al. 2017; Haidar et al. 2016; Trotel-Aziz et al. 2019).

GTD outbreak: a matter of fungal ecology rather than pathogen incidence

Unlike other grapevine diseases, such as Downy Mildew, or Powdery Mildew, the GTDs is far from elucidated. Classically, pathogens are identified if they are necessary and sufficient for symptomatics, an approach known as Koch Postulates (Loeffler, 1884). This classical approach fails for GTDs, because symptoms can rarely be attributed to absence and presence of a given fungus. This has led to the provocative suggestion that Esca disease may not be a fungal disease in the classical sense (Hofstetter et al. 2012), but rather an ecological syndrome in which fungi harming the plants compromised by other stressors.

Consistent with this view, most GTD-associated taxa, including those linked to black foot disease, did not show significant abundance shifts between symptomatic and asymptomatic vines (Fig. 2c). *Coprinellus* was the only apparent exception, yet evidence suggests it is a saprotrophic hitchhiker potentially feeding on decayed tissues after the outbreak rather than a driver of disease (Brown et al. 2020; Cui et al. 2024). In contrast, interaction dynamics within the fungal community changed markedly after disease outbreak. GTD-associated taxa with pathotrophic potential showed strongly increased mutual correlations, particularly among Esca-associated fungi such as *Phaeomoniella with Fomitiporia, Stereum with Fomitiporia, and Stereum with Phaeomoniella* (Suppl. Table 1).

These patterns support a model in which fungal taxa engage in cooperative interactions through co-colonisation or metabolic cross-feeding once host resilience is compromised. Whether such interaction shifts are triggered by loss of beneficial bacteria, altered root exudation, or direct stress signals remains to be tested experimentally. Nevertheless, the amplification of fungal interaction networks clearly supports a contextual, ecology-driven model of GTD pathogenesis that transcends pathogen incidence alone.

outlook: GTDs as a hypothesis-driven ecological disease model

Taken together, our results provide a coherent, hypothesis-driven model for understanding GTDs as an emergent outcome of plant–microbe–environment interactions. The data support the hypothesis that GTD outbreak is not associated with increased abundance of GTD-associated fungi, but rather with restructuring of microbial communities and interactions. Bacterial rhizomicrobiome structure was strongly linked to soil micronutrient availability, while disease outbreak was characterised by loss of beneficial bacterial taxa and reduced network connectivity. In parallel, fungal communities responded primarily through altered interaction dynamics rather than abundance shifts.

While this study is correlative and represents a static snapshot during disease outbreak, it identifies statistically robust associations that are clearly linked to the stated hypotheses. Future work integrating temporal sampling and functional assays will be required to establish causality, particularly regarding microbial drivers of plant immunity and fungal lifestyle switching. Nevertheless, the present findings provide a strong conceptual basis for developing rhizomicrobiome-based strategies to enhance grapevine resilience and to complement existing GTD management approaches.

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Data availability Sequencing rawdata: NCBI bioproject (Vineyards_rhizomicrobiome), Accession: PRJNA1328367, ID: 1,328,367 (https://www.ncbi.nlm.nih.gov/bioproject/1328367). **Bioinformatics: Github respiratory, "Vineyards_rhizomicrobiome_Upper_Rhine" (https://github.com/Khattab2022/Vineyards_rhizomicrobiome_Upper_Rhine).**

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