UNIVERSITÉ DU QUÉBEC À TROIS-RIVIÈRES

MYCOREMÉDIATION DES MICRO-ORGANISMES PATHOGÈNES PRÉSENTS DANS LES EAUX D'IRRIGATION AGRICOLES DU DISTRICT DE LA RAMADA, DANS LE DÉPARTEMENT DE CUNDINAMARCA, EN COLOMBIE

MYCOREMEDIATION OF PATHOGENIC MICROORGANISMS IN AGRICULTURAL IRRIGATION WATER IN LA RAMADA DISTRICT, CUNDINAMARCA, COLOMBIA

THÈSE PRÉSENTÉE

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DOCTORAT EN BIOLOGIE CELLULAIRE ET MOLECULAIRE

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RÉSUMÉ

L'eau constitue l'une des ressources non renouvelables les plus menacées en raison des activités anthropiques, et sa pollution représente une préoccupation majeure pour l'environnement et la santé publique dans le monde entier. En Colombie, environ un million d'hectares sont consacrés à la culture de fruits et légumes. La rivière Bogotá, qui s'étend sur plus de 300 kilomètres et draine environ 590 000 hectares, est utilisée à des fins agricoles et industrielles. Cependant, au cours de son parcours, elle reçoit des déversements d'eaux usées insuffisamment ou non traitées, compromettant gravement la qualité de l'eau destinée à l'irrigation. Dans le district d'irrigation de La Ramada, les sites Venecia et Lucero ont été identifiés comme des points critiques de pollution : Venecia reçoit des décharges agricoles et domestiques, tandis que Lucero est influencé par des décharges industrielles et urbaines. Les deux sites ont signalé la présence de coliformes totaux, *E. coli*, de champignons filamenteux opportunistes et d'autres micro-organismes à risque pour la santé. Bien que ces contaminants biologiques aient déjà été signalés, le potentiel de la mycoremédiation à modifier les communautés microbiennes indigènes dans le cadre de son application en tant que stratégie de traitement biologique alternative n'a pas encore été exploré. Par conséquent, l'objectif principal de cette recherche était d'évaluer le potentiel de la mycoremédiation des champignons macromycètes *Trametes* sp., *Ganoderma* sp. et *Pleurotus ostreatus* pour lutter contre les micro-organismes pathogènes dans les eaux d'irrigation agricoles du district de La Ramada, en Colombie. Pour atteindre cet objectif, les buts spécifiques suivants ont été établis : caractériser la diversité taxonomique et fonctionnelle des microbiomes bactériens et fongiques aux sites de Venecia et de Lucero ; et évaluer l'efficacité de la mycoremédiation des trois macromycètes contre les bactéries entéropathogènes et les champignons opportunistes.

Les prélèvements ont été effectués le 11 janvier 2023 sur deux sites situés dans le district d'irrigation de La Ramada, dans les municipalités de Funza et de Madrid, Cundinamarca. Les paramètres physico-chimiques de l'eau ont été mesurés selon des méthodes standard (APHA, 2017). Le traitement par mycoremédiation a consisté à inoculer individuellement des disques de mycélium issus de *Trametes* sp. (FDH-00018), *Ganoderma* sp. (FDH-00023) et *Pleurotus ostreatus* (FDH-00008), provenant de la Collection de Macromycètes et Micromycètes de l'Université Nationale de Colombie. Les champignons ont été incubés pendant 30 jours à 25°C avec agitation continue à 125 rpm. L'ADN total des micro-organismes retenus sur des membranes de filtration a été extrait à l'aide du kit DNeasy PowerSoil Pro (Qiagen) et séquencé sur la plateforme Illumina MiSeq en amplifiant la région 16S rRNA pour les bactéries et la région ITS2 pour les champignons. Le traitement bio-informatique a utilisé DADA2, avec des analyses statistiques portant sur les indices de diversité alpha et bêta pour évaluer la composition et la structure des communautés microbiennes ; une analyse de l'abondance différentielle avec ANCOM-BC2 pour détecter les changements liés aux traitements de macromycètes ; et une annotation fonctionnelle avec FAPROTAX pour les bactéries et FUNGalTraits pour les champignons afin d'inférer leurs

rôles écologiques potentiels.

L'application de cette méthodologie a montré que les analyses physico-chimiques indiquaient que les eaux des deux sites dépassaient les normes colombiennes d'irrigation. Lucero a connu la plus forte dégradation, avec les niveaux de contamination les plus élevés, tandis que Venecia, bien que moins impacté, dépassait également les limites acceptables. Le jour 0, les communautés bactériennes à Venecia étaient dominées par Exiguobacteraceae, Moraxellaceae et Pseudomonadaceae, alors qu'à Lucero, Moraxellaceae et Pseudomonadaceae étaient plus présentes. Les Enterobacteriaceae, principalement du genre *Klebsiella*, étaient les plus abondants à Lucero. Bien que leur présence ait diminué après les traitements, cette réduction a également été observée au jour 30, il n'est donc pas possible de lier de façon définitive cette baisse au traitement par macrofungi. De plus, le faible taux de survie des boutures de mycélium suggère qu'une contamination sévère a entravé leur établissement et pourrait avoir contribué à l'augmentation de la diversité alpha bactérienne dans les traitements avec Ganoderma sp. et *Pleurotus ostreatus*.
Dans la communauté fongique, le jour 0 se caractérisait par la dominance de Sporidiobolaceae et du genre opportuniste *Rhodotorula* dans les deux sites, en particulier à Lucero. Les traitements avec des macrofungi ont modifié cette communauté de manière différentielle, en réduisant les levures opportunistes ; en particulier, *Trametes* sp. a montré la plus grande diminution des champignons classés comme pathogènes animaux à Lucero, tandis que *Ganoderma* sp. et *Trametes* sp. ont significativement réduit l'abondance du genre *Apiotrichum*. De plus, l'annotation fonctionnelle a révélé une réorganisation des profils écologiques, avec une augmentation des fonctions associées au métabolisme hétérotrophe et à la dégradation de la matière organique, ainsi qu'une réduction partielle des fonctions liées aux risques pour la santé, bien que le parasitisme intracellulaire ait augmenté dans tous les traitements. Globalement, les résultats montrent que les eaux de Venecia et Lucero abritent des communautés bactériennes et fongiques distinctes, cohérentes avec leurs sources de contamination. L'analyse fonctionnelle a révélé que ces deux communautés exercent des fonctions à risque pour la santé à partir de leur état de référence. Les trois macrofungi ont modifié différemment les communautés microbiennes natives selon les espèces et les sites : *Trametes* sp. a été le plus efficace à Venecia pour réduire les champignons opportunistes, tandis que *Ganoderma* sp. et *Pleurotus ostreatus* ont été efficaces à Lucero. Le taux de survie global du mycélium, de 49 %, avec seulement 11 % pour *Pleurotus ostreatus*, suggère que la contamination a entraîné un stress élevé chez les macrofungi dans les deux localités. En résumé, ces macrofungi montrent un potentiel de mycoremédiation, partielle et spécifique à chaque site, des communautés microbiennes dans l'eau d'irrigation du district de La Ramada, *Ganoderma* sp. étant le plus efficace de manière constante sur les deux sites.

Mots-clés : diversité microbienne, abondance différentielle, annotation fonctionnelle, mycoremédiation, eau d'irrigation agricole

ABSTRACT

Water constitutes one of the most threatened non-renewable resources due to anthropogenic activities, and its pollution poses a significant concern for environmental and public health worldwide. In Colombia, approximately one million hectares are dedicated to the cultivation of fruits and vegetables. The Bogotá River, which extends over 300 kilometers and drains approximately 590,000 hectares, is utilized for agricultural and industrial purposes. However, during its course, it receives discharges of inadequately treated or untreated wastewater, thereby severely compromising water quality intended for irrigation. In the La Ramada Irrigation District, the sites Venecia and Lucero have been identified as critical pollution points: Venecia receives agricultural and domestic discharges, while Lucero is influenced by industrial and urban discharges. Both sites have reported Total coliforms, *E. coli*, opportunistic filamentous fungi, and other health-risk microorganisms. Although these biological contaminants have been reported previously, the potential of mycoremediation to modify native microbial communities in the context of its application as an alternative biological treatment strategy has not yet been explored. Consequently, the primary objective of this research was to evaluate the mycoremediation potential of the macromycete fungi *Trametes* sp., *Ganoderma* sp., and *Pleurotus ostreatus* to combat pathogenic microorganisms in agricultural irrigation waters in the La Ramada District, Colombia. To achieve this objective, the following specific aims were established: to characterize the taxonomic and functional diversity of bacterial and fungal microbiomes at the Venecia and Lucero sites; and to assess the mycoremediation efficacy of the three macromycetes against enteropathogenic bacteria and opportunistic fungi.

Sampling was conducted on January 11, 2023 in the two sites within the La Ramada Irrigation District, located in the municipalities of Funza and Madrid, Cundinamarca. Water physicochemical parameters were measured using standard methods (APHA, 2017). Mycoremediation treatment involved inoculating plugs of mycelium from fungi *Trametes* sp. (FDH-00018), *Ganoderma* sp. (FDH-00023), and *Pleurotus ostreatus* (FDH-00008) individually, obtained from the Collection of Macromycetes and Micromycetes at the National University of Colombia. The fungi were incubated for 30 days at 25°C with continuous agitation at 125 rpm. Total DNA from microorganisms retained on filtration membranes was extracted using the DNeasy PowerSoil Pro kit (Qiagen) and sequenced on the Illumina MiSeq platform by amplifying the 16S rRNA for bacteria and the ITS2 region for fungi. Bioinformatics processing used DADA2, with statistical analyses covering alpha and beta diversity indices to assess microbial community composition and structure; differential abundance analysis with ANCOM-BC2 to detect changes related to macromycetes treatments; and functional annotation with FAPROTAX for bacteria and FUNGalTraits for fungi to infer potential ecological roles.

The application of this methodology showed that physicochemical analyses indicated waters from both sites surpassed Colombian irrigation standards. Lucero experienced the most deterioration, with the highest contamination levels, while Venecia, although less impacted, also exceeded acceptable limits. On day 0, the bacterial communities in Venice were dominated by Exiguobacteraceae, Moraxellaceae, and Pseudomonadaceae, whereas in Lucero, Moraxellaceae and Pseudomonadaceae were more prevalent. Enterobacteriaceae, primarily genus *Klebsiella*, were the most abundant in Lucero. Although its presence decreased after the treatments, this reduction was also observed on day 30, so it cannot be definitively linked to macrofungi treatment. Additionally, the low survival rate of mycelium plugs suggests that severe contamination hindered their establishment and might have contributed to increased bacterial alpha diversity in treatments with *Ganoderma* sp. and *Pleurotus ostreatus*.

In the fungal community, day 0 was characterized by the dominance of Sporidiobolaceae and the opportunistic genus *Rhodotorula* at both sites, especially at Lucero. Treatments with macrofungi differentially altered this community, reducing opportunistic yeasts; in particular, *Trametes* sp. showed the greatest decrease in fungi classified as animal pathogens at Lucero, while *Ganoderma* sp. and *Trametes* sp. significantly reduced the abundance of the genus *Apiotrichum*. Additionally, functional annotation revealed a reorganization of ecological profiles, with an increase in functions associated with heterotrophic metabolism and organic matter degradation, and a partial reduction in functions linked to health risks, although intracellular parasitism increased in all treatments.

Overall, the results show that the waters from Venecia and Lucero host distinct bacterial and fungal communities, consistent with their sources of contamination. Functional analysis revealed that both communities perform health-risk functions from their baseline state. The three macrofungi altered native microbial communities differently across species and sites: *Trametes* sp. was most effective in Venecia at reducing opportunistic fungi, whereas *Ganoderma* sp. and *Pleurotus ostreatus* were effective in Lucero. The global mycelial survival rate of 49%, with *Pleurotus ostreatus* accounting for only 11%, suggests that contamination created high stress for the macrofungi at both locations. Overall, these macrofungi show potential for partial, site-specific mycoremediation of microbial communities in irrigation water in the La Ramada District, with *Ganoderma* sp. being the most consistently effective across both sites.

Keywords: microbial diversity, differential abundance, functional annotation, mycoremediation, agricultural irrigation water

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LIST OF ABBREVIATIONS

ABTS	2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
ASVs	Amplicon Sequence Variants
BOD	Biochemical Oxygen Demand
CFU	Colony-Forming Units
COD	Chemical Oxygen Demand
DNA	Deoxyribonucleic Acid
H_2O_2	Hydrogen Peroxide
ITS	Internal Transcribed Spacer
LIA	Environmental Engineering Laboratory
LiP	Lignin Peroxidase
LPB	Lucero Sampling Point
MnP	Manganese Peroxidase
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PES	Polyethersulfone
rRNA	Ribosomal Ribonucleic Acid
SM	Standards methods
TAE	Tris-Acetate-EDTA
UN	Universidad Nacional de Colombia
UV	Ultraviolet
VPA	Venecia Sampling Point
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

Water is one of the most threatened non-renewable resources by anthropogenic activities due to increasing demand and continuous population growth, making water pollution a primary global environmental and public health concern. According to the World Health Organization (WHO), over 2.2 billion people use contaminated water, and in 2021, more than 2 billion people resided in water-stressed regions. It is estimated that by 2050, water demand could increase by 20% to 30%, exceeding 6,000 km^3 per year (Bose et al., 2024). Water used for domestic, agricultural, and industrial purposes must be collected and treated for safe reuse. However, in low-income countries, only 8% of wastewater is treated, whereas in developed countries, 70% of sewage is treated to meet the quality standards required for safe reuse *(UNESCO, 2022).*

This natural resource plays a crucial role in agriculture, as crop yields, the safety of agricultural products, and long-term soil preservation are all directly affected by its quality. In rural and peri-urban regions of Latin America, poor water infrastructure, insufficient access to water resources, and fragmented government management have been identified as key factors contributing to deteriorating water quality. This, in turn, has a direct and negative impact on agricultural productivity (Ruttan, 2002). In addition, Linderhof et al. have shown that irrigation water can harbor high microbial loads, including pathogens, as well as chemicals and other toxic substances (Linderhof et al., 2021). This can jeopardize the food safety of agricultural products, and a review has shown that irrigation water can act as an

important vehicle for human pathogens and a major source of pre-harvest contamination of fresh produce, thereby compromising food safety (Gurtler & Gibson, 2022).

In agriculture, this natural resource is of great importance, as its quality directly influences crop yields, long-term soil preservation, and the safety of agricultural products. In rural and peri-urban regions of Latin America, poor water infrastructure, insufficient access to water resources, and fragmented government management have been identified as key factors contributing to deteriorating water quality. This, in turn, has a direct and negative impact on agricultural productivity (Ruttan, 2002).

1. Irrigation Water Quality in Colombia

In Colombia, approximately one million hectares are dedicated to cultivating fruits and vegetables for both the domestic and export market. The department of Cundinamarca -where the capital of Colombia, Bogotá, is located- accounts for about 28% of this production. National vegetable consumption reaches 1 494 400 tons per year. Several studies have found that contaminated water is used for irrigation in many regions of Colombia (ASOHOFRUCOL, 2025). In the western savannah of Cundinamarca, which spans approximately 18 000 hectares, it has been observed that around 1 000 people discharge domestic wastewater, industrial effluents, and septic tank contents directly into the water system. This situation compromises the quality of water intended for irrigation, mainly due to the risk posed by phytopathogenic microorganisms that affect agricultural productivity and the presence of opportunistic pathogens that threaten human health (Corrales et al., 2018; Arguello & Bustos-Lopez., 2018);

The Bogotá River, which extends 347 kilometers and drains an area of approximately 589 143 hectares, is used in productive activities such as agriculture and industry (Millán, 2019). However, along its channel, it receives continuous discharges of domestic wastewater, industrial effluents, and agro-industrial waste. Consequently, an increase in biological contamination has been observed, with values reaching up to 40% of fecal coliforms, *Escherichia coli*, and other pathogens (Sánchez-Alfonso et al., 2020). Additionally, significant chemical contamination, including heavy metals such as cadmium, lead, mercury, and chromium (Mahecha et al., 2017) has been detected, exceeding the limits set by Colombian regulations, which are based on the legal framework established in the 1991 Constitution, Law 99 de 1993 and Decree 1729 de 2002.

2. Biological pollutants in irrigation water

Water used in agricultural irrigation systems is often contaminated with various pathogenic microorganisms, posing a direct threat to crop safety and public health. International research has revealed that, in the absence of adequate treatment, these waters can harbor bacteria such as *E. coli*, as well as *Salmonella* and *Shigella* species, along with protozoa, viruses, and, to a lesser extent, opportunistic fungi (Pachepsky et al., 2011; Steele & Odumeru, 2004).

In 2018, Corrales and colleagues published research indicating the concurrent presence of multiple enterobacteria, including *E. coli*, *Proteus vulgaris*, and *Enterobacter cloacae*, along with various filamentous fungi from the genera *Aspergillus, Penicillium, Fusarium, Mucor*, and *Rhizopus*, in the waters of the middle basin of the Bogotá River (Corrales et al., 2018). These findings suggest limited implementation of effective treatment and risk-reduction

measures for removing biological contaminants from water resources. Concurrent studies conducted in irrigation canals in the same area have identified elevated concentrations of total and fecal coliforms that exceed the limits set by Colombian Decree 1594 of 1984 and the WHO guidelines (Dreschsel et al., 2022; Corrales Ramirez et al., 2014; Echeverry et al., 2024). In a sampling study conducted across 10 locations within the district, levels of up to 1 × 108 CFU/100 mL for total coliforms and 1 × 107 CFU/100 mL for fecal coliforms were observed, particularly in sectors with urban and industrial influences (Arguello & Bustos-Lopez, 2018). This condition is a substantial microbiological risk, as several pathogens have the potential to proliferate on or within agricultural products irrigated with this type of water (Cubides, 2018), and multiple gastrointestinal disease outbreaks have been documented related to the ingestion of vegetables irrigated with contaminated water, evidencing the transmission of pathogens from the water source to the consumer (Pachepsky et al., 2011; Steele & Odumeru, 2004).

2.1. Pathogenic microorganisms

Enteropathogens are a group of bacteria identified as causative agents of various diseases affecting humans and animals. One of the critical water quality criteria is the presence or absence of these pathogens, which are associated with gastrointestinal diseases such as dysentery and salmonellosis (Mogane & Momba, 2025). Diarrhea is among the top twenty causes of death globally and is the fifth leading cause of mortality in lower-income countries (WHO, 2021). According to the WHO, approximately 1.1 billion people worldwide consume unsafe water, and diarrheal diseases or gastroenteritis cause between 5000 and 8000 deaths

annually worldwide, constituting the most predominant cause of mortality among children under five years old.

2.1.1. Enterobacteria

In surface waters, the detection of fecal coliforms indicates fecal contamination. When these bacteria exceed quality standards, they become a potential threat to public health. Their detection is of particular concern because they are associated with microorganisms that can cause gastrointestinal illnesses, including severe gastroenteritis, especially in vulnerable populations (supplementary table 5).

According to Allocati et al., (2013) *E. coli* is classified in the Enterobacteriaceae family and is a Gram-negative, facultative anaerobic, non-spore-forming bacillus. This species naturally inhabits the intestinal tract of humans and other warm-blooded animals. However, under immunosuppressive conditions or when the intestinal mucosa is compromised, *E. coli* can cause infections after ingestion of contaminated food or water. These opportunistic pathogenic strains have been linked to various diseases, including urinary tract infections, sepsis, meningitis, and diarrhea (Nataro & Kaper, 1998). In addition, Allocati warns of the emergence of antibiotic-resistant strains to beta-lactams, quinolones, and aminoglycosides, which are commonly prescribed drugs worldwide. Therefore, the presence of antibiotic remnants in waters with high levels of microorganisms can lead to bacterial resistance due to continuous exposure and interaction with the bioactive compound (Kümmerer, 2009; Posada-Perlaza et al., 2019).

2.1.2. Opportunistic Filamentous Fungi

Filamentous fungi in irrigation water pose a major global challenge due to their impact on food security and agricultural sustainability. Some studies have even documented the presence of ubiquitous environmental fungi, including the genus *Fusarium, Aspergillus, Rhizopus,* and *Penicillium,* in irrigation systems, thereby correlating their presence with crop losses of up to 30% (Pitt & Hocking, 2009). These phytopathogens cause various plant diseases, such as wilts and rots, and produce mycotoxins that contaminate food. In countries with water scarcity or without efficient treatment plants, where wastewater is reused for irrigation, there is a high risk of transmission and spread of these organisms in agricultural activities (Disciglio et al, 2015).

In the middle basin of the Bogotá River, research conducted by Corrales and collaborators (2018) identified a high presence of phytopathogenic fungi in agricultural irrigation sources, including *Aspergillus fumigatus* and *A. flavus*, which predominate in stagnant water or water with high suspended solids content, increasing the risk of contaminating grains and seeds (Bennett & Klich, 2003). In addition, the presence of *Fusarium* species, which cause vascular wilt and root rot in potato (*Solanum tuberosum)* and strawberry (*Fragaria vesca*) crops, as well as species of *Mucor* and *Rhizopus*, responsible for soft rot under high humidity conditions, has been documented (Seifert, 2008).

2.2. Microbiological characterization methods: classical techniques vs. Metabarcoding

Traditional methods for the microbiological characterization of irrigation water, such as plate culture assays, membrane filtration, and colony-forming unit (CFU) count, rely on selective media, differential staining, and biochemical tests to directly quantify microbial presence. However, these techniques are limited to detecting culturable organisms and fail to account for most microorganisms that are non-culturable under standard laboratory conditions (Amann et al., 1995; Stewart, 2012). In contrast, the development of molecular tools, particularly the amplification and sequencing of genetic markers such as 16S rRNA for bacteria and ITS regions for fungi have broadened the understanding of microbial community structure and composition. Metabarcoding enables high-resolution identification of microbial taxa, including those present at low abundance, and facilitates exploration of complex and diverse communities (Lindahl et al., 2013; Knight et al., 2018). When coupled with robust bioinformatics pipelines, this approach provides a more comprehensive and unbiased profile of microbial ecosystems, enabling the detection of potential pathogens, contamination indicators, and ecologically relevant taxa that may be overlooked with traditional culture-based methods (Thompson et al., 2017).

Despite its advantages, metabarcoding has methodological limitations that constrain the interpretation of its results. Amplification is not quantitatively neutral: PCR efficiency varies among taxa according to GC content, marker copy number, and primer affinity, a bias documented for both the 16S rRNA gene and the ITS region (Beaudry et al., 2021; Kyle & Klassen, 2025). In addition, marker conservation and amplicon length restrict taxonomic resolution, so that closely related taxa often cannot be distinguished beyond the genus level,

and taxonomic assignment depends on the coverage of reference databases (Kumar et al., 2024).

3. Bioremediation

The use of contaminated water in agricultural irrigation not only jeopardizes crop productivity but also spreads infectious diseases to the population. The coexistence of pathogenic microorganisms that threaten public health and phytopathogenic agents in contaminated water bodies underscores the need for integrated remediation approaches that consider not only human health protection but also the preservation of the ecological functioning of agricultural systems. Today, several conventional processes are used to mitigate the effects of pollutants in effluent discharges. These processes integrate physical, chemical, and biological techniques to transform, incorporate, or remove solids, organic matter, microorganisms, and, in some cases, nutrients from wastewater (Sonune & Ghate, 2004). According to Gehlot et al., (2022), bioremediation is defined as a strategy that uses microorganisms or their enzymes to eliminate xenobiotics. These are transformed through metabolic or co-metabolic processes into simpler, safer substances, or bioaccumulated for subsequent removal from the environment.

The classification of bioremediation approaches is based on the organisms or biological mechanisms used to remove contaminants from the environment. Phytoremediation is a process that uses plants to absorb, accumulate, or immobilize toxic substances through phytoextraction or phytostabilization, with the potential for wide-area application (Vaksmaa et al., 2023). However, the effectiveness of phytoremediation depends on factors such as plant growth rate and bioavailability of the contaminants (Ali et al., 2013). Microbial

bioremediation employs specialized bacteria and archaea to transform toxic compounds through bioventilation, biostimulation, or bioaugmentation (Yaashikaa et al., 2022). This process exhibits high removal rates; however, it is susceptible to variations in pH, temperature, and compound concentration (Gavrilescu, 2022). Moreover, mycoremediation uses fungi as bioremediation agents, given their ability to utilize organic and inorganic contaminants as substrates, bioaccumulate certain heavy metals, and/or secrete substances that can inhibit or remove pathogenic microorganisms from their environment (Harms et al., 2011).

3.1. Mycoremediation

Most fungi with bioremediation potential are classified within the phyla Ascomycota and Basidiomycota, as well as the subphylum Mucoromycotina, among others. In general, the efficacy of mycoremediation can be attributed mainly to the extracellular and absorptive systems of fungi, as well as the low substrate specificity of their enzymes, which allow them to metabolize or bioaccumulate various compounds, making them excellent decontaminating agents (Akhtar & Mannan, 2020).

One of the fungal degradation pathways is extracellular, carried out by oxidoreductases acting on recalcitrant compounds such as dyes, agrochemicals, and per- and polyfluorinated alkyl substances - PFAS (Khan et al., 2023). Bacteria can also utilize these aromatic and xenobiotic compounds; however, the radial growth of fungal mycelium provides them with an ecological advantage by increasing the surface area available for contaminant exposure and, consequently, their capacity for enzymatic degradation. The most widely studied

enzymes in basidiomycetes and ascomycetes are laccases and peroxidases, which oxidize contaminants by producing free radicals (Harms et al., 2011).

3.1.1. Mycofiltration

Mycofiltration is a bioremediation process that uses fungi as filters to remove toxic compounds through their mycelium (Taylor et al., 2015). This biotechnology allows the removal of contaminants and pathogens in watershed models. The large surface area ratio of the mycelium and the production of bioactive metabolites, such as antibiotics, can reduce bacterial populations (Al-Fatimi et al., 2007; Krupodorova et al., 2016). Some fungi can prey on and digest bacteria under nutritionally limiting conditions, representing another response of fungi to varying environmental conditions (Taylor et al., 2015). Although this technology can guarantee a percentage of bacterial removal, only some fungal species have the physical strength in their mycelia to be used as biological filters with different densities and pore sizes. Later studies showed that mycofiltration with white rot fungi successfully removed multiple water contaminants, including surfactants, nutrients, and aromatic xenobiotics from water, resulting in cost reductions compared to other processes with similar objectives (Malik et al., 2023; Mnkandla & Otomo, 2021). In agreement with these findings, preliminary studies conducted by our research group at the National University of Colombia have also produced promising results for the application of white rot fungi in water treatment (unpublished data).

3.1.2. Mycoaccumulation

Fungi, through their mycelium, have been shown to biosorb and bioaccumulate heavy metals, making them promising candidates for the decontamination of these pollutants (Latha et al.,

2012; Dusengemungu et al., 2020). Fungi frequently accumulate metal ions in their mycelia and spores via their cell walls, with chitin enhancing metal uptake and binding. Furthermore, studies conducted by Dhankhar et al. demonstrated that some fungi isolated and purified directly from contaminated soils and waters can bind these pollutants even under high variations in temperature, pH, oxygen availability, and nutrient concentration (Dhankhar & Hooda, 2011).

3.1.3. Mycodegradation

Fungi play a crucial role in the decomposition of organic matter, as they possess the ability to break down or mineralize pollutants through extracellular oxidases and intracellular enzymes (Syed & Yadav, 2012).

In recent years, the scientific community has shown interest in the enzymatic system of white rot fungi, particularly in nonspecific enzymes such as lignin peroxidase, manganese peroxidase, and laccase (Dashtban et al., 2010). These oxidoreductases have demonstrated potential for oxidizing persistent pollutants, facilitating the transformation of complex compounds into more biodegradable metabolites and even their complete mineralization, thus obviating the need for separate treatments for each pollutant (Mir-Tutusaus et al., 2018).

The lignin peroxidase (LiP) is a hemodependent glycoprotein that catalyzes the oxidation of aromatic substrates through the formation of H_2O_2-dependent cation radicals, allowing it to act on compounds with high reduction potential, such as polycyclic aromatic hydrocarbons, synthetic dyes, and emerging contaminants (Wong, 2009). Meanwhile, manganese peroxidase (MnP) operates via an indirect mechanism, oxidizing Mn^{2+} to Mn^{3+} and

interacting with phenolic and non-phenolic structures in lignin and other resistant polymers. This kinetic strategy is especially useful in heterogeneous matrices, such as soils and sediments, where direct contact between the enzyme and substrate is limited (Hofrichter, 2002). Finally, laccase catalyzes the oxidation of a wide variety of phenolic and non-phenolic substrates through the tetravalent reduction of molecular oxygen to water, without requiring H_2O_2. Its spectrum of action is significantly expanded in the presence of redox mediators such as 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) - ABTS, thereby enabling the oxidation of substrates with high redox potential (Wong, 2009).

Olicón-Hernández in its 2017 publication (Figure 1), describes the biotransformation of pharmaceutically active compounds (PAC) through physiological mechanisms such as adsorption on the cell wall due to its ability to immobilize contaminants without necessarily degrading them, the synthesis of biosurfactants, such as glycoproteins and glycolipids, which improve the solubilization of substances by reducing interfacial tension and the extracellular secretion of enzymes such as peroxidases and laccases, capable of oxidizing a wide variety of compounds, even in the presence of H_2O_2, which can also catalyze epoxidation, aromatic peroxygenation and sulfoxidation reactions. Intracellularly, cytochrome P450 is notable for facilitating the transformation of contaminants via hydroxylation and epoxidation reactions, and for storing certain compounds in organelles that enable their subsequent elimination without degradation (Olicón-Hernández et al., 2017).

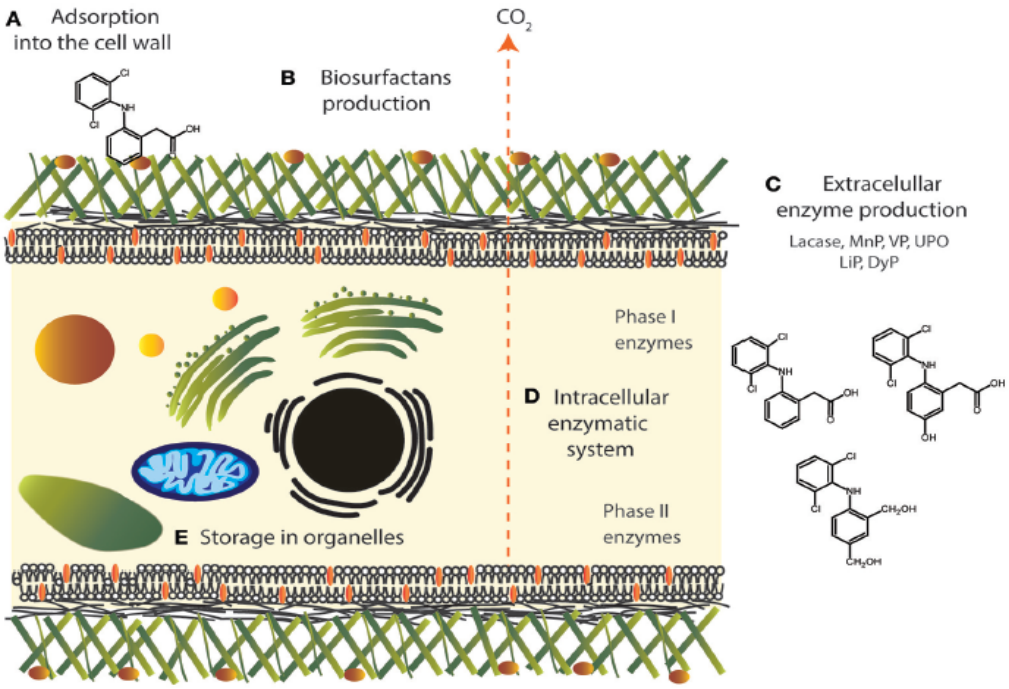


Figure 1: Fungal cell mycoremediation pathways: A. Adsorption into the cell wall; B. Biosurfactant production; C. Extracellular enzymes: MnP: Manganese Peroxidase, VP: Versatile Peroxidase, UPO: Unspecific Peroxygenase, LiP: Lignin Peroxidase, DyP: Dye-decolorizing Peroxidase; D. Intracellular enzymes. E. Storage in organelles. Taken from Olicón-Hernández et al. (2017).

4. Mycoremediation in the world and in Colombia

Mycoremediation has gained global importance as a sustainable alternative for the cleanup of soil and water bodies contaminated with hydrocarbons, heavy metals, industrial dyes, and pathogens (Akpasi et al., 2023). This technique has been implemented in countries such as India, China, the United States, and Brazil, where various macromycete species have demonstrated biodegradation capacity in both controlled and natural environments (Psurtseva et al., 2023). Some *Trametes* species have been utilized in bioreactors to treat industrial effluents, achieving reductions in herbicides such as diuron and bentazone through the action of both intracellular and extracellular enzymes (Hu et al., 2020). Efenudu reported

Gas Chromatography – Mass Spectrometry (GC–MS) based degradation values of up to 90% for certain Polycyclic aromatic hydrocarbons PAHs after treatment with *Pleurotus ostreatus (*Efenudu, 2024).

Although research in mycoremediation is still in its initial stages in Colombia, there are reports where preliminary studies have been carried out on the degradation of contaminants by native fungi, particularly in the treatment of domestic wastewater and agricultural leachates (Castro Fernández et al., 2022), as well as work in bioremediation, applying macromycetes in the biodegradation of hydrocarbons (Boada Hurtado et al., 2018), removal of pharmaceutical waste (Vargas, 2022), decolorization of Gram stain dyes (Hernández-Sáenz et al., 2020) and the mycoremediation of cigarette butts (Vera Marmanillo et al., 2024) and mycoremediation of microplastics (Sagar et al., 2022).

However, most studies have been conducted at the laboratory scale; the scarcity of financial resources, a lack of knowledge about the enzymatic properties of native fungi, and limited communication among academia, rural communities, and government decision-makers have slowed the development of pilot tests of this biotechnology as an alternative in the country.

5. Macromycete fungi with mycoremediation potential

Fungi producing visible sporomes, mainly within the phyla Basidiomycota and Ascomycota, are commonly referred to as macromycetes or macrofungi and can often be recognized in the field based on the morphology of their fruiting bodies (Laessøe & Petersen, 2019; Deng et al., 2024). In the field of mycoremediation, the mycelium (Figure 2) of these fungi is characterized by their wide fungal diversity and well-documented enzymatic capabilities.

Among the saprophytic macromycete fungi, some species offer considerable potential for implementation in pilot scale mycoremediation systems in rural contexts, as they exhibit high biological efficiency, environmental adaptability, and local availability (H.M Kasuni & Ekanayake, 2026).



Figure 2. Mycelial colony of *Ganoderma* sp (FDH-00023) subcultured on potato dextrose agar (PDA) medium. The strain was obtained from the Living Macromycetes and Micromycetes Biological Collection (FDHUN) of the Universidad Nacional de Colombia and maintained at 24°C until active mycelial growth was achieved prior to the mycoremediation experiment. Photograph by the author.

Among saprophytic macrofungi, white-rot fungi have received particular attention because of their exceptional ligninolytic system, which is based on the secretion of highly oxidative, largely nonspecific extracellular enzymes. Among the most studied genera, *Pleurotus*, *Trametes*, and *Ganoderma* have demonstrated remarkable biodegradation capabilities.

5.1. *Pleurotus* sp.

Pleurotus sp., more commonly known as the oyster mushroom, is an edible species that, in addition to being used as food, has been utilized in the field of mycoremediation. It is distinguished by its taxonomic diversity, rapid growth on various substrates, and its broad enzymatic capacity to produce laccase and manganese peroxidases (Pérez Montiel et al., 2021; Vera Marmanillo et al., 2024). The extracellular enzymatic system has been molecularly characterized in various strains, demonstrating an isoenzymatic diversity that extends its ability to act on a wide range of structurally diverse contaminants (Riley et al., 2014). The mycelium has demonstrated high efficiency in the biosorption of lead and cadmium in industrial waters (Goligar et al., 2023) and a robust enzymatic metabolome capable of biotransforming emerging pollutants, organic micropollutants, and aromatic compounds from wastewater (Hu et al., 2020).

Beyond its environmental applications, *Pleurotus ostreatus* also exhibits antimicrobial properties (Gashaw et al., 2020) and it has been postulated that bioactive extracts of the fungus exhibit the ability to inhibit microbes such as *Pseudomonas sp., Klebsiella sp., Salmonella sp., E. coli, Bacillus subtilis,* and *Saccharomyces cerevisiae* (Sagar et al., 2022).

5.2. *Trametes* sp.

Trametes sp. are saprotrophic fungi of the family Polyporaceae and represent one of the most widely distributed genera of white rot fungi in forested ecosystems of temperate and tropical climates (Olou et al., 2020); it has been attributed medicinal benefits and is reported in several studies in the field of bioremediation (Dinakarkumar et al., 2024). In particular, *T. versicolor* and *T. hirsuta* are distinguished by their ability to degrade organic and inorganic contaminants in soil and wastewater through the action of extracellular enzymes, the versatility in the degradation of contaminants such as polycyclic aromatic hydrocarbons (PAHs), industrial dyes, phenolic compounds, and certain heavy metals is linked to the non-specific nature of ligninolytic enzymes, which do not require specific induction but react in a generalized manner to aromatic and heterocyclic structures (Tavares et al., 2017). According to Margot (2015), the versatility in degrading organic and inorganic contaminants is linked to the non-specific nature of ligninolytic enzymes, which do not require specific induction but instead react in a generalized manner to aromatic and heterocyclic structures (Margot et al., 2015).

Immobilization of *T. versicolor* on wood or its application in trickling-bed reactors has demonstrated up to 90% removal efficiency of hydrophobic pesticides, such as chlorpyrifos, dicofol, and cypermethrin, after two weeks of treatment (García-Vara et al., 2021; Hu et al., 2020). In addition, several studies have demonstrated its effectiveness in the biological treatment of wastewater from the textile and paper industries, particularly in decolorizing effluents containing complex dyes. This capability is mainly attributed to the synergistic activity of laccase, manganese peroxidase (MnP), and lignin peroxidase (LiP), which catalyze the oxidative cleavage of dye chromophoric groups, generating low-molecular-

weight aromatic compounds that can be further degraded and, under favorable conditions, completely mineralized to CO_2 and H_2O. Nevertheless, the composition and toxicity of intermediate metabolites may vary with dye chemical structure, highlighting the importance of ecotoxicological assessment of treated effluents (Thampraphaphon et al., 2022).

5.3. *Ganoderma* sp.

The genus *Ganoderma*, particularly *Ganoderma lucidum*, is a wood-decaying polypore that has been used in traditional Chinese medicine for over two millennia. It is one of the most extensively studied medicinal mushrooms due to its rich content of bioactive metabolites, including triterpenoids, polysaccharides, sterols, and peptidoglycans. These compounds are widely recognized for their immunomodulatory, hepatoprotective, anti-inflammatory, and antioxidant properties (Boh et al., 2007; Ma et al., 2024; Wasser, 2011). Recent studies have demonstrated that *G. lucidum* can remove more than 70% of chemical oxygen demand (COD) and ammonia nitrogen (NH_3) from domestic wastewater under controlled conditions. Optimal agitation levels of 100 rpm and a favorable carbon-nitrogen ratio in the reactors have been shown to facilitate this removal (Ma et al., 2024). The regulation of the enzyme system's expression is contingent upon nutrient availability and the presence of inducers within the medium. Research, such as that conducted by Palazzolo et al. (2019), has indicated that the biochemical characterization of these enzymes reveals significant thermal stability and tolerance to a wide range of pH levels. A laboratory study was conducted using mining water, and the results demonstrated that preconditioned *G. lucidum* pellets exhibited a remarkable efficacy in reducing chromatic contaminants in water. Furthermore, the fungus genus *Ganoderma* sp. has been shown to possess protective mechanisms against toxic metal ions,

including Cu^{2+}, Cd^{2+}, Mn^{2+}, and Ni^{2+}, through the production of antioxidant polysaccharides. This ability to produce antioxidant polysaccharides enables *Ganoderma sp.* to survive in contaminated environments (Ma et al., 2024).

6. Microbial diversity changes and functional analysis as indicators of remediation

The characterization of microbial diversity and its functional potential in aquatic systems is essential not only for assessing resource quality and the effectiveness of remediation strategies but also for understanding ecological dynamics. In this context, metabarcoding has proven to be an effective approach for characterizing both the composition of microbial communities, defined as the identity of the taxa present, and their structure, which encompasses both the identity and relative abundance of taxa, including their distribution and dominance patterns. This approach also enables the taxonomic identification of even low-abundance taxa (Sansupa et al., 2021). When performed with bioinformatics pipelines such as DADA2 and supported by curated taxonomic databases, including SILVA for bacteria and UNITE for fungi, this approach enables robust and high-resolution microbial classification (Callahan et al., 2016, Bolyen et al., 2019). Furthermore, integrating taxonomic data with functional inference tools, such as FAPROTAX for prokaryotes and FUNGalTraits for fungi, enables the prediction of potential ecological roles and metabolic functions of microbial consortia, providing a broader understanding of how these communities respond to environmental stresses and bioremediation processes (Sansupa et al., 2021).

Bioinformatics tools, such as DADA2, Phyloseq, and ANCOMBC, used in metabarcoding studies, have significantly optimized the analysis of microbial communities in complex environments. DADA2 enables processing of sequences from massive sequencing data and

the generation of amplicon sequence variants (ASVs) with high accuracy (Callahan et al., 2016). These ASVs can then be integrated into Phyloseq, along with taxonomic information and experimental metadata. This integration facilitates the development of diversity analyses and the construction of ecological visualizations (McMurdie & Holmes, 2013). To compare microbial communities across treatments, ANCOMBC and its most recent version, ANCOM-BC2, employ statistical models that are correct for compositional bias, read depth, and sampling variability, enabling the detection of taxa with significant differences between groups (Lin & Peddada, 2020; Huang et al., 2023). The combination of these tools enables transparent, reproducible study of changes in microbial communities induced by processes such as mycoremediation applied to irrigation water treatment (Lin & Peddada, 2020, 2024).

7. The context of la Ramada, Venecia, and Lucero

The La Ramada Irrigation District is situated in the middle basin of the Bogotá River (Figure 3), encompassing approximately 6 200 hectares under the jurisdiction of Funza and Mosquera. The district is primarily used for agricultural activities and is moderately populated, with industries present as well. It benefits approximately 1 000 farmers and users. According to Espinosa, 2022 the predominant crops include fruits, vegetables, and tubers. However, the district's canals capture water from the Bogotá River, whose quality is compromised by high levels of salts, organic contaminants, and heavy metals, which impacts the healthiness of horticultural production and generates hydrobiological risks (Miranda et al., 2008).

In La Ramada, the areas called Venecia and Lucero are identified as agricultural and industrial influence zones, critical points of contamination. In Venice, identified as a zone of agricultural influence and a critical contamination point (Supplementary figure 1), vegetable crops such as spinach, cauliflower, celery, and cilantro are predominant and are consumed directly in salads; agricultural leachate and domestic waste have been observed. In Lucero, located adjacent to urban areas, an additional load of industrial and domestic waste is generated by plastics, paints, detergents, and the recycling of vehicle tires (Supplementary figure 2). High concentrations of indicator pathogenic microorganisms, including total and fecal coliforms, have been detected at both sites (Heliodoro & Martha., 2018; Ramírez Espinosa, 2022). These concentrations exceed the national standards established by the Colombian Ministry of Environment and Sustainable Development (2017) for agricultural irrigation waters.

Torres (2004) has reported that the water used for irrigation in La Ramada exhibits high loads of total coliforms and *E. coli*, which serve as indicators of microbiological contamination in anaerobic environments. This phenomenon has the potential to exacerbate eutrophication processes in water bodies, significantly reducing the availability of water suitable for agricultural irrigation and posing a potential risk to the health of farmers and neighboring communities. This issue has gained relevance in the context of reports documenting the presence of pharmaceutical residues, including antihypertensives, analgesics, and antibiotics, in these effluents. This observation highlights the critical concern of continued exposure of enterobacteria to antibiotics, which can lead to the development of antibiotic-resistant strains, a priority for the WHO (Torres, 2024).

The La Ramada Irrigation District has sparked academic interest at several Colombian universities, especially in Bogotá, due to concerns about the microbiological and chemical quality of its water resources. A review of the literature identified 17 studies conducted by five institutions between 2005 and 2024, covering five main areas: nine on coliform contamination and bacteriological water quality; two on parasites; two on fungal communities; three on pharmaceutical waste; and one on mycoremediation as a pharmaceutical waste treatment strategy. Although bacterial contamination is well documented in the district, studies on fungal communities and biotechnological remediation approaches remain largely unexplored. Details of each study, including year, institution, and focus, are summarized in Table 1.

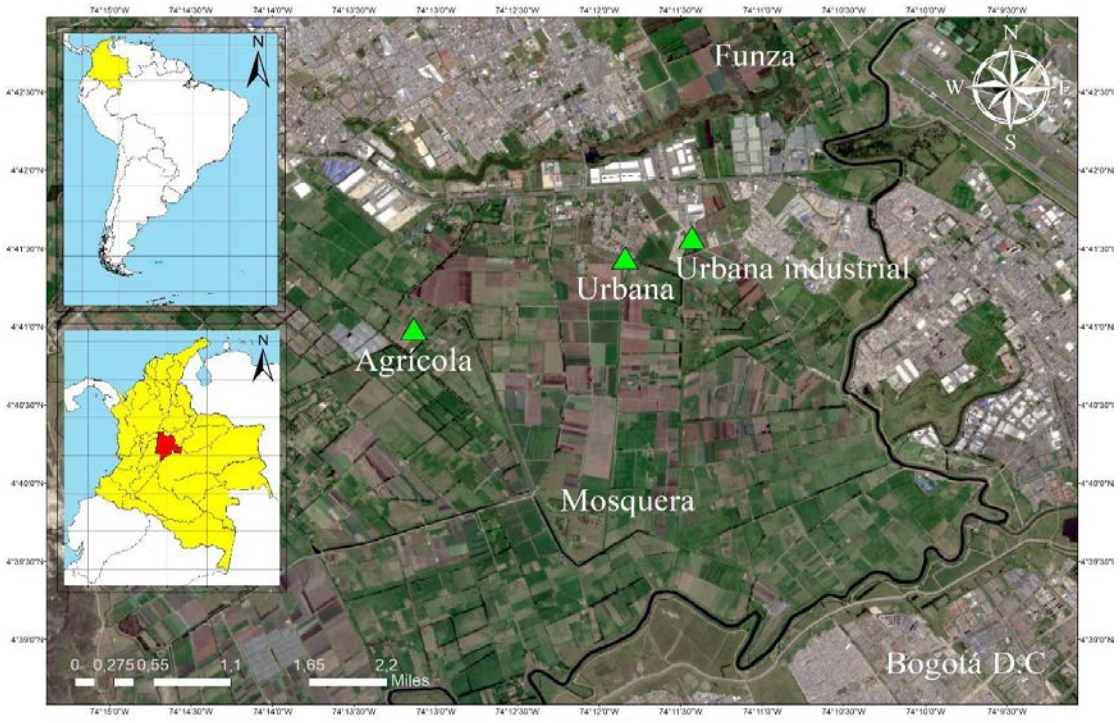


Figure 3: The La Ramada Irrigation District. Taken from Sánchez, C. A. (2020). Ilustración donde se ubica los tipos de actividades que afectan las aguas en la sabana de Bogotá ubicado en el centro de Colombia sur América

Table 1: Research on the La Ramada Irrigation District by Colombian Universities in Bogotá

Year	Colombia Institution	Authors	Research
2024	Catholic University of Colombia	Vesga FJ, et, al	Estimating risk of *Salmonella* related illness from lettuce irrigated with contaminated water
2024	Andes University	Bulla-Marín NY, Henao L, Husserl J	Risk assessment of *Salmonella* sp. and antibiotic-resistant *Salmonella* in lettuce irrigated with water from La Ramada Irrigation District
2024	National University of Colombia	Ospina Santos et al.	Occurrence of parasites in waters used for irrigation of crops and vegetables from La Ramada irrigation district
2022	National University of Colombia	Espitia J.A., Bustos-López MC	Evaluation of a laboratory-scale treatment system to reduce the concentration of organic load, bacteria and/or parasites using synthetic wastewater, with a view to its applicability in crop irrigation in the western savanna of Bogotá - Colombia
2022	National University of Colombia	Vargas, I., Fung, Y.W	Capacity of fungi to remove pharmaceuticals present in irrigation waters from the La Ramada irrigation district
2022	Rosario University	Urrea, VP, Muñoz, M	Description of bacterial communities in tributaries of the Bogotá River using a metagenomic approach.
2021	Andes University	Ramirez et al.	Analysis of irrigation and drainage practices in the La Ramada irrigation district 1939-2000
2020	National University of Colombia	Sánchez, C.A, Bustos-López MC	Valuation of toxicity in sediments from the channels of La Ramada Irrigation District using two bioassays

2019	National University of Colombia	Martinez, P., Bustos-López MC	Evaluation of azithromycin removal dissolved in synthetic wastewater in a laboratory-scale UASB reactor
2018	National University of Colombia	Cubides PA, Bustos	Evaluation of a treatment to improve the quality of water used for irrigation in the western Savanna of Cundinamarca
2018	National University of Colombia	Argüello H, Bustos-López MC	Contamination by Pathogenic Microorganisms in Water used for Agricultural Irrigation on the Sabana de Bogotá, Colombia
2018	El Bosque University	Morales, D.	Comprehensive Solid Waste Management Plan for a floriculture company
2018	Colegio Mayor de Cundinamarca University	Corrales LC et al.	Potentially phytopathogenic microorganisms in irrigation water from the middle basin of the Bogotá River
2015	National University of Colombia	Hernández et al.	C-QTOF MS screening of more than 1,000 licit and illicit drugs and their metabolites in wastewater and surface waters from the area of Bogotá, Colombia
2009	National University of Colombia	Husserl J, Corrales LC, Matiz A	Estimating the risk of illness from contaminated irrigation water
2009	National University of Colombia	Carranza et al.	Analysis of lettuce (*Lactuca sativa*) 'Batavia' growth irrigated with water from La Ramada Irrigation District
2005	Andes University	Alarcon et al.	Evaluation of parasitological water quality

Table 2: Research on the La Ramada Irrigation District by Colombian Universities in Bogotá

Previous local studies, including the university research presented in Table 1, have documented the presence of enteropathogenic bacteria, opportunistic fungi, and parasites in irrigation water from the La Ramada district and have assessed physical and chemical remediation methods. However, no studies have examined the use of lignocellulosic macrofungal mycelium as a bioremediation strategy to remove microbial pathogens from agricultural irrigation water in Colombia. This is a significant gap because, despite the worldwide evidence of ligninolytic macromycetes bioremediation potential, their impact on pathogenic microbial community structures in Colombian irrigation water remains unstudied. To fill this gap, this study investigates whether *Trametes* sp., *Ganoderma* sp., and *Pleurotus ostreatus* can alter pathogenic microbial communities in contaminated irrigation water from La Ramada, along with the related objectives and hypotheses.

8. Research Objectives:

8.1. General Objective:

To evaluate the mycoremediation potential of *Trametes* sp., *Ganoderma* sp., and *Pleurotus ostreatus* against pathogenic microorganisms in agricultural irrigation waters of the La Ramada district, Colombia

8.2. Specific Objectives:

O1. To characterize the taxonomic and functional diversity of the bacterial and fungal microbiomes in irrigation waters at the Venecia and Lucero sites of the La Ramada district

O2. To evaluate the mycoremediation potential of *Trametes* sp., *Ganoderma* sp., and *Pleurotus ostreatus* on enteropathogenic bacteria and opportunistic fungi in irrigation waters at the Venecia and Lucero sites.

9. Research Hypotheses

9.1. General Hypotheses

GH: The ligninolytic macromycetes *Trametes* sp., *Ganoderma* sp., and *Pleurotus ostreatus* exhibit differential mycoremediation potential in agricultural irrigation waters of the La Ramada district, modifying the pathogenic microbial communities' structure.

9.2. Specific Hypotheses

H1: The irrigation waters of Venecia and Lucero harbor different bacterial and fungal community structures, reflecting differences in the nature of their respective contamination sources.

H2: The application of mycelium from *Trametes* sp., *Ganoderma* sp., and *Pleurotus ostreatus* under controlled experimental conditions induces shifts in the overall structure and composition of bacterial and fungal communities in the treated water.

CHAPTER 2

MATERIALS AND METHODS

10.1. Water sampling and study sites

Sampling was conducted on January 11, 2023, at two sites in the La Ramada Irrigation District (Supplementary Figures 1 and 2), located in the middle basin of the Bogotá River, within the jurisdiction of the municipalities of Funza and Mosquera, Cundinamarca, Colombia. The Venecia site (VPA) (N 04°41.369', W 074°14.807') primarily receives discharges associated with agricultural and horticultural activities, while the Lucero site (LPB) (N 04°41.581', W 074°11.443') is exposed to discharges of industrial and urban origin. At each site, 5 liters of water were aseptically collected in sterile containers and transported to the Environmental Engineering Laboratory (LIA) at the National University of Colombia (UN) Bogota campus. The samples were filtered using 0.22 μm polyethersulfone (PES) membranes via vacuum filtration to concentrate the microorganisms present in the water at the time of sampling on day 0 (Control 0). The membranes were stored at –20 °C until shipment and molecular processing at the Université du Québec à Trois-Rivières (UQTR).

10.2. Physicochemical and microbiological characterization

The physicochemical parameters were determined according to standard methods for water analysis (APHA, 2017), pH, electrical conductivity, temperature, and dissolved oxygen were measured *in situ* with a HACH HQ40D multiparameter device. In the laboratory, chemical oxygen demand (COD) was quantified using the standard methods protocol number SM 5220-C, biochemical oxygen demand (BOD) by SM 5210-B, and suspended solids by SM

2540-D. The determination of total coliforms and *E. coli* was performed through membrane filtration with 0,45 µm filters and counting on Chromocult® Coliform Agar chromogenic medium, which simultaneously detects β-galactosidase activity for total coliforms and β-glucuronidase activity for *E. coli*, in accordance with method SM 9223-B (APHA, 2017). All analyses were conducted in accordance with the LIA's internal laboratory protocols.

10.3. Mycoremediation Experiment

The mycelium of the macromycetes used as experimental treatments, *Trametes* sp. (FDH-00018), *Ganoderma* sp. (FDH-00023), and *Pleurotus ostreatus* (FDH-00008), was obtained from the Living Macromycetes and Micromycetes Biological Collection (FDHUN) at the National University of Colombia. The strains were subcultured on potato dextrose agar (PDA) medium and incubated at 24°C until active mycelial growth was achieved. The mycoremediation experiment was conducted in Erlenmeyer flasks containing 200 mL of water from each sampling site, and each treatment was evaluated in triplicate by independently inoculating a 1 cm^2 plug of mycelium corresponding to each macromycete. Three types of controls were established, all in triplicate: water controls without mycelium on day 0 (Control 0), water controls without mycelium incubated for 30 days under the same experimental conditions (Control 30), and viability controls of the mycelium of the macromycetes used as treatment, maintained in malt extract medium to verify survival during the experimental period. All treatments and controls were kept under constant agitation at 125 rpm and 20 ± 2 °C for 30 days. At the end of the experiment, each mycelium plug was cultured on PDA medium to assess viability, and the water from each flask was vacuum filtered through 0,22 µm polyethersulfone (PES) membranes to concentrate the

microorganisms. The membranes were stored in Eppendorf tubes at –20 °C until shipment to UQTR for molecular analysis.

10.4. DNA Extraction

Total DNA from microorganisms retained on filtration membranes was extracted at the Plant Biology Laboratory of the UQTR using the Qiagen DNeasy PowerSoil Pro Kit, following the manufacturer's instructions. Cell lysis was achieved through combined mechanical and chemical disruption using PowerBead Pro tubes in a TissueLyzer II system (Qiagen), and purified DNA was eluted in 10 mM Tris solution. DNA quality and integrity were assessed by electrophoresis on 1.5% agarose gels prepared in 1× TAE buffer stained with RedSafe, using a 100-bp DNA ladder (FroggaBio). Electrophoresis was performed at 90 V for 60 min, and DNA was visualized under UV light using a gel documentation system.

10.5. Library Preparation, Amplification and Sequencing

10.5.1. Bacterial 16S rRNA Gene

Extracted DNA served as a template for amplifying the bacterial 16S rRNA gene using the IDT Technologies USA primer pair 515f-CS1 (ACACTCTTTCCCTACACGACGCTCT-TCCGATCTGTGYCAGCMGCCGCGGTAA) and 806r-CS2 (GTGACTGGAGTTCA-GACGTGTGCTCTTCCGATCTGGACTACNVGGGTWTCTAAT), which included CS1 and CS2 adapter sequences at the 5′ end for Illumina library preparation (Supplementary figure 6). PCR reactions were conducted in a total volume of 25 µL containing 12.5 µL of OneTaq Quick-Load 2X Master Mix, 0.5 µL of each primer (10 µM), 5 µL of template DNA,

and 6.5 µL of nuclease-free water. The amplification program involved an initial denaturation at 95 °C for 3 minutes, followed by 33 cycles of 95 °C for 30 seconds, 55 °C for 30 seconds, and 68 °C for 30 seconds, with a final extension at 68 °C for 5 minutes. Amplicons were verified on 1.5% agarose gels stained with RedSafe, using a 100-bp DNA ladder (FroggaBio), and run at 90 V for 60 minutes. After confirming the expected fragment size, 63 amplicons were sent to Genome Québec for library preparation and sequencing on an Illumina MiSeq paired-end platform.

10.5.2. Fungal ITS Region

The same DNA extracts were used to amplify the fungal internal transcribed spacer (ITS) region. To minimize bias, primers with degenerate positions (nBITS2f/n58A2r), designed to broaden taxonomic coverage, were used (Bokulich et al., 2013). IDT Technologies USA primer pair nBITS2f-CS1 (ACACTCTTTCCCTACACGACGCTCTTCCGATCT-RCCWGYGGRGGATCA) and n58A2r-CS2 (GTGACTGGAGTTCAGACGTGTGCTC-TTCCGATCTCTRYGTTCTTCATCGAT), which also incorporated CS1 and CS2 adapter sequences at the 5′ end for Illumina library preparation (Supplementary figure 6). PCR reactions were carried out in a final volume of 25 µL containing 12.5 µL of OneTaq Quick-Load 2X Master Mix, 0.5 µL of each primer (10 µM), 5 µL of template DNA, and 6.5 µL of nuclease-free water. Thermal cycling consisted of an initial denaturation at 95 °C for 3 min, followed by 35 cycles of 95 °C for 30 s, 52 °C for 30 s, and 68 °C for 30 s, with a final extension at 68 °C for 5 min. Amplicons were verified on 1.5% agarose gels stained with RedSafe, using a 100-bp DNA ladder (FroggaBio), and were run at 90 V for 60 min. After

confirmation of the expected fragment size, 63 amplicons were submitted to Genome Québec for library preparation and sequencing on an Illumina MiSeq paired-end platform.

10.6. Sequence analyses and downstream processing

Raw paired-end reads generated on the Illumina MiSeq platform were demultiplexed and adapter-trimmed using Cutadapt, and subsequently quality-filtered with Trimmomatic (Bolger et al., 2014). Bioinformatic processing was performed in R using DADA2 (v1.20.0) (Callahan et al. 2016), according to the standard workflow for Illumina paired-end amplicon data. Bacterial reads targeting the 16S rRNA V4 region were filtered with maxEE = c(1, 1) and truncLen = c(260, 230), whereas fungal ITS2 reads were filtered with maxEE = c(0.5, 0.5), truncQ = 2, and minLen = 50, with no length truncation applied because of the intrinsic size variability of the ITS region, preserving the natural length and increasing coverage to reduce biases due to amplicon length. In both datasets, error learning, denoising, and chimera removal were performed to infer amplicon sequence variants (ASVs). Taxonomy was assigned against SILVA v138.2 for bacterial sequences and UNITE v9.0 for fungal sequences. The resulting taxonomic tables were integrated with the ASV table and the sample metadata into a single synchronized phyloseq object for reproducible microbiome analyses and downstream analyses (McMurdie et al., 2013).

10.7. Statistical analysis

Alpha diversity was assessed using the Observed Species, Shannon, Simpson, and Faith's PD indices, whereas beta diversity was evaluated with NMDS based on Bray–Curtis dissimilarities and tested with PERMANOVA using a Benjamini–Hochberg correction and Tukey HSD for pairwise comparisons. Group differences were analyzed using the Kruskal–

Wallis test and Dunn's post hoc test with a Bonferroni correction. Differentially abundant taxa between each fungal treatment and day 30 Control were identified with ANCOM-BC2, with significance defined as $q < 0.05$ after passing the stochastic sensitivity filter (Lin & Peddada, 2020). Functional annotation of bacterial communities was performed with FAPROTAX, and fungal functional guilds were assigned with FUNGalTraits (Põlme et al., 2020). All statistical analyses were conducted with the phyloseq, vegan, picante, microbiome, ANCOMBC, and microbiomeutilities packages in R 4.1.0. (R Core Team. 2021).

CHAPTER 3

RESULTS

11. Water physicochemical characterization and sequencing yield

Physicochemical analyses at the Venecia and Lucero sites revealed differences between them. In Lucero, chemical and biological oxygen demand reached 1,037 mg/L and 360 mg/L, respectively. In Venecia, they were 190 mg/L and 112 mg/L, respectively. Electrical conductivity was 791 µS/cm in Lucero and 459 µS/cm in Venecia. Dissolved oxygen levels were 0,71 mg/L in Lucero and 0,53 mg/L in Venecia. Regarding microbiological load, Lucero recorded 126 000 CFU/100 mL of total coliforms and 20 000 CFU/100 mL of *E. coli*, while Venecia reported 7 000 CFU/100 mL and 4 000 CFU/100 mL, respectively. The complete set of parameters is detailed in Table 2. The assessment of mycelial viability at the end of the experiment revealed an overall survival rate of 49%. Of the surviving fraction, *Trametes* sp. accounted for 23%, *Ganoderma* sp. for 17%, and *Pleurotus ostreatus* for 11%. By site, *Trametes* sp. had equal survival at both sites (50% in Lucero and 50% in Venecia), *Ganoderma* sp. survived more in Venecia (67%) than in Lucero (33%), and *Pleurotus ostreatus* had the lowest survival in Lucero (25%) compared to Venecia (75%), the values are in Table 3. Global mycelial survival. The sequencing analysis yielded a total of 1,925,503 high-quality reads for bacteria (16S rRNA, V4 region) and 3,969,086 for fungi (ITS2), with an average retention percentage of 75% and 64%, respectively, after quality filtering with DADA2. The distribution of reads per sample is found in supplementary table 3-1 for bacteria and supplementary table 3-2 for fungi

	Total colifoms UFC	Fecal coliforms UFC	COD mg/L	BOD mg/L	TDS mg/L	pH	Cond µS cm-1	OD mg L-1	TH ppm	TA ppm
Resolución 1076/2015	< 5,000	< 1,000	<125	< 25	-	4.5 - 9.0	< 450-2	> 3-5	<60-180	<60-180
Venecia	7.30E+03	4.00E+03	190	112	273	6.6	459	0.53	250	50
lucero	1.26E+05	2.00E+04	1037	360	512	7.06	791	0.71	180	180

Table 3: Physicochemical and microbiological parameters of irrigation water sampled at the Venecia and Lucero sites of the La Ramada Irrigation District, Cundinamarca, Colombia (January 11, 2023). COD: chemical oxygen demand; BOD: biochemical oxygen demand; TDS: total dissolved solids; EC: electrical conductivity; DO: dissolved oxygen; TH: total hardness; TA: total alkalinity; TC: total coliforms; FC: fecal coliforms (E. coli). Values in bold red exceed the regulatory limits established by Colombian Resolution 1076 of 2015. —: no regulatory limit established for this parameter. Analyses were performed in accordance with the Standard Methods for the Examination of Water and Wastewater (APHA, 2017) at the Environmental Engineering Laboratory (LIA), Universidad Nacional de Colombia.

Macromycete	% Global survival	Venecia % surviving fraction	Lucero % surviving fraction
Trametes sp.	22%	50%	50%
Ganoderma sp.	16%	67%	33%
Pleurotus ostreatus	11%	75%	25%

Table 4:Global mycelial survival after 30 days of mycoremediation treatment in irrigation water from the La Ramada District. Survival was assessed by culturing each mycelial plug on PDA medium at the end of the experimental period. Global survival represents the percentage of plugs that remained viable among those inoculated at both sites. Species distribution reflects the proportion of each macromycete within the surviving fraction. Site-specific survival indicates the proportion of surviving plugs of each species recovered from each sampling site. *Trametes* = *Trametes* sp. (FDH-00018); *Ganoderma* = *Ganoderma* sp. (FDH-00023); *Pleurotus* = *Pleurotus ostreatus* (FDH-00008).

12. Composition and structural characterization of microbial communities

12.1. Bacterial community composition

On day 0, the bacterial communities in Venecia and Lucero differed in composition. Venecia was characterized by a high relative abundance of Exiguobacteraceae (21,71%), Moraxellaceae (15,83%), and Pseudomonadaceae (13,80%), from a total of 240 151 reads, while Lucero was prevalent in Moraxellaceae (37,46%), Pseudomonadaceae (15,45%), and Exiguobacteraceae (11,52%), with 84 778 reads and top 20 most abundance families (Table 4; Fig. 4A, 4B). The family Enterobacteriaceae ranked 13th in overall relative abundance; however, when analyzed by site, it ranked seventh in Lucero (8,69%) and 27th in Venecia (2,56%). The genus *Klebsiella* was the most abundant, representing 97,5% of the Enterobacteriaceae reads in Venecia and 83,8% in Lucero (Fig. 5A, 5B; Table 5). After 30 days of treatment, changes in the bacterial community were observed at both sites. In Lucero, total reads per sample decreased relative to day 0 control, whereas in Venecia, the most abundant family shifted from Exiguobacteriaceae to Micrococcaceae. Finally, a decrease in the abundance of Enterobacteria was observed from day 30 onward across all treatments and controls (Supplementary Table 3-3).

Family	**Venecia (%)**	**Reads**	**Lucero (%)**	**Reads**
Exiguobacteraceae	21,71	52 133	11,52	9 765
Moraxellaceae	15,83	38 010	37,46	31 760
Pseudomonadaceae	13,8	33 138	15,45	13 098
Xanthomonadaceae	6,92	16 623	4,43	3 752
Micrococcaceae	5,34	12 817	—	—
Caulobacteraceae	5,2	12 483	—	—
SRhodanobacteraceae	3,48	8 356	—	—
Carnobacteriaceae	—	—	10,19	8 635
Enterobacteriaceae	2,56	6 139	8,69	7, 63
Total reads		**240 151**		**84 778**

Table 5: Dominant bacterial families at Day 0 by site (relative abundance). (—) = not among top 7 families

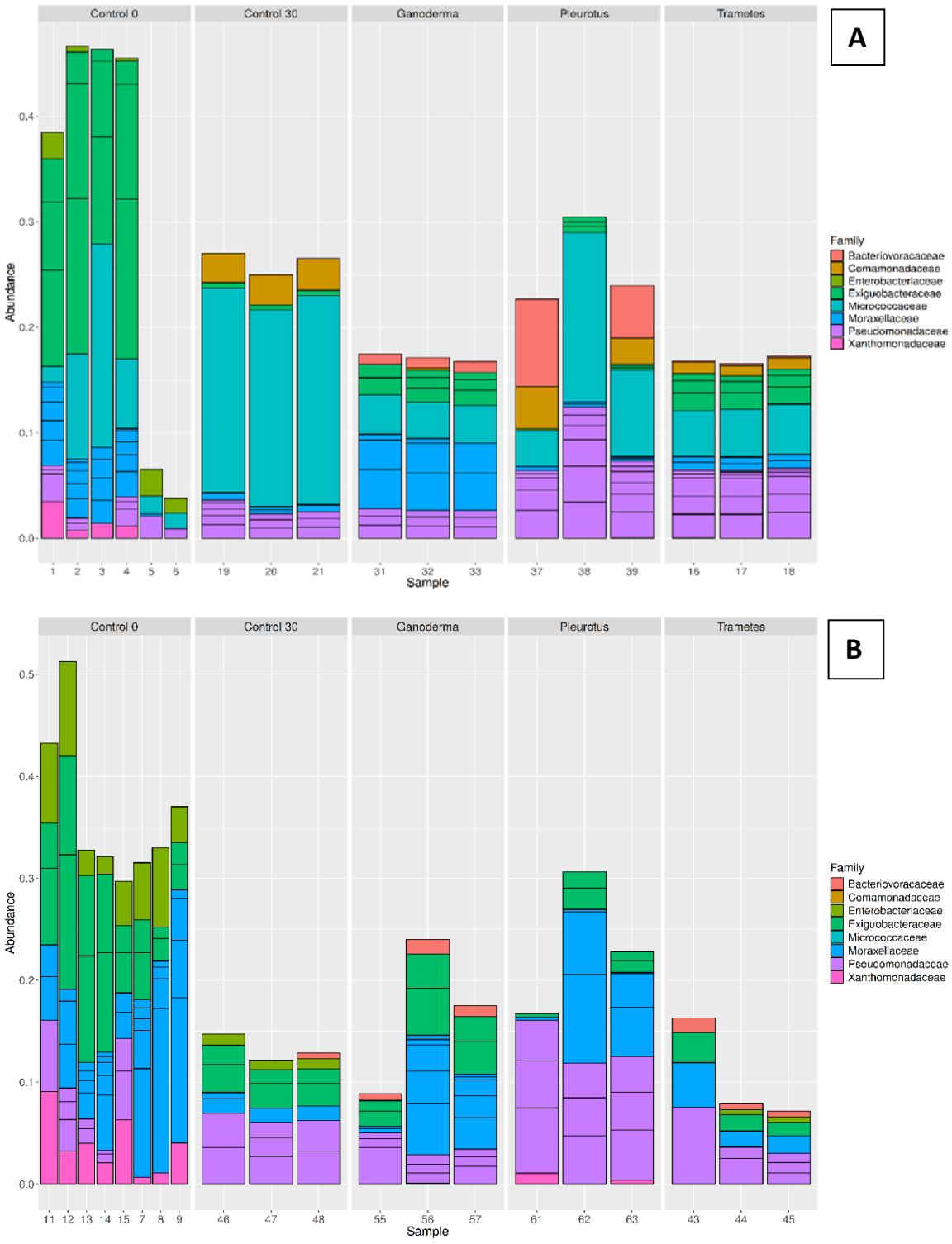


Figure 4: Relative abundance of the 20 most abundant bacterial families per sample and treatment in Venecia (A) and Lucero (B).

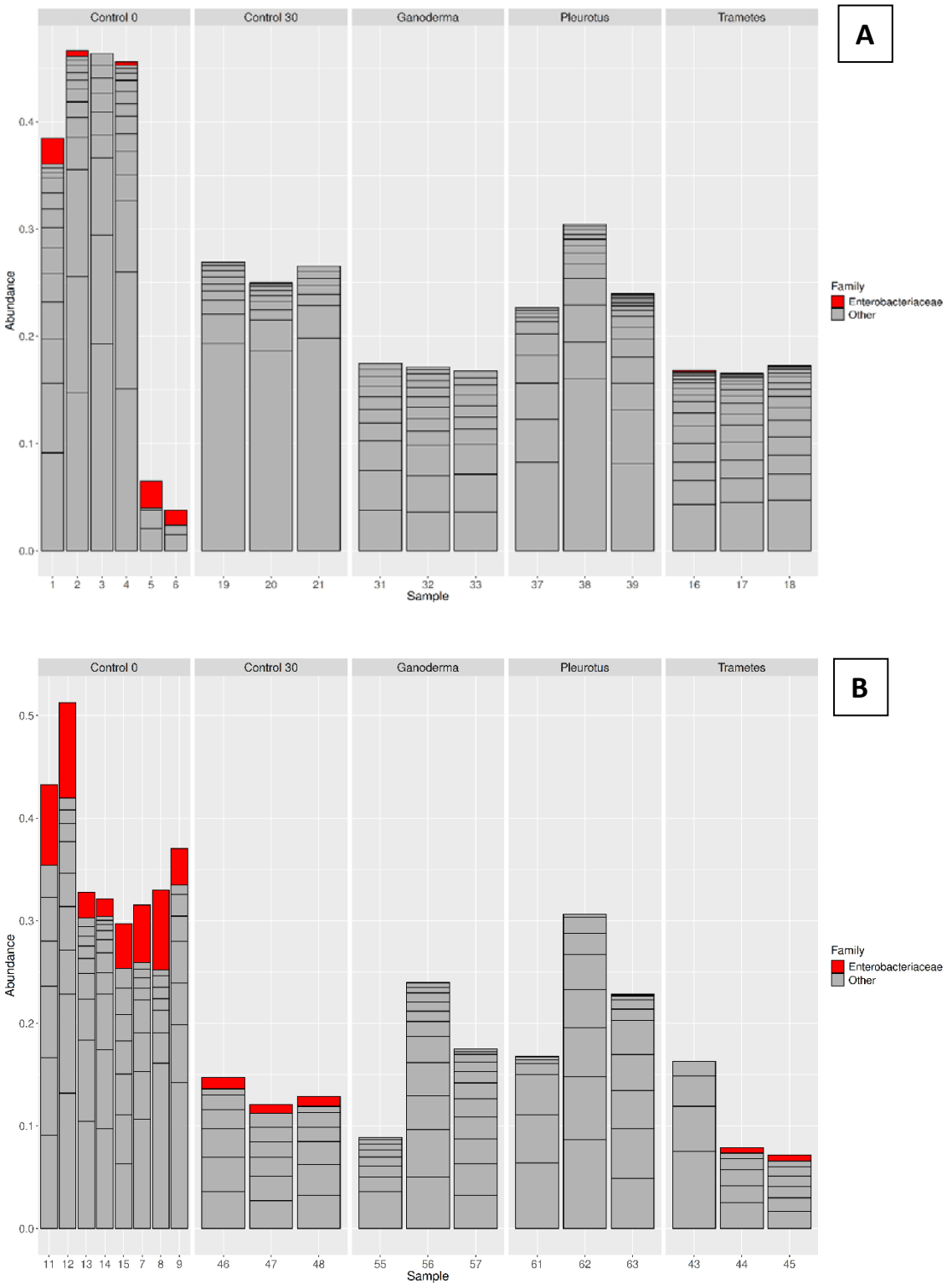


Figure 5 Highlight the relative abundance of Enterobacteriaceae (red) compared to the rest of the community (gray) per sample and treatment in Venecia (A) and Lucero (B).

Genus	Venecia reads	Venecia %	Lucero reads	Lucero %
Klebsiella	5 988	97,50%	6 172	83,80%
Escherichia-Shigella	29	0,50%	—	—
Buttiauxella	—	—	276	3,70%
Kosakonia	—	—	58	0,80%
Total Enterobacteriaceae	**6 139**	**2,56%***	**7 363**	**8,69%***

Table 6: Enterobacteriaceae composition at Day 0 by site. *Percentage of total bacterial reads per site. (—) = not detected.

12.2. Bacterial alpha and beta diversity

On day 0, Venecia reported higher bacterial diversity values than Lucero across all evaluated indices: Shannon (4,17 ± 0,27 vs 3,35 ± 0,32) and Faith's PD (31,55 ± 7,01 vs 8,17 ± 2,83) (Mann-Whitney, $p < 0,05$). After the treatments, the values increased at both sites. In Lucero, *Ganoderma* sp. and *Pleurotus* sp. were significantly different from the control 0 in the Shannon and Faith's PD indices, and in Venecia, *Trametes* sp. reported the highest values in the Shannon index 6,08 ± 0,14 and Faith's PD 191,33 ± 72,76, being the only significant treatment (letter b, table 6). The NMDS showed differences between the Venecia and Lucero groups on day 0. PERMANOVA confirmed a significant difference after multiple corrections ($p = 0,001$; BH = 0,043) when comparing Venecia control day 0 vs. Lucero control day 0. The treatment and control groups on day 30 were positioned closer together (Figure 6).

	Treatment	n	Observed ASVs	Sig.	Shannon	Sig.	Faith's PD	Sig.
Lucero	Control Day 0	8	56,38 ± 26,34	a	3,35 ± 0,32	a	8,17 ± 2,83	a
Lucero	Control Day 30	3	119,33 ± 47,65	ab	4,06 ± 0,27	ab	29,21 ± 8,99	ab
Lucero	*Trametes* sp.	3	101,67 ± 72,45	ab	3,83 ± 0,62	ab	25,38 ± 13,99	ab
Lucero	*Ganoderma* sp.	3	361,00 ± 258,17	ab	4,86 ± 0,37	b	58,30 ± 34,18	b

Lucero	*Pleurotus ostreatus*	3	596,00 ± 641,73	b	4,92 ± 0,49	b	81,53 ± 74,18	b
	Treatment	n	Observed ASVs	Sig.	Shannon	Sig.	Faith's PD	Sig.
Venecia	Control Day 0	6	211,67 ± 80,69	a	4,17 ± 0,27	a	31,55 ± 7,01	a
Venecia	Control Day 30	3	406,00 ± 211,00	ab	4,55 ± 0,28	ab	73,68 ± 31,48	ab
Venecia	*Ganoderma*sp.	3	422,00 ± 171,68	ab	5,18 ± 0,21	ab	83,25 ± 30,10	ab
Venecia	*Pleurotus ostreatus*	3	730,33 ± 779,51	ab	4,92 ± 0,61	ab	110,20 ± 103,58	ab
Venecia	*Trametes* sp.	3	1400,00 ± 566,24	b	6,08 ± 0,14	b	191,33 ± 72,76	b

Table 7 Alpha diversity indices of bacterial communities by site and fungal treatment. Values are expressed as mean ± standard deviation. Different letters within each column indicate statistically significant differences (Kruskal-Wallis, Dunn post hoc test with Bonferroni correction, $p < 0,05$). *Trametes* = *Trametes* sp.; *Ganoderma* = *Ganoderma* sp.; *Pleurotus* = *Pleurotus ostreatus*.

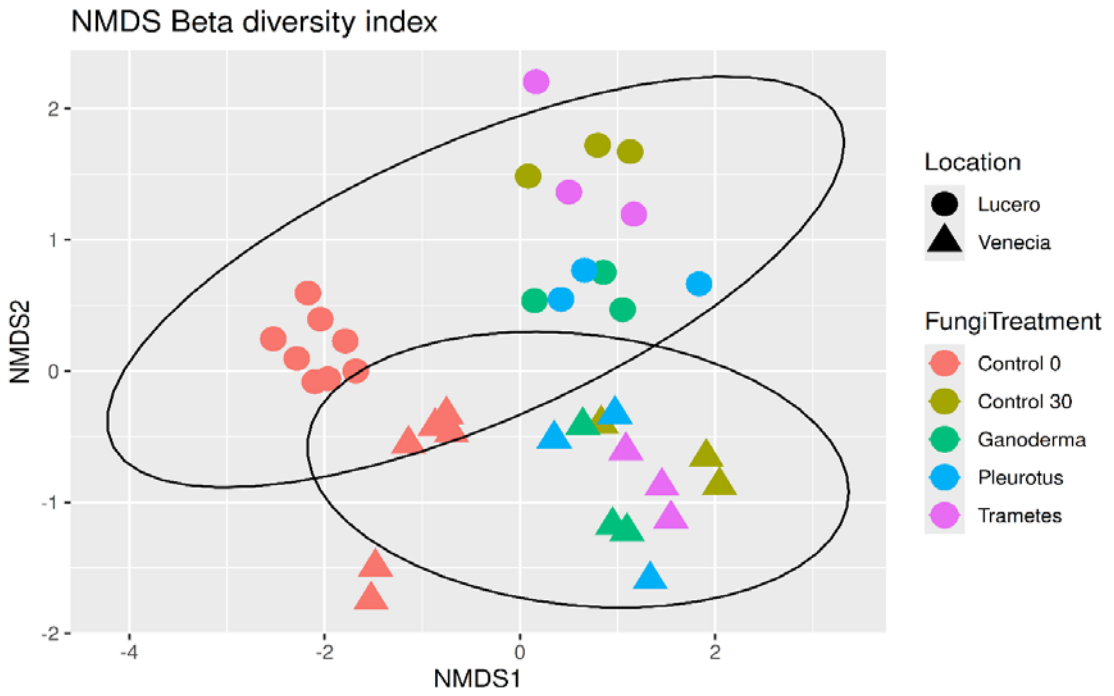


Figure 6: Bacterial Community composition. MDS ordination of bacterial communities based on Bray–Curtis dissimilarity distances. Circles represent Lucero samples and triangles represent Venecia samples. Ellipses correspond to 95% confidence intervals per site. The only statistically significant pairwise comparison after Benjamini–Hochberg correction was Control Day 0 Venecia vs Control Day 0 Lucero (PERMANOVA, p = 0,001; BH-adjusted p = 0,043). C0 = Control Day 0; C30 = Control Day 30; Gan = *Ganoderma* sp.; Ple = *Pleurotus ostreatus*; Tra = *Trametes* sp.

12.3. Fungal community composition

On day 0, the fungal community at the Lucero site was characterized by a high relative abundance of Sporidiobolaceae (99,2%) from a total of 747 340 reads. In Venecia, the composition included Sporidiobolaceae (60,0%), Rhynchogastremaceae (11,9%), Cryptococcaceae (4,1%), Cystobasidiaceae (3,4%), and Trimorphomycetaceae (3,3%). Among the opportunistic genera, *Rhodotorula* and *Apiotrichum* were identified at both sites, with higher relative abundances recorded in Lucero (Tables 7 and 8; Figure 7). By day 30, shifts in the fungal composition were observed across all experimental and control groups. In Lucero, Trichosporonaceae became the most abundant family, replacing Sporidiobolaceae in the Day 30 Control (93,9%) and the treatments with *Ganoderma sp.* (81,7%), *Pleurotus ostreatus* (87,0%), and *Trametes sp.* (69,3%). In Venecia, the Ganodermataceae family, which accounted for 0,02% on day 0, reached 99,4% in the Day 30 Control. Finally, samples from the *Trametes sp.* treatment in Venecia were excluded from the fungal analysis due to insufficient sequencing depth (Supplementary table 3-4)

Family	**Venecia (%)**	**Reads**	**Lucero (%)**	**Reads**
Sporidiobolaceae	60	17 800	99,22	741 499
Rhynchogastremaceae	11,91	3,532	—	—
Cryptococcaceae	4,11	1,218	0,04	269
Cystobasidiaceae	3,37	999	0,04	285
Trimorphomycetaceae	3,33	987	—	—
Bulleribasidiaceae	3,2	950	—	—
Trichosporonaceae	2,45	728	0,36	2,698
Microbotryomycetes fam. Incertae sedis	1,24	369	0,24	1,818
Leucosporidiaceae	—	—	0,07	545
Mrakiaceae	—	—	0,01	74
Total reads		**29,668**		**747,340**

Table 8; Dominant fungal families at Day 0 by site (relative abundance). (—) = not among top 7 families. Total reads correspond only to Control Day 0 samples.

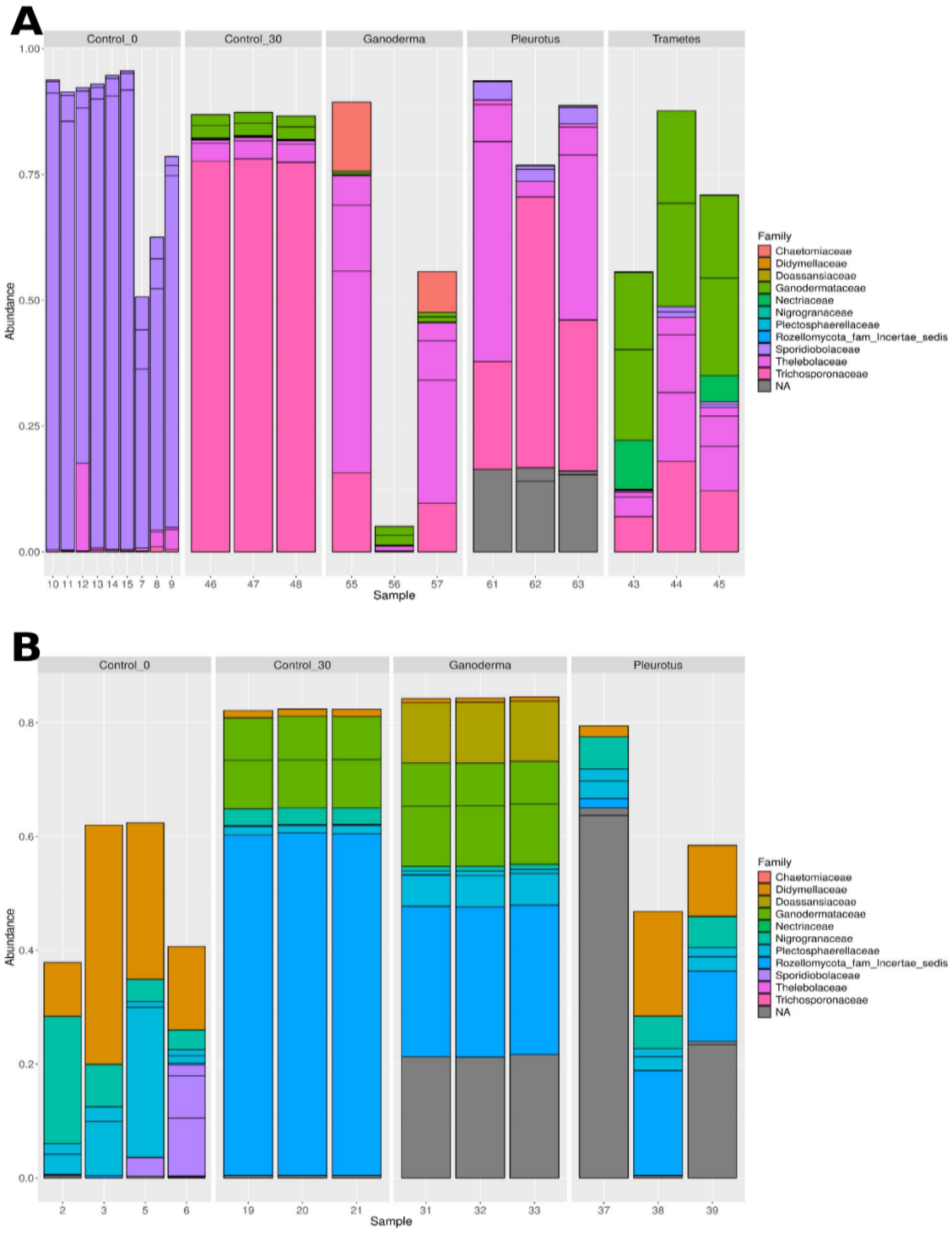


Figure 7: Relative abundance of the 20 most abundant fungal families by sample and treatment at Lucero (A) and Venecia (B). C0 = Control Day 0; C30 = Control Day 30; Gan = *Ganoderma* sp.; Ple = *Pleurotus ostreatus*; Tra = *Trametes* sp. *Trametes* sp. samples from Venecia were excluded due to insufficient sequencing depth.

Genus	Family	Venecia reads	Venecia (%)	Lucero reads	Lucero (%)
Rhodotorula	Sporidiobolaceae	17 795	**59,98**	741 499	**99,22**
Papiliotrema	Rhynchogastremaceae	3 532	11,91	7	0,001
Cryptococcus	Cryptococcaceae	1 218	4,11	259	0,035
Apiotrichum	Trichosporonaceae	728	2,45	2 669	0,357
Naganishia	Cryptococcaceae	26	0,09	—	—
Cutaneotrichosporon	Trichosporonaceae	—	—	12	0,002
Trichosporon	Trichosporonaceae	—	—	3	0
Total opportunistic		**23 299**	**78,53%***	**744 449**	**99,61%***

Table 9: Opportunistic fungal genera at Day 0 by site (relative abundance). (—) = not detected. *Percentage of total fungal reads at Day 0 per site.

12.4. Fungal alpha and beta diversity

On day 0, the fungal communities in Venecia and Lucero showed different diversities. Venecia had a higher significance than Lucero in Fisher's indices (26,26 vs. 11,11) and in Faith's PD (Mann-Whitney, $p = 0,045$ and $p = 0,022$, respectively). After treatment in Lucero, Shannon diversity values were significantly different according to Dunnett's test ($p < 0,05$) between the control 30 (1,14) and the three treatment fungi: *Ganoderma* sp. (2,79), *Trametes* sp. (2,82), and *Pleurotus ostreatus* (1,99). In Venecia, only the *Ganoderma* sp. treatment was reported as significant, with a Fisher's index of 26,26 compared to 9,89 for the control 0 (Dunn-Bonferroni, $p = 0,036$) (letter b, Table 9). The fungal communities of Venecia and Lucero maintained distinct structures throughout the experiment, regardless of the treatment applied. The NMDS showed separation between the groups, and PERMANOVA revealed differences among the initial site controls ($p = 0,002–0,036$). Within Lucero, *Ganoderma* sp.

was the only treatment with a significant structural difference compared to Control 0 (TukeyHSD, p = 0,044), while in Venecia, no treatment with *Ganoderma* and *Pleurotus* significantly modified the community structure (TukeyHSD, p = 0,882–0,999) (Figure 8).

Site	Treatment	n	Observed ASVs	Sig.	Shannon	Sig.	Fisher	Sig.	Faith's PD	Sig.
Lucero	Control Day 0	9	96,56 ± 50,59	a	1,29 ± 0,93	a	11,11 ± 6,82	—	36,29 ± 19,88	—
Lucero	Control Day 30	3	87,33 ± 23,01	a	1,14 ± 0,02	a	9,28 ± 2,58	—	33,12 ± 6,61	—
Lucero	*Ganoderma* sp.	3	105,33 ± 65,03	**b**	2,79 ± 0,86	**b**	11,73 ± 7,27	—	50,21 ± 27,61	—
Lucero	*Pleurotus ostreatus*	3	183,33 ± 130,09	**b**	1,99 ± 0,23	**b**	23,44 ± 16,58	—	262,23 ± 238,65	—
Lucero	*Trametes* sp.	3	174,00 ± 79,83	**b**	2,82 ± 0,38	**b**	19,94 ± 8,84	—	56,23 ± 18,86	—
Site	**Treatment**	**n**	**Observed ASVs**	**Sig.**	**Shannon**	**Sig.**	**Fisher**	**Sig.**	**Faith's PD**	**Sig.**
Venecia	Control Day 0	4	206,75 ± 28,71	—	3,05 ± 0,47	—	26,26 ± 4,78	a	91,32 ± 6,77	—
Venecia	Control Day 30	3	146,33 ± 32,25	—	1,84 ± 0,00	—	17,66 ± 4,15	ab	70,16 ± 10,74	—
Venecia	*Ganoderma* sp.	3	89,67 ± 9,29	—	2,42 ± 0,01	—	9,89 ± 0,92	**b**	49,80 ± 2,73	—
Venecia	*Pleurotus ostreatus*	3	172,67 ± 86,96	—	2,94 ± 0,95	—	21,92 ± 10,82	ab	73,54 ± 27,12	—
Venecia	*Trametes* sp.	—	—	—	—	—	—	**	—	—

Table 10: Alpha diversity indices of fungal communities. Values expressed as mean ± standard deviation. Different letters within each column indicate statistically significant differences (Kruskal-Wallis, Dunnett test, p < 0,05). Lucero: Dunnett reference = Control Day 30. Venecia: Dunnett reference = Control Day 0, confirmed by Dunn–Bonferroni for *Ganoderma* sp. (—) not applicable. ** excluded due to insufficient sequencing depth.

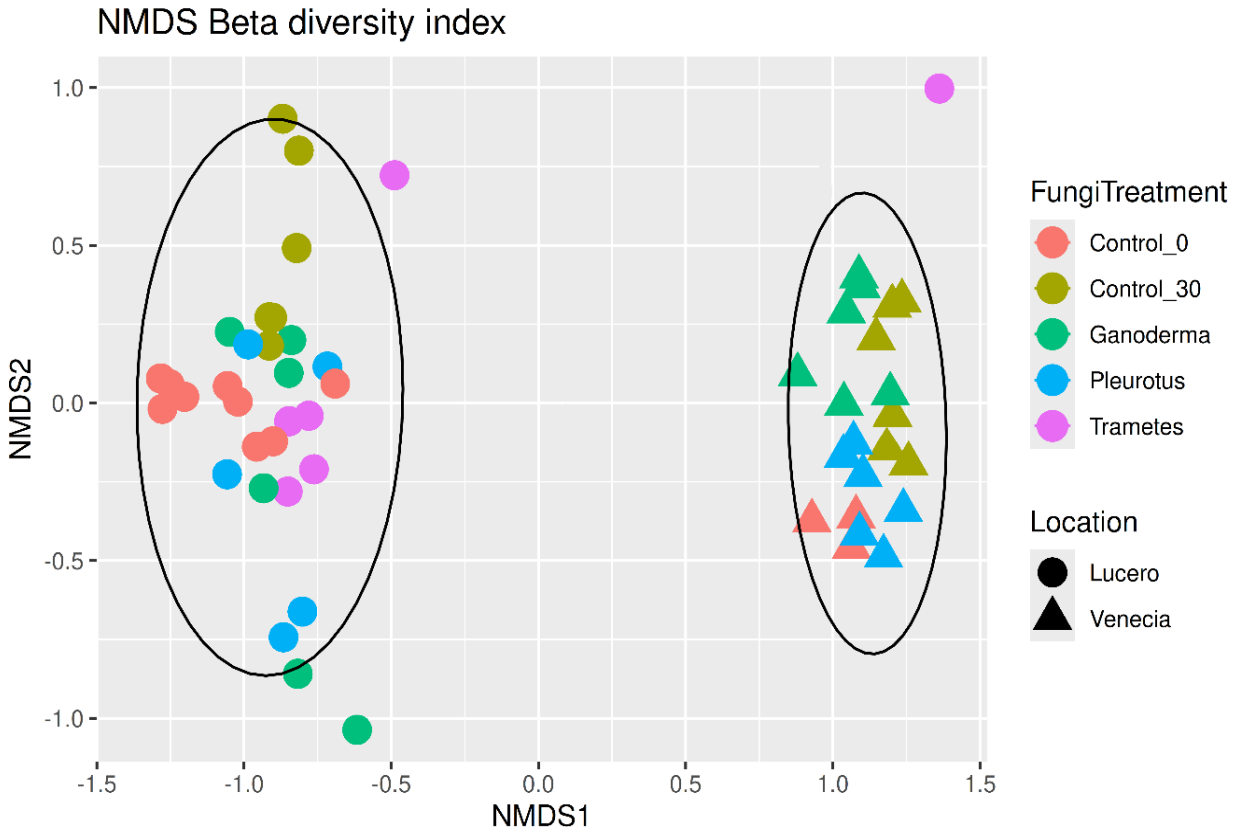


Figure 8 Fungal community composition. NMDS ordination of fungal communities based on Bray–Curtis dissimilarity distances. Circles represent Lucero samples and triangles represent Venecia samples. Ellipses correspond to 95% confidence intervals per site. Pairwise PERMANOVA indicated separation between sites at Day 0 (p = 0,002–0,036); no comparisons remained significant after multiple testing correction. Within Lucero, only *Ganoderma* sp. produced a significant structural difference relative to Control Day 0 (TukeyHSD, p = 0,044). No treatment significantly modified community structure in Venecia (TukeyHSD, p = 0,882–0,999). *Trametes* sp. samples from Venecia were excluded due to insufficient sequencing depth

CHAPTER 4

RESULTS

13. Differential abundance and functional annotation of microbial taxa

13.1. Bacterial Differential Abundance

The differential bacterial abundance analysis using ANCOM-BC2 identified taxa with significant changes (q < 0,05; passed_ss = TRUE). In Lucero, the differential bacterial abundance analysis of the Day 0 Control versus the Day 30 Control identified 20 significant taxa *Massilia, Klebsiella, Pseudomonas*, and *Stenotrophomona*s increased, and 16 genera decreased, including *Caulobacter, Legionella, Aquicella, Luteimonas, Devosia, Ancylobacter, Xanthobacter, Methylotenera, Polynucleobacter*, and *Sphingopyxis* (Figure 9A). Compared to the Day 30 Control, *Ganoderma sp.* showed 20 significant taxa, all of which increased in abundance, including *Rhodococcus, Galbitalea, Marmoricola, Hydrogenophaga, Variovorax, Arenimonas, Edaphobaculum, Iamia, Mycobacterium, Prosthecobacter, Rickettsiella, and Sphingorhabdus* (Figure 9B). *Pleurotus ostreatus* generated the highest number of significant taxa (25), all of which increased, including *Aeromicrobiaceae, Arenimonas, Edaphobaculum, Galbitalea, Kaistia, Rhodococcus, Polynucleobacter, Pseudomonas, Mesorhizobium, Paenibacillus, Xanthobacter*, and *Limnohabitans* (Figure 9C). *Trametes* sp. showed the lowest number of significant taxa (6) *Galbitalea* and *Terrimicrobium* increased, while *Pirellula, Sphingopyxis* and *Luteimonas* decreased (Figure 9D). In Venecia, the Day 0 Control versus the Day 30 Control identified 20 significant taxa, 13 genera increased, including *Pedobacter, Methylotenera, Mycobacterium, Sphingomonas, Aeromicrobium, Caulobacter, Aquicella, Zoogloea,*

Luteimonas, Flavobacterium, Nocardioides, and *Klebsiella*, and 7 genera decreased: *Massilia, Gemmobacter, Marmoricola, Sphingorhabdus, Arenimonas, Ensifer*, and *Edaphobaculum* (Figure 10A). *Ganoderma* sp. showed 12 significant taxa, only *Coxiella* increased, while 11 genera decreased: *Limnohabitans, Rhodococcus, Prosthecobacter, Fimbriiglobus, Marmoricola, Xanthobacter, Iamia, Pseudomonas, Arenimonas,* and *Pseudarthrobacter* (Figure 10B). *Pleurotus ostreatus* presented 5 significant taxa *Methylotenera* and *Coxiella* increased, and *Arenimonas, Edaphobaculum*, decreased (Figure 10C). *Trametes* sp. identified 9 significant taxa *Luteimonas, Sphingopyxis, Sphingomonas, Pirellula,* IMCC26207, *Coxiella, Klebsiella*, and *Fimbriiglobus* increased, and *Pseudarthrobacter* decreased (Figure 10D).

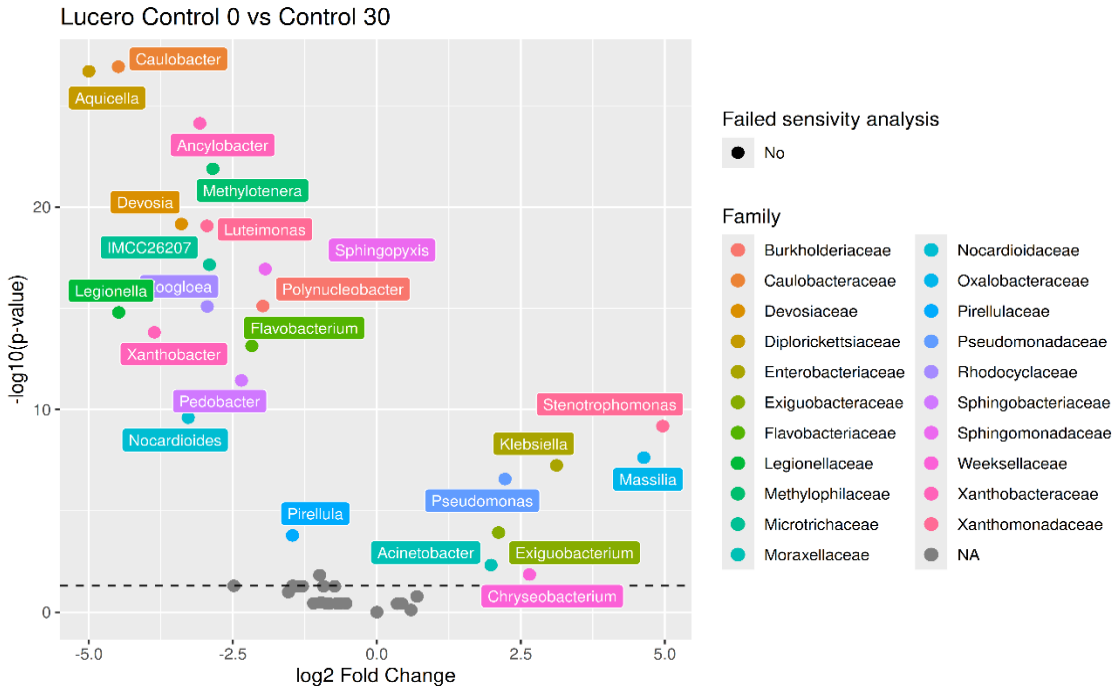


Figure 9A: Differential abundance of bacterial taxa at Lucero Control Day 0 vs Control Day 30, Filled circles indicate taxa that passed the stochastic sensitivity filter (q < 0,05, ANCOM-BC2). Open circles indicate taxa that did not pass this filter. Positive log2 fold change values indicate increased abundance relative to Control Day 30; negative values indicate decreased abundance. The dashed line represents the significance threshold.

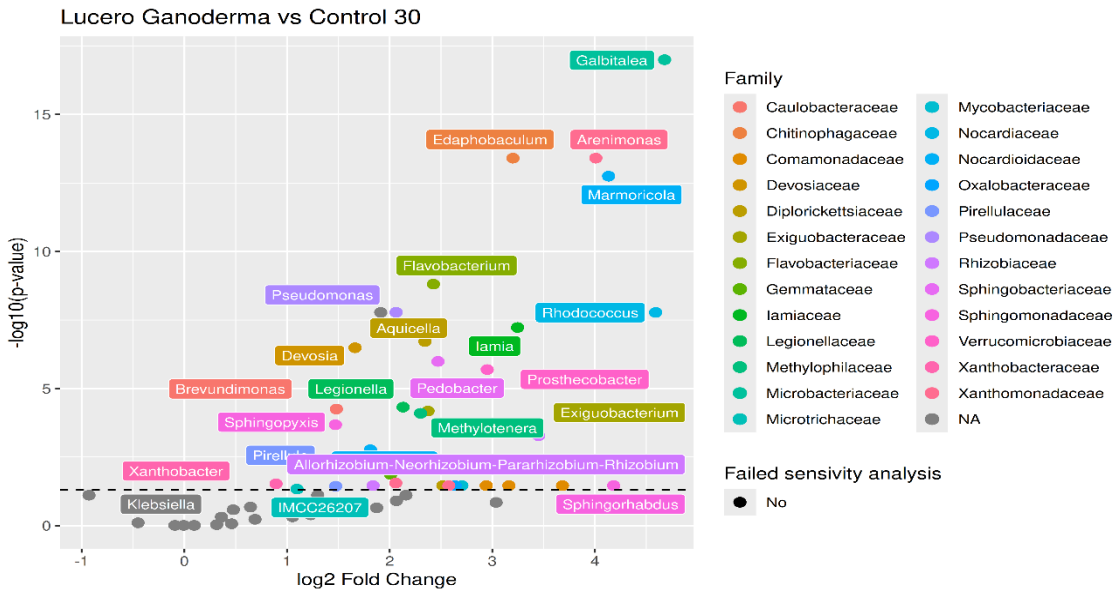


Figure 9B: Differential abundance of bacterial taxa at Lucero *Ganoderma* sp. vs Control Day 30, Filled circles indicate taxa that passed the stochastic sensitivity filter (q < 0,05, ANCOM-BC2). Open circles indicate taxa that did not pass this filter. Positive log2 fold change values indicate increased abundance relative to Control Day 30; negative values indicate decreased abundance. The dashed line represents the significance threshold.

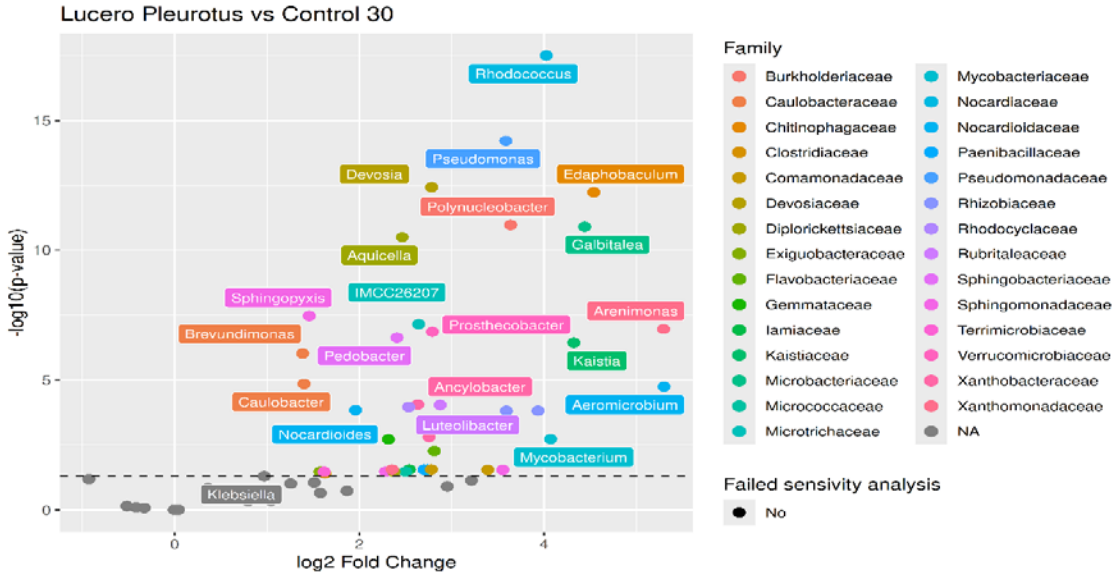


Figure 9C: Differential abundance of bacterial taxa at Lucero *Pleurotus ostratus* vs Control Day 30, Filled circles indicate taxa that passed the stochastic sensitivity filter (q < 0,05, ANCOM-BC2). Open circles indicate taxa that did not pass this filter. Positive log2 fold change values indicate increased abundance relative to Control Day 30; negative values indicate decreased abundance. The dashed line represents the significance threshold.

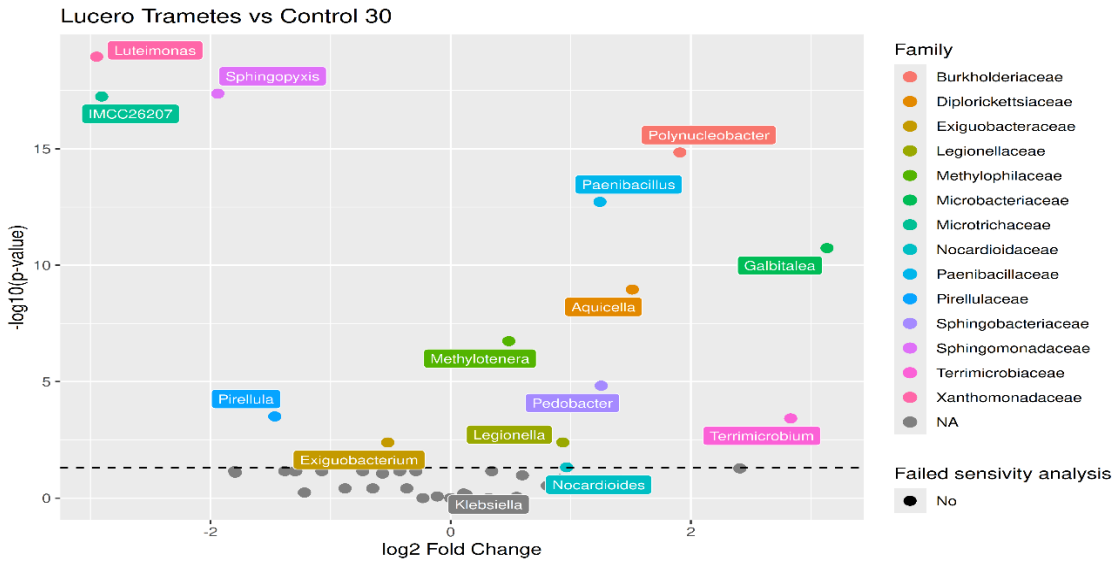


Figure 9D: Differential abundance of bacterial taxa at Lucero *Trametes* vs Control Day 30, Filled circles indicate taxa that passed the stochastic sensitivity filter (q < 0,05, ANCOM-BC2). Open circles indicate taxa that did not pass this filter. Positive log2 fold change values indicate increased abundance relative to Control Day 30; negative values indicate decreased abundance. The dashed line represents the significance threshold.

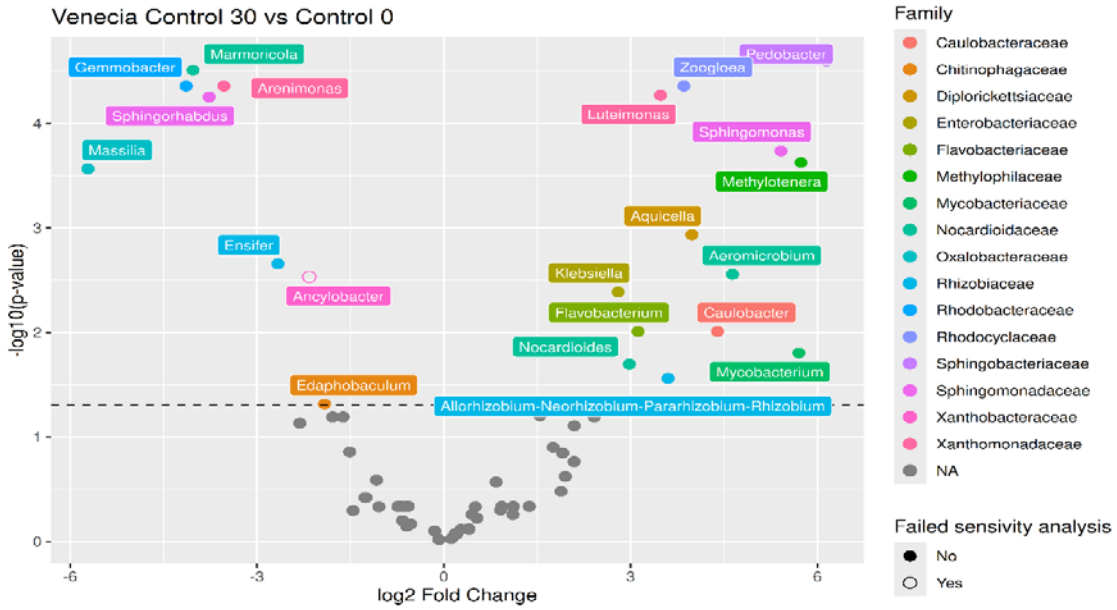


Figure 10A: Differential abundance of bacterial taxa at Venecia Control Day 0 vs Control Day 30, Filled circles indicate taxa that passed the stochastic sensitivity filter (q < 0,05, ANCOM-BC2). Open circles indicate taxa that did not pass this filter. Positive log2 fold change values indicate increased abundance relative to Control Day 30; negative values indicate decreased abundance. The dashed line represents the significance threshold

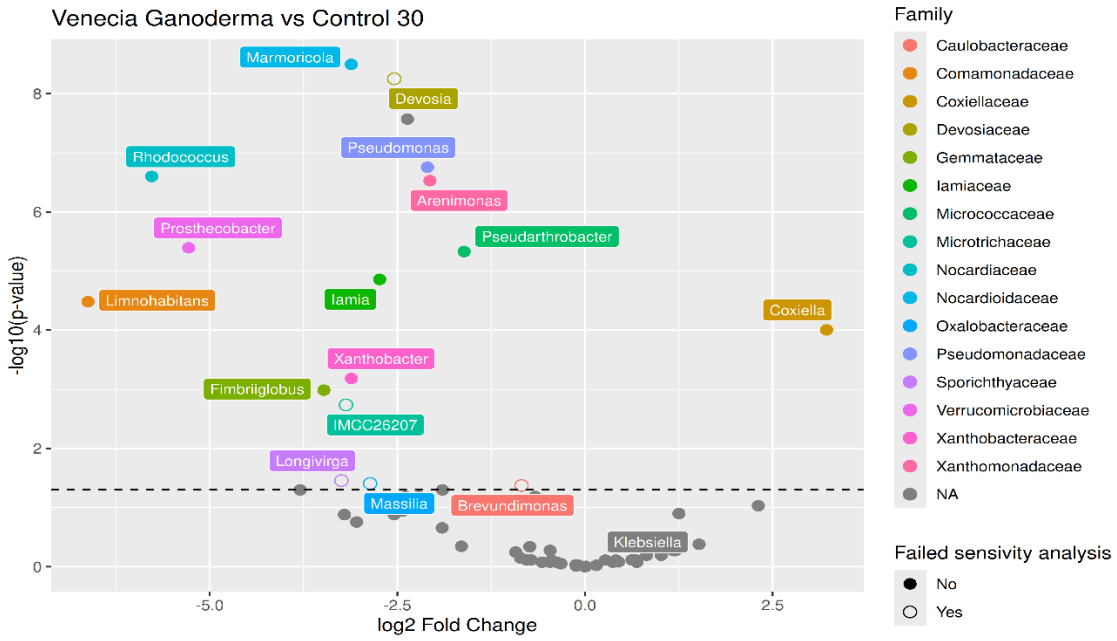


Figure 10B. Differential abundance of bacterial taxa at Venecia *Ganoderma* sp. vs Control Day 30, Filled circles indicate taxa that passed the stochastic sensitivity filter (q < 0,05, ANCOM-BC2). Open circles indicate taxa that did not pass this filter. Positive log2 fold change values indicate increased abundance relative to Control Day 30; negative values indicate decreased abundance. The dashed line represents the significance threshold.

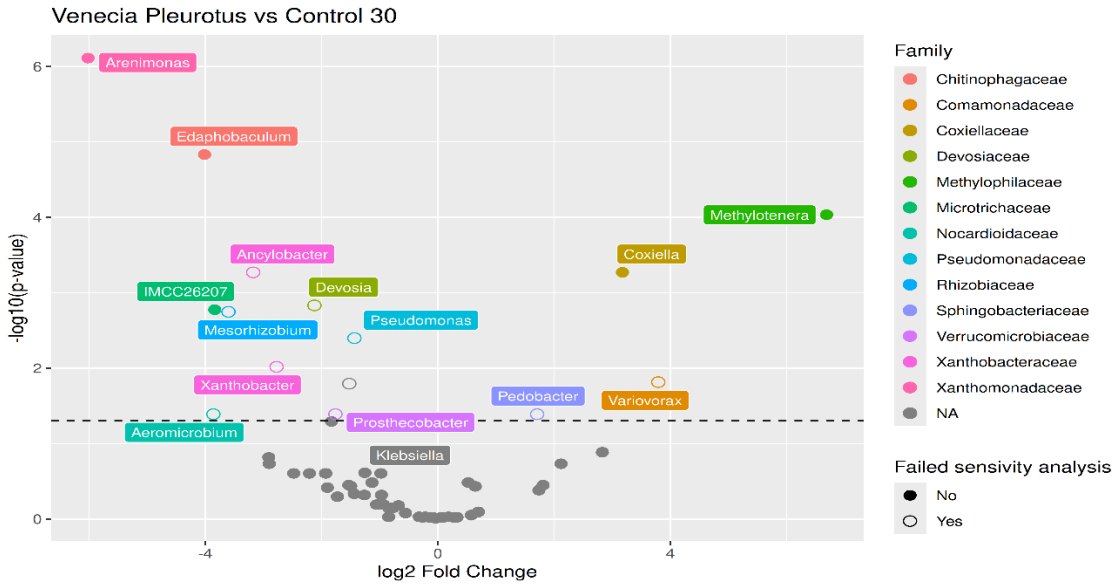


Figure 10C. Differential abundance of bacterial taxa at Venecia *Pleurotus ostratus* vs Control Day 30, Filled circles indicate taxa that passed the stochastic sensitivity filter (q < 0,05, ANCOM-BC2). Open circles indicate taxa that did not pass this filter. Positive log2 fold change values indicate increased abundance relative to Control Day 30; negative values indicate decreased abundance. The dashed line represents the significance threshold.

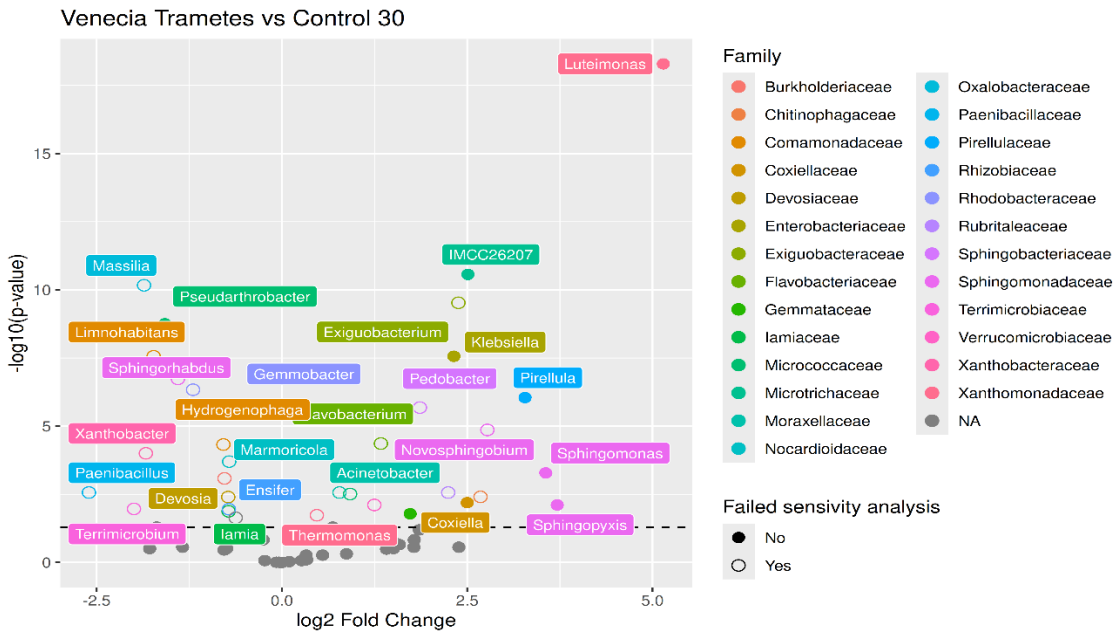


Figure 10D Differential abundance of bacterial taxa at Venecia *Trametes* sp. vs Control Day 30, Filled circles indicate taxa that passed the stochastic sensitivity filter (q < 0,05, ANCOM-BC2). Open circles indicate taxa that did not pass this filter. Positive log2 fold change values indicate increased abundance relative to Control Day 30; negative values indicate decreased abundance. The dashed line represents the significance threshold

13.2. Fungal Differential Abundance

The differential fungal abundance analysis using ANCOM-BC2 identified taxa with significant changes (q < 0,05; passed_ss = TRUE). In Lucero, the differential fungal abundance analysis comparing Day 0 Control versus Day 30 Control identified 8 significant taxa: *Rhodotorula, Meyerozyma, Clavispora, and Candida* increased, while *Ganoderma*, *Apiotrichum, Rozellomycota*, and an unidentified taxon decreased (Figure 11A). *Ganoderma* sp., compared to the day 30 Control, showed only 1 significant taxon: *Apiotrichum* decreased (Figure 11B). *Pleurotus ostreatus* identified 6 significant taxa: *Talaromyces, Meyerozyma, Geotrichum, Rhodotorula,* and *Candida* increased, and *Ganoderma* decreased (Figure 11C). *Trametes* sp. showed 3 significant taxa: *Geotrichum* increased, and *Apiotrichum* decreased

(Figure 11D). In Venecia, the differential fungal abundance analysis of the Day 0 Control versus the Day 30 Control identified 8 significant taxa: *Sarocladium, Xenopyrenochaetopsis, Plectosphaerella*, and *Didymella* increased, while *Ganoderma* sp., *Rozellomycota, Metschnikowia* (Figure 12A). *Ganoderma* sp., compared to the Day 30 control, showed 10 significant taxa: *Plectosphaerella, Tolypocladium, Papiliotrema,* and *Tuber* increased, and *Metschnikowia, Phaeosphaeria, Sarocladium, Nigrograna*, and *Dictyosporiaceae* decreased (Figure 12B). *Pleurotus ostreatus* showed the fewest significant taxa *Plectosphaerella* and *Tuber* increased, while *Ganoderma* and *Metschnikowia* decreased (Figure 12C). The treatment with *Trametes* sp. in Venecia was excluded from the fungal analysis due to insufficient sequencing depth.

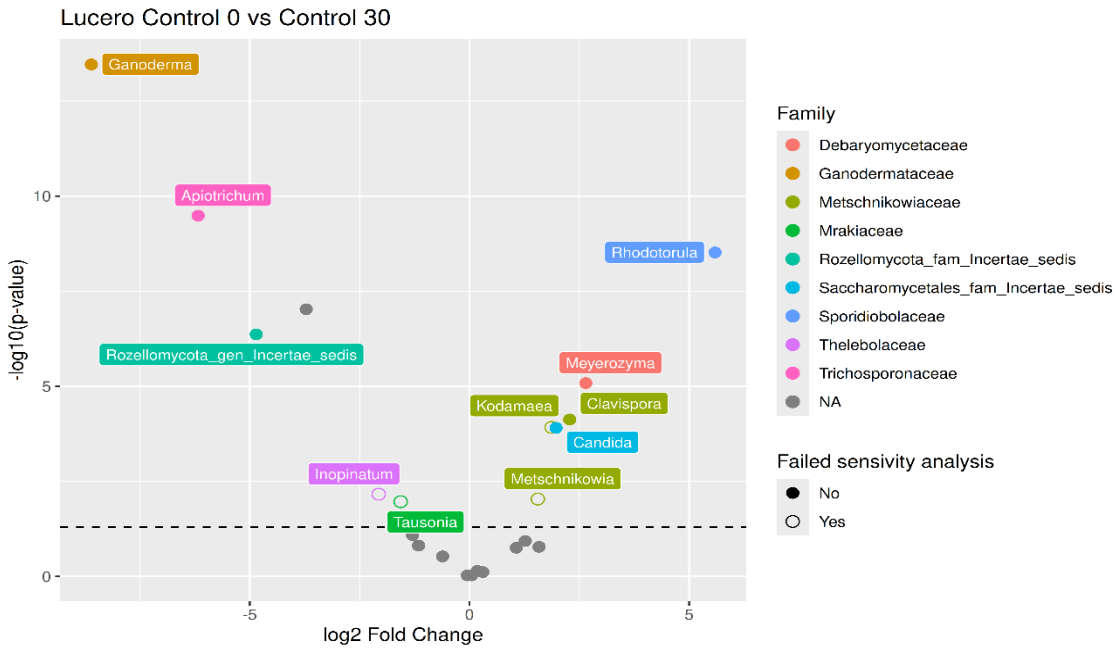


Figure 11A Differential abundance of fungi taxa at Lucero Control Day 0 vs Control Day 30, Filled circles indicate taxa that passed the stochastic sensitivity filter (q < 0,05, ANCOM-BC2). Open circles indicate taxa that did not pass this filter. Positive log2 fold change values indicate increased abundance relative to Control Day 30; negative values indicate decreased abundance. The dashed line represents the significance threshold

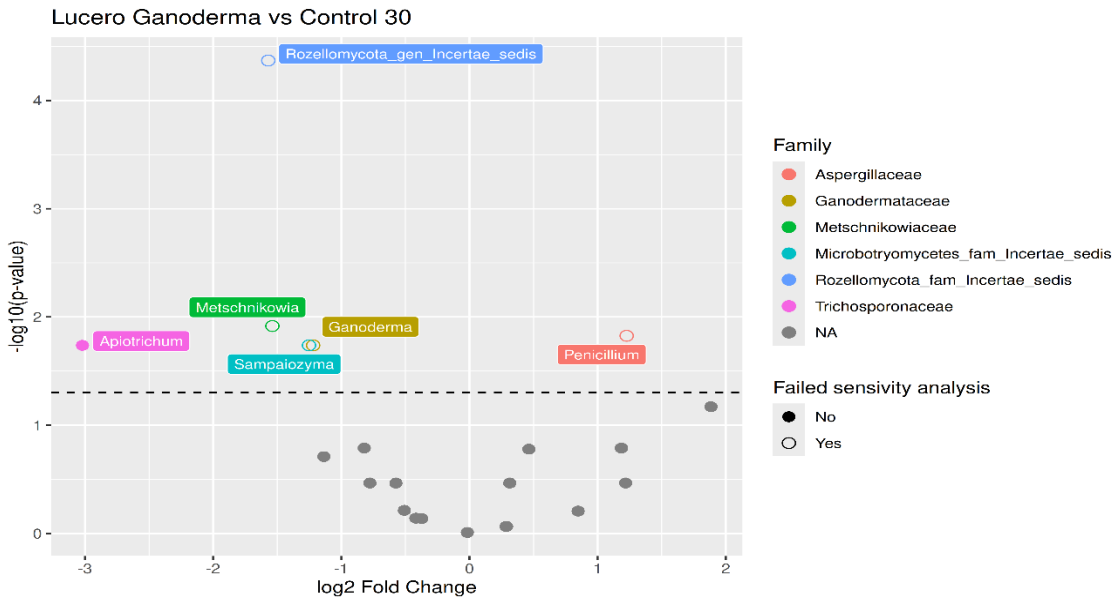


Figure 11B Differential abundance of fungi taxa at Lucero *Ganoderma* sp. vs Control Day 30, Filled circles indicate taxa that passed the stochastic sensitivity filter (q < 0,05, ANCOM-BC2). Open circles indicate taxa that did not pass this filter. Positive log2 fold change values indicate increased abundance relative to Control Day 30; negative values indicate decreased abundance. The dashed line represents the significance threshold

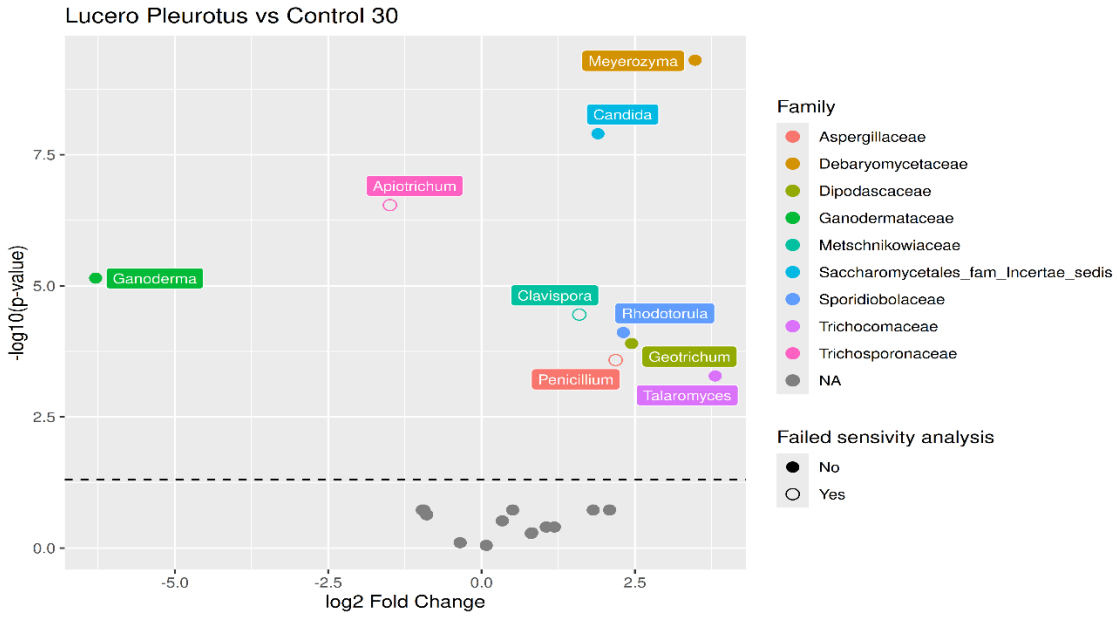


Figure 11C Differential abundance of fungi taxa at Lucero *Pleurotus ostreatus* vs Control Day 30, Filled circles indicate taxa that passed the stochastic sensitivity filter (q < 0,05, ANCOM-BC2). Open circles indicate taxa that did not pass this filter. Positive log2 fold change values indicate increased abundance relative to Control Day 30; negative values indicate decreased abundance. The dashed line represents the significance threshold

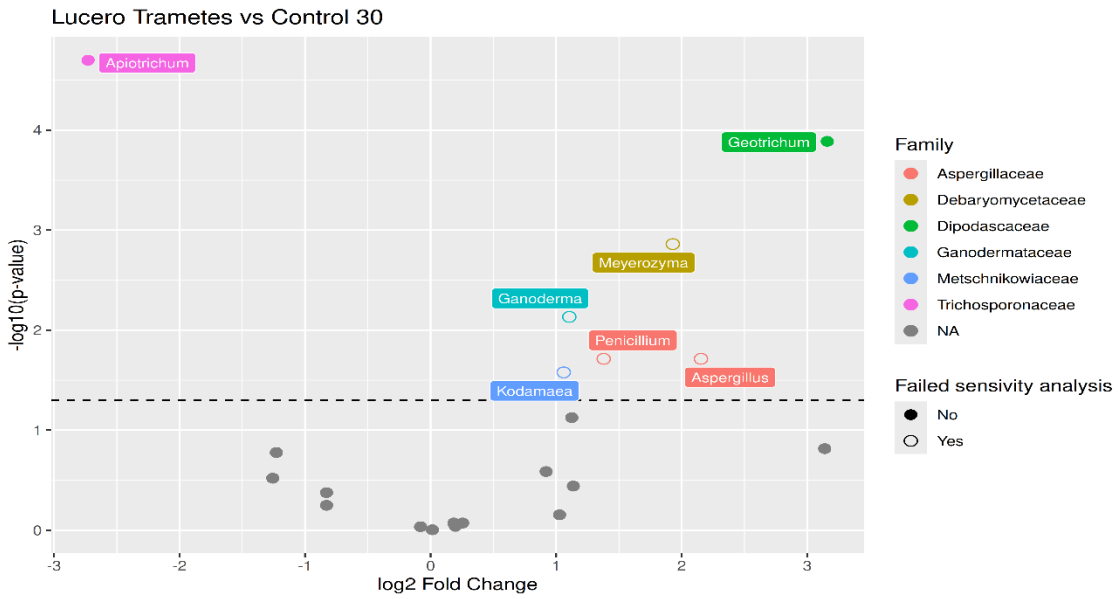


Figure 11D Differential abundance of fungi taxa at Lucero *Trametes sp.* vs Control Day 30, Filled circles indicate taxa that passed the stochastic sensitivity filter (q < 0,05, ANCOM-BC2). Open circles indicate taxa that did not pass this filter. Positive log2 fold change values indicate increased abundance relative to Control Day 30; negative values indicate decreased abundance. The dashed line represents the significance threshold



Figure 12A Differential abundance of fungi taxa at Venecia Control Day 0 vs Control Day 30, Filled circles indicate taxa that passed the stochastic sensitivity filter (q < 0,05, ANCOM-BC2). Open circles indicate taxa that did not pass this filter. Positive log2 fold change values indicate increased abundance relative to Control Day 30; negative values indicate decreased abundance. The dashed line represents the significance threshold

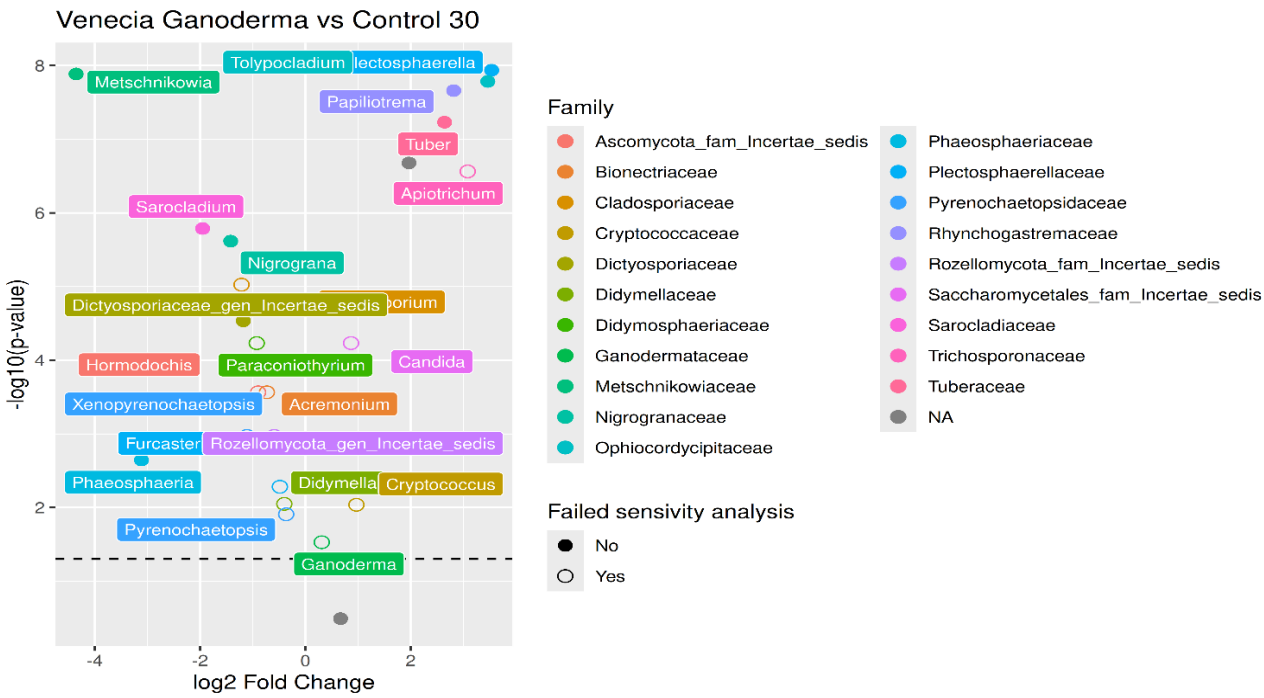


Figure 12B Differential abundance of fungi taxa at Venecia *Ganoderma* sp. 0 vs Control Day 30, Filled circles indicate taxa that passed the stochastic sensitivity filter (q < 0,05, ANCOM-BC2). Open circles indicate taxa that did not pass this filter. Positive log2 fold change values indicate increased abundance relative to Control Day 30; negative values indicate decreased abundance. The dashed line represents the significance threshold

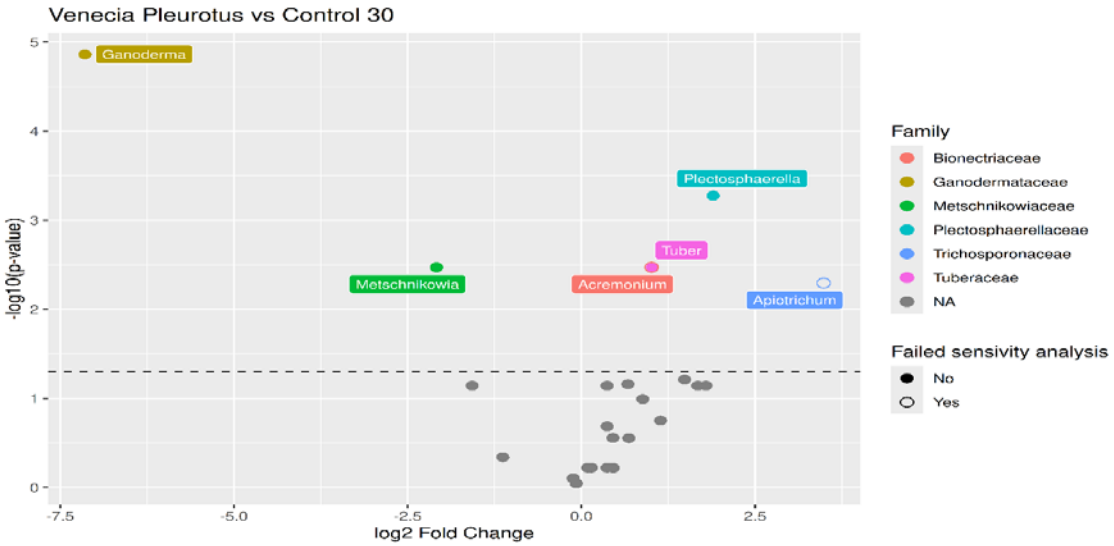


Figure 12C Differential abundance of fungi taxa at Venecia *Pleurotus ostreatus*. 0 vs Control Day 30, Filled circles indicate taxa that passed the stochastic sensitivity filter (q < 0,05, ANCOM-BC2). Open circles indicate taxa that did not pass this filter. Positive log2 fold change values indicate increased abundance relative to Control Day 30; negative values indicate decreased abundance. The dashed line represents the significance threshold

13.3. Bacterial Functional Annotation

Bacterial functional annotation using FAPROTAX revealed distinct patterns between sites and treatments. In Lucero, aerobic chemoheterotrophy was higher in *Ganoderma* sp. (5,28%) than Day 0 Control (0,35%), methylotrophy was higher in *Ganoderma* sp. (1,40%) and in the Day 30 Control (1,00%). Intracellular parasites, which are organisms that invade and survive within the cells of a host, reached their maximum value in *Trametes* sp. (13,40%), doubling the Day 30 Control (6,78%) and exceeding the Day 0 Control (0,007%). In Venecia, total chemoheterotrophy was highest in *Pleurotus ostreatus* (9,15%), and methylotrophy was highest in the same treatment (3,99%). *Trametes* sp. showed the highest nitrification value (0,39%) and *Ganoderma* sp. the highest value of predator or ectoparasitic organisms (3,09%). Intracellular parasites increased in all fungal treatments compared to the Day 0 Control at both sites. The full results are presented in Figures 13 and 14 and Supplementary Table 4-1.

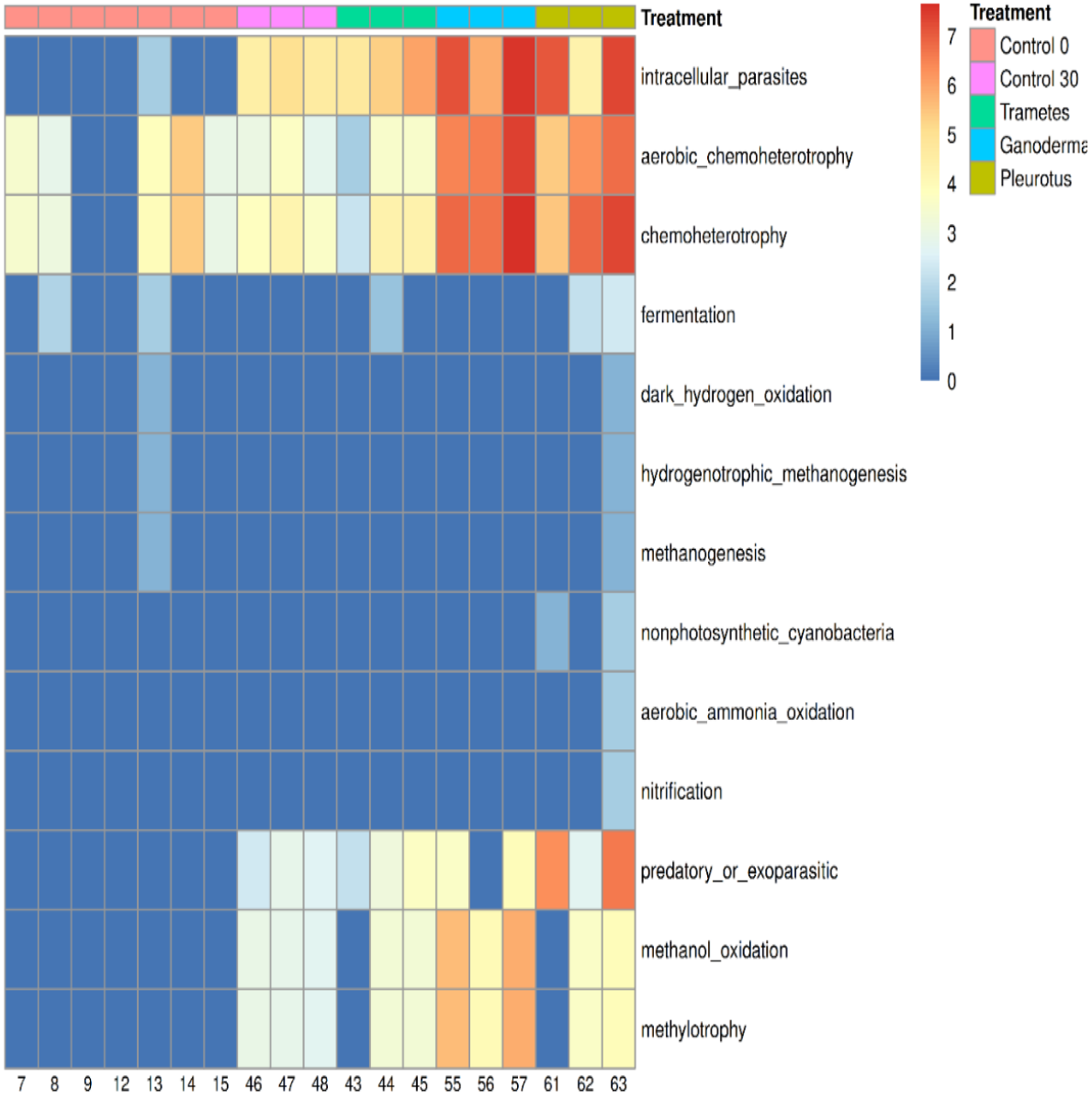


Figure 13. Functional profile of bacterial communities at Lucero inferred by FAPROTAX annotation. Each row represents a functional category and each column a sample. Color scale represents the relative abundance (%) of reads assigned to each functional category. L = Lucero; C0 = Control Day 0; C30 = Control Day 30; Tra = *Trametes* sp.; Gan = *Ganoderma* sp.; Ple = *Pleurotus ostreatus*

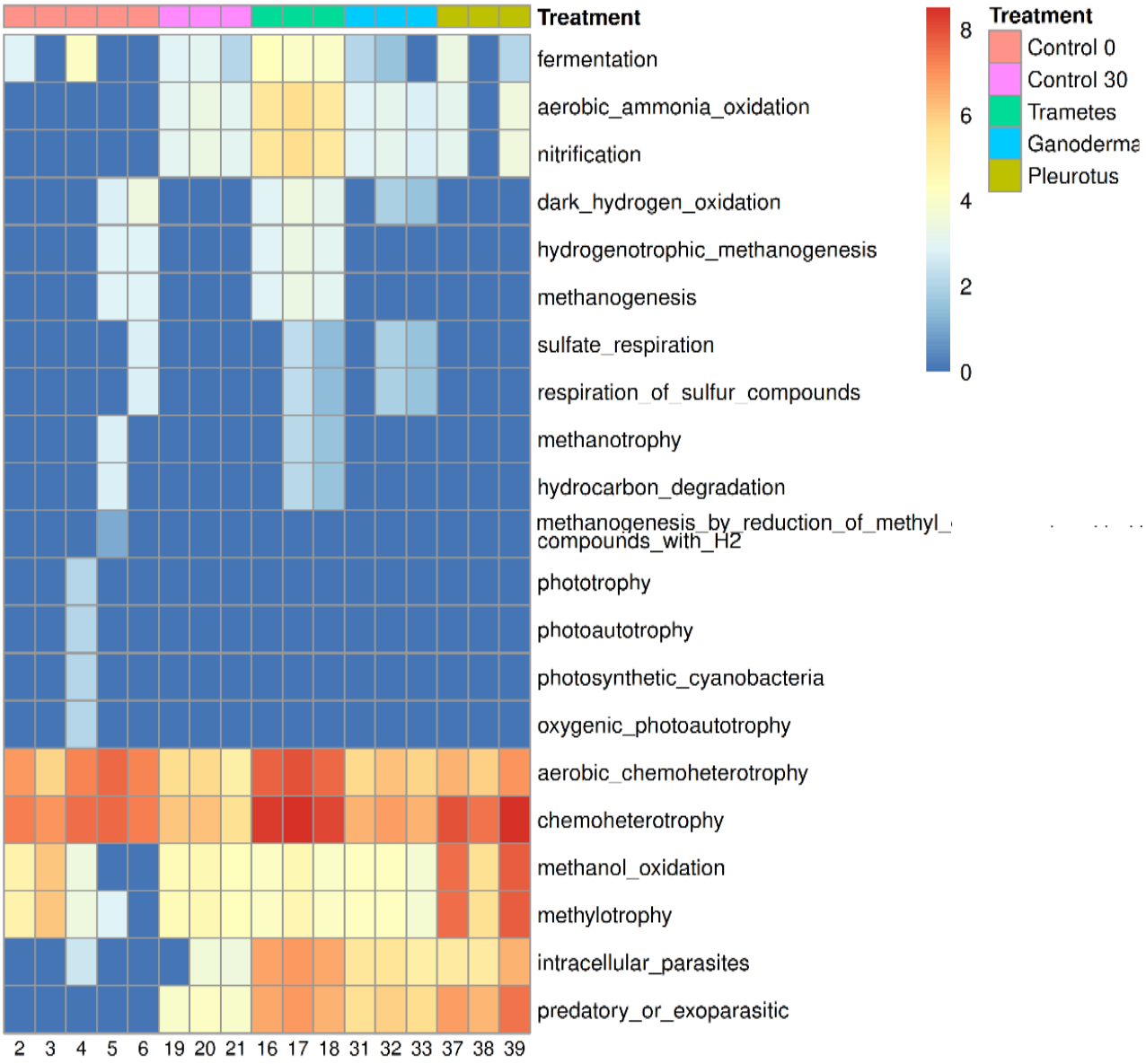


Figure 14 Functional profile of bacterial communities at Venecia inferred by FAPROTAX annotation. Each row represents a functional category and each column a sample. Color scale represents the relative abundance (%) of reads assigned to each functional category. V = Venecia; C0 = Control Day 0; C30 = Control Day 30; Tra = *Trametes* sp.; Gan = *Ganoderma* sp.; Ple = *Pleurotus ostreatus.*

13.4. Fungal Functional Annotation

Fungal functional annotation using FUNGalTraits revealed changes in ecological groups at both sites. At Lucero, the day 0 control was dominated by undefined saprotrophs (67,34%) and animal pathogens (15,83%). The day 30 control and treatments with *Trametes* sp. (92,82%) and *Ganoderma* sp. (67,64%) showed dominance of the Plant Pathogen-Wood Saprotroph group, whereas *Pleurotus ostreatus* exhibited greater diversity across groups, including Plant Pathogen (20,58%), Undefined Saprotroph (35,85%), and Endophyte (8,30%). At Venecia, the day 0 control was dominated by Plant Pathogen (44,54%) and Animal Pathogen (14,97%). Day 30 control and *Ganoderma* sp. showed dominance of the Plant Pathogen-Wood Saprotroph group (85,01% and 79,71%, respectively). *Pleurotus ostreatus* in Venecia displayed greater diversity: Endophyte, Plant Pathogen, Wood Saprotroph (25,47%), Plant Pathogen (20,98%), and Endophyte-Fungal Parasite-Plant Pathogen (9,16%). The most abundant group in *Ganoderma* sp. from Venecia was Fungal Parasite (14,39%). The complete results are presented in Figures 15 and 16 and in Supplementary Table 4-2.

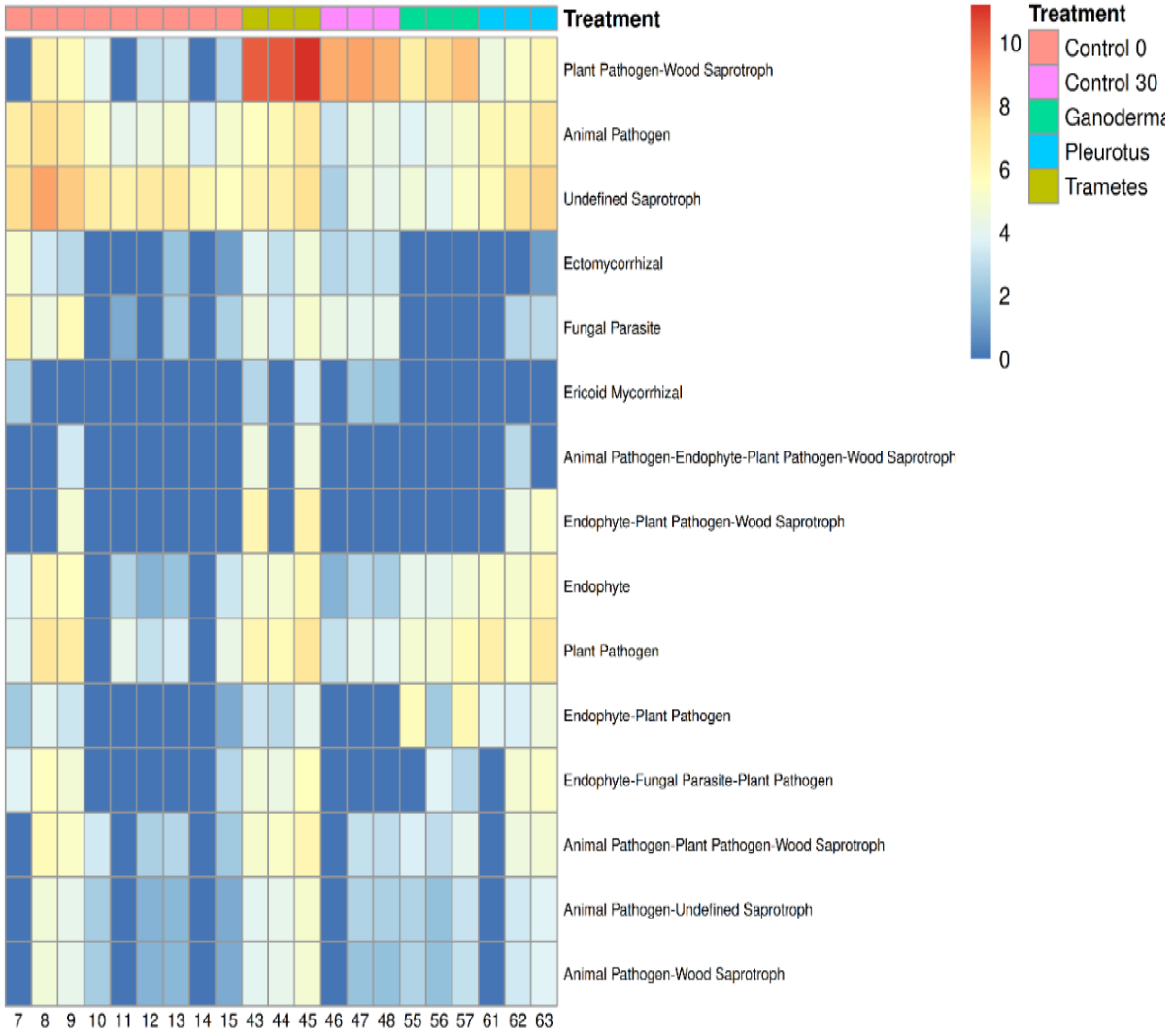


Figure 15 Functional guild profile of fungal communities inferred by FUNGalTraits annotation at Lucero. Each row represents a functional guild and each column an individual sample. Color scale represents the relative abundance (%) of reads assigned to each guild.

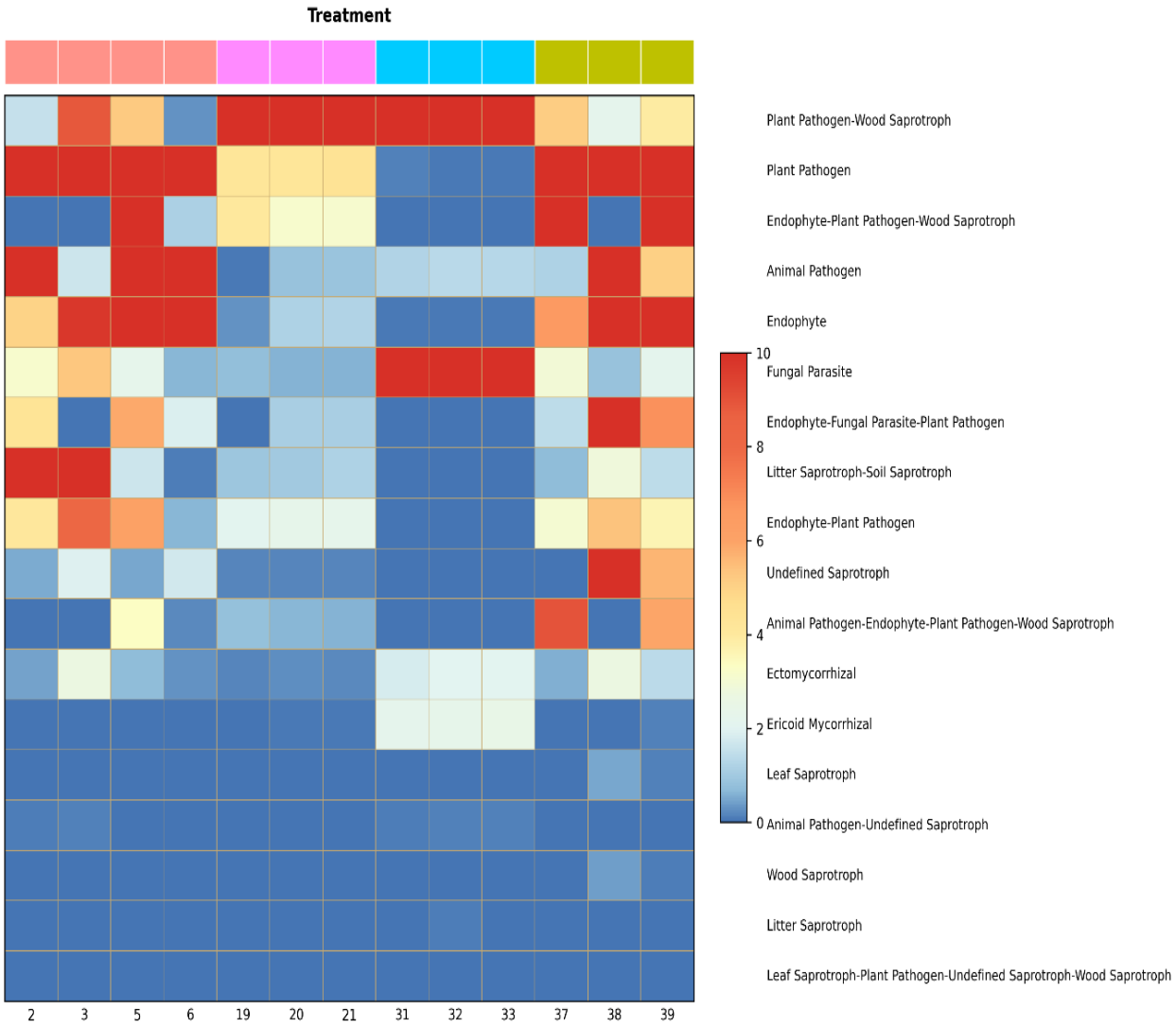


Figure 16 Functional guild profile of fungal communities inferred by FUNGalTraits annotation at Venecia. Each row represents a functional guild and each column an individual sample. Color scale represents the relative abundance (%) of reads assigned to each guild. *Trametes* sp. data are not available for Venecia due to insufficient sequencing depth.

DISCUSSION

14. Physicochemical and traditional microbiological characterization

The physicochemical and microbiological results obtained at the Venecia and Lucero sites in the La Ramada Irrigation District exceed the limits established by Resolution 1076 of 2015 of the Colombian Ministry of Environment, reflecting the heterogeneity of the sources of anthropogenic pressure at each site. Lucero showed BOD values of 360 mg/L and COD of 1,037 mg/L, which are 14 and 8 times the permitted thresholds, respectively, while Venecia, although less contaminated, also exceeded both parameters, with 112 mg/L of BOD and 190 mg/L of COD. This difference in organic load is consistent with the nature of the discharges received at each point, since Venecia mainly receives agricultural runoff and domestic waste of horticultural origin, while Lucero is exposed to more complex industrial and urban effluents, as also described in the characterization and treatment studies of La Ramada waters in the theses, where systematic sampling during dry and rainy seasons reports a high load of total and fecal coliforms (Cubides, 2018).

The electrical conductivity values recorded at the two sampling points indicate significant saline loading, particularly in Lucero (791 µS/cm), which exceeds the threshold of 0.45 dS/m, frequently used in international guidelines to identify salinity restrictions in irrigation waters, while in Venecia (459 µS/cm) the reading was lower, though indicative of appreciable mineralization. Additionally, dissolved oxygen was extremely low at both sites (0,71 mg/L in Lucero and 0,53 mg/L in Venecia), which, along with high COD and BOD values, indicates an anoxic environment in which scarce oxygen availability favors eutrophication

and the proliferation of facultative and strict anaerobic microorganisms. Many of these are relevant to health. Complementarily, in accordance with Bremer's 2019 report, the increase in dissolved salts exerts osmotic pressure on microorganisms, requiring them to maintain cellular hydration, turgor, and physiological integrity, thereby further stressing them (Bremer & Krämer, 2019).

From a microbiological perspective, the total coliform and *E. coli* loads recorded at Lucero are 126 000 CFU/100 mL and 20 000 CFU/100 mL, respectively, exceeding the limits established by current Colombian standards by more than 25- and 20-fold, respectively, confirming the health risk associated with this site. Venecia, although close to the total coliform range, exceeded the *E. coli* limit by 4×, at 4 000 CFU/100 mL. These findings are consistent with studies reported in the La Ramada Irrigation District, which documented high loads of total coliforms and *E. coli* in irrigation waters, associated with untreated domestic and industrial discharges. They also align with multitemporal analyses of the middle section of the Bogotá River basin, where unacceptably high concentrations of these sanitary indicators were recorded, accumulating throughout the water system (Castro Fernández et al., 2022b) and Corrales et al., (2018) reports have identified in the waters of the middle Bogotá River basin the simultaneous presence of bacteria such as *E. coli*, *Proteus vulgaris*, and *Enterobacter cloacae*, as well as filamentous fungi from the genera *Aspergillus, Fusarium, Mucor, and Rhizopus*, indicating a high risk of contamination and dispersal of phytopathogenic microorganisms in the crops. Overall, these results support that both bodies of water were under multiple environmental stresses characterized by high organic loads, severe hypoxia, and elevated salinity, conditions that restrict biological activity and reflect an advanced state of water quality deterioration.

The differences in these parameters reveal that Lucero and Venecia face different pressures: chronic and mixed pollution in the first, and mainly agricultural and domestic loads in the second. These variations are not just numbers but features that reinforce the conditions for differential selection among native microbial communities, as supported by studies in the middle section of the Bogotá River, where *E. coli* concentrations of up to 10^6 and 10^8 CFU/L indicate deficiencies in sanitation processes. In this context, the predominance of opportunistic Enterobacteriaceae within the characterized communities is of clinical relevance. Although antibiotic resistance was not directly assessed in this study, they are recognized by the World Health Organization as a priority pathogen owing to their capacity to develop and disseminate resistance to multiple antimicrobials, and it has been documented in irrigation water and agricultural soil carrying multidrug resistance phenotypes and resistance genes (Iwu et al., 2020). This concern is compounded by reports of antibiotic residues in wastewater and reclaimed water used for agricultural irrigation, where they can exert subinhibitory selective pressure, favoring the emergence and co-selection of resistant bacteria (Ashworth et al., 2023).

This differential contamination profile between Venecia and Lucero lays the groundwork for understanding the divergences observed in the structure and composition of their microbial communities, which are discussed in the following section.

15. Microbial composition and differential abundance

Based on an analysis of physicochemical characteristics and the environmental stress conditions resulting from human activities at the two water points, it is proposed that these

conditions act as an ecological filter. This filter selects for stress-tolerant microbial communities, thereby directly shaping the dominant and displaced taxa over time. Additionally, it affects the survival and metabolic activity of macromycetes used in bioremediation.

The results of the characterization of bacterial and fungal communities confirm that the microbial profile of each site is ecologically consistent with its contaminant load and differs from each other: Lucero, with higher industrial pressure, showed more impoverished bacterial and fungal communities dominated by a few tolerant taxa such as *Moraxellaceae* and *Sporidiobolaceae*, while in Venecia, with predominantly agricultural and domestic pollution, it hosted more diverse communities with the presence of fungal phytopathogens (*Fusarium, Cladosporium*, among other microfungi), a pattern consistent with what was reported by Corrales et al. (2018) for waters of the middle basin of the Bogotá River and the thesis works of Cubides (2018), Martinez (2019), Sánchez (2020), and Espitia (2022), which report the presence of total and fecal coliforms at levels above permissible limits, while other studies such as those by Vargas (2022) show the presence of fungi at different points in the middle basin of the Bogotá River.

These results are particularly significant, as they reveal the presence of pathogenic bacteria and opportunistic fungi within the microbial communities, thereby posing a public health risk in accordance with WHO guidelines. The bacterial genus *Klebsiella* was identified with a prevalence of 97,5%, alongside other opportunistic bacteria, including *Pseudomonas* and *Acinetobacter.* All of these are listed by the WHO (2017) as priority bacterial pathogens requiring the development of new antibiotics, with levels classified as critical or high priority.

This underscores the global clinical significance of biological contamination resulting from direct domestic discharges into water bodies used for agricultural irrigation. Regarding fungal characterization, a substantial presence of yeasts with opportunistic potential is noted, primarily from the genera *Rhodotorula, Cryptococcus*, and *Apiotrichum / Trichosporon*, detected at both sites. These genera, included in the WHO (2022) list of priority pathogenic fungi, are known to cause fungemia, *Cryptococcal meningitis*, and invasive infections in immunocompromised patients, thus representing a health concern often neglected in the assessment of irrigation water quality.

The comparison between Venecia and Lucero was the only one that remained statistically significant after applying the strictest correction for both bacteria and fungi (PERMANOVA p=0,002–0,036), suggesting that the inherent conditions of each site exert a predominant effect on both microbial communities, beyond incubation time and fungal treatment. However, this difference is not only compositional but also structural in both domains. In bacteria and fungi, Venecia showed greater richness, higher diversity, and greater phylogenetic diversity than Lucero. However, Lucero exhibited fungal abundance dominated almost exclusively by *Sporidiobolaceae* (99,2%). The divergence between high abundance and low diversity in Lucero represents a classic pattern of dominance in ecosystems subjected to severe stress, similar to what was reported by Crump et al. (2012) in studies of bacterial communities in contaminated river environments, and by Shade et al. (2014), who demonstrate microbial dominance. This pattern is also observed in the characterization of aquatic fungi in altered rivers, as reported by Pascoal et al. (2003). The association with this trend is linked to the heterogeneous conditions of each site, where the organic load in Lucero, ten times higher than in Venecia, and the extremely low dissolved oxygen in both, favored

certain stress-tolerant organisms (*Moraxellaceae* 37,5% bacteria; *Rhodotorula* 99,2% fungi), while Venecia, with lower organic pressure and predominantly agricultural pollution, supported more diverse communities with a higher presence of fungal phytopathogens (Plant Pathogen guild 57,4%) and bacteria from agricultural runoff (Exiguobacteraceae 21,7%, with *Klebsiella* genus as an indicator of fecal contamination) Fei et al. (2020) Chen et al. (2019).

The efficacy of the macromycetes *Trametes* sp., *Ganoderma* sp., and *Pleurotus ostreatus* as bioremediation agents was influenced by environmental characteristics. The high mortality rates among the macromycetes, with combined survival rates of only 49% in the plugs and 11% in *P. ostreatus*, suggest that the physicochemical conditions at both locations exceeded the inoculum's tolerance thresholds. This phenomenon was more pronounced in Lucero than in Venecia, as evidenced by the mortality percentages across the three fungal species. Such unexpected outcomes resemble findings reported in a study involving *Pleurotus* sp., in which the demise of the bioremediation agent was documented at the conclusion of the treatment (Cotter, 2014). The heterogeneity observed among sampling points in microbial communities, physicochemical parameters, and mycelial mortality pressure is significant for interpreting the extent and nature of microbial community alterations. Additionally, it is noteworthy that, within the *Trametes* sp. treatment in Venecia, results were insufficient for definitive interpretation.

After the treatment period, the three macromycetes altered the structure of the bacterial community relative to the Day 0 control, *Ganoderma* sp., and *P. ostreatus*, with significant increases in alpha diversity in Lucero and *Trametes* sp. in Venecia, as evidenced by Shannon and Faith's indices ($p<0,05$). However, Day 30 control also shifted relative to the initial state,

indicating that part of the observed change was attributable to the community's natural temporal dynamics rather than solely to the treatment. This aligns with work on newly colonized aquatic microcosms, where bacterial composition changes over time due to the interplay of stochastic and deterministic processes, allowing temporary reorganization even without attributing these changes exclusively to a specific biotic intervention (Langenheder & Székely, 2011). In the native fungal community, only *Ganoderma* sp. significantly altered fungal community structure in Lucero, with Tukey HSD p=0.044 compared to day 0. This suggests that the native fungal community, dominated by yeasts, was more resilient to perturbation by exogenous mycelium than the bacterial community, as evidenced by studies of the ecological distribution and persistence of yeasts in soils (Slávikovᡠ& Vadkertiová, 2003) and by Linkind and colleagues (2009), who report the presence of yeasts in high-altitude areas subjected to intense environmental stress.

The relative abundance of Enterobacteriaceae at both sites decreased across all treatments compared to the control 0, from 8,69% to 0,16–0,51% in Lucero and from 2,56% to 0–0,09% in Venecia. However, in Venecia, Day 30 Control showed no evidence of Enterobacteriaceae (0%), indicating that the reduction at that site is not solely attributable to the fungal treatment but also to the community's temporal dynamics, while in Lucero, the Day 30 control remained at 1,08%, and reductions were observed in the fungal treatments. After conducting statistical analyses, none of these reductions were significant compared to Day 30 control, so the decrease in Enterobacteriaceae cannot be attributed to any of the macromycetes. These results differ from those reported by Chirnside et al. (2013), in which *Pleurotus ostreatus* reduced *E. coli* in wastewater and simulated rain runoff. They also differ from studies that document the presence of *Klebsiella* in surface waters and discuss key traits for its environmental

persistence, such as antimicrobial resistance and biofilm formation (Araújo et al., 2024). The difference between my study's results and those reported in the literature may be due to the different physicochemical conditions under which the studies were conducted, since my research used non-sterile water samples with the full contaminant load, reflecting the real conditions of irrigation waters at the sampled points, unlike other studies involving surface waters or simulation wastewaters.

Contrary to the expected results of microbial reduction in a bioremediation process, a significant increase in critically prioritized pathogenic bacteria was observed in Venecia with *Trametes* sp., in the genera *Klebsiella* (lfc=+2,3, q<0,05) and *Acinetobacter* (lfc=+0,8, q<0,05). This result, combined with the fact that *Trametes* sp. was the fungus with the highest overall survival (23% of all survivors), suggests that its metabolic activity may have created conditions that favored the proliferation of these opportunistic taxa in the specific physicochemical environment of Venecia. The persistence of *Klebsiella* and *Acinetobacter* in disturbed aquatic environments appears to be facilitated by human-associated conditions, including wastewater discharge, nutrient availability, surfaces for adhesion, and biofilm formation (Araújo et al., 2024; Barati et al., 2016; Hubeny et al., 2022). It is also noteworthy that the significant increase of the genus *Coxiella* in all three treatments (*Trametes* sp. lfc=+2,5; *Ganoderma* sp. lfc=+3,2; *Pleurotus* sp. lfc=+3,2, q<0,05) suggests that this is not a specific bacteria – macromycete fungi effect, but rather a response of the native bacterial community in Venecia to the disturbance caused by exogenous fungal biomass in an environment influenced by agriculture and livestock activity, as explained in the study by (D'Ugo et al., 2017), which reports the presence of *C. burnetii* in river water, supporting the idea that this microorganism can reach surface waters through contaminated discharges.

In the fungal component, opportunistic yeasts decreased differentially depending on the fungus and site, and the priority fungal pathogenic genera reported by the WHO in 2022, with higher opportunistic potential and known to cause infections: *Rhodotorula, Cryptococcus, Apiotrichum*, and *Trichosporon*, responded differently to each treatment. *Trametes* sp. in Lucero produced the greatest reduction of opportunistic fungi, being the fungus with the highest tolerance to industrial stress and reported in other studies as an excellent remediator of heavy metals and chemical substances (Yalcinkaya et al., 2002). Meanwhile, in Venecia, *Ganoderma* sp. achieved the most pronounced reduction of native fungi, suggesting that the production of extracellular lignolytic enzymes can remove contaminants from water (Yang et al., 2013) and produce secondary metabolites with antimicrobial and antifungal activity, which could interfere with the growth of some native fungi (Rašeta et al., 2023).

Just like in the analysis of bacteria, an increase in some native fungi was observed in treatments with macromycetes, where *Pleurotus ostreatus* in Lucero increased to *Rhodotorula* (lfc=+2,31) and *Candida* (lfc=+1,90). This, in relation to the 97% mortality rate of the plugs in Lucero and an 89% overall mortality, suggests that the necromass of *Pleurotus ostratus* released fungal biomass rich in chitin, β-glucans, and proteins into the aquatic environment. This served as a source of nitrogen and organic carbon, stimulating the proliferation of opportunistic and degrading taxa. Although the plug's necromass was small relative to the water volume, the ecological significance of an organic carbon input is not determined by its absolute mass but by its availability relative to the system's nutrient status. In a closed microcosm without nutrient replenishment, labile carbon becomes progressively

limiting over the 30-day incubation. Under these increasingly oligotrophic conditions, even a modest input of labile carbon released via mycelial lysis could disproportionately affect the remaining heterotrophic community, particularly copiotroph taxa that exploit nutrient pulses. This inference aligns with the findings reported by Cotter in 2014, which showed that long-term observations revealed an increase in the bacteria initially intended to be reduced.

On the other hand, it is worth noting that in Lucero, a significant decrease in the genus *Apiotrichum* (Trichosporon) was observed by *Ganoderma sp.* (lfc = -3,02) and *Trametes* sp. (lfc = -2,73), making it the only native fungal taxon consistently displaced by two independent treatments. The reduction of *Apiotrichum* is clinically relevant, as species of Trichosporonaceae related to this group have been associated with invasive trichosporonosis in immunocompromised patients; therefore, its reduction is one of the most noteworthy health-related mycoremediation effects observed in this study. (Martínez-Herrera et al., 2021)

The analysis of differential abundance in treatments with macromycetes allows us to suggest that the results of reduction or increase are the convergence of various effects of the fungus as a bioremediation agent, starting with extracellular activity as a mechanism of action during the active survival phase: greater resistance is observed in *Trametes* sp., with uniform tolerance across different sites, and less in *Pleurotus* sp., which shows greater sensitivity, with only 25% survival in Lucero's. Several studies have demonstrated that enzymes such as laccases, manganese peroxidases, and lignin peroxidases, characteristics of white-rot fungi, act on recalcitrant organic compounds present in water, modifying the physicochemical conditions of the microenvironment and altering the availability of substrates for the native

bacterial and fungal community (Hatakka, 1994; Janusz et al., 2013; Kersten & Cullen, 2007; Leonowicz et al., 1999; Yang et al., 2013). In addition to enzymes, the physical micellar structure of fungi could act as a bioadsorbent for suspended solids and ionic contaminants present in water, a mechanism documented in ligninolytic fungi in contaminated aquatic systems. (Maurya et al., 2006). This physical action, independent of enzymatic activity, may have contributed to the reduction of available organic load in the microenvironment of the microcosms, creating more favorable conditions for strict aerobic bacterial taxa that would otherwise be suppressed by the system's hypoxia (Chen et al., 2022; Maurya et al., 2006; Yalcinkaya et al., 2002). The direct influence of high mycelial mortality, in which necromass may have acted as a source of nitrogen and carbon post-mortem, restructured bacterial and fungal communities after the 30-day treatment.

A finding that warrants consideration is the detection of the family Ganodermataceae in the non inoculated controls, despite not having been added as a treatment. In the Day 30 Control at Venecia, Ganodermataceae rose from 0.02% at Day 0 to an apparent 99.4%; however, this figure does not reflect genuine biological proliferation but rather a compositional artifact arising from the very nature of metabarcoding, which reports relative rather than absolute abundances. In that sample, the fungal community collapsed, and the sequencing depth dropped sharply, such that a few residual Ganodermataceae reads came to represent almost the entire relative profile: as the rest of the detectable community collapsed, any remaining taxon was artificially inflated to the point of appearing dominant. This behavior contrasts with the Day 30 Control at Lucero, which retained an adequate sequencing depth and in which Ganodermataceae accounted for only 5.6%, a low value consistent with a background presence. This background presence is, in turn, explained by the fact that *Ganoderma* is a

cosmopolitan basidiomycete and a prolific spore producer, whose basidiospores disperse massively through the air and are routinely deposited on water bodies; their airborne abundance has been documented in the Colombian context by López-Vásquez et al. (2024), and their transport from forest environments toward agricultural areas by Sadyś et al. (2014). As an open system, irrigation water acts as a passive reservoir of this pre-existing environmental inoculum. Taken together, the detection of Ganodermataceae in the controls is attributable neither to cross contamination from the treatment nor to genuine growth in the absence of inoculum, but rather to the coexistence of a background environmental inoculum with a compositional artifact amplified by the low sequencing depth in samples from an impoverished community. This observation underscores the importance of interpreting relative abundances considering each sample's depth and richness and reinforces the compositional caution adopted throughout this work.

16. Microbial functional profiles

The analysis of the functional profile revealed a differential reorganization of ecological roles, with gains in degradation functions and partial losses in irrigation functions, though significant exceptions occurred.

Although taxonomic characterization showed that environmental families are predominant, their health significance remains unclear at the family level. The functional inference analysis, using FAPROTAX for bacteria and FUNGalTraits for fungi based on taxonomy (Kersten & Cullen, 2007; Louca et al., 2016; Põlme et al., 2021), identified intracellular parasites at nearly undetectable levels on Day 0 at both sites (0,007% Lucero; 0,003%

Venecia) and chemoheterotrophic bacteria, which were 21 times more prevalent in Venecia (8%) than in Lucero (0,4%). Among fungi, Undefined Saprotrophs (50%) and Animal Pathogens (17%) predominated in Lucero, whereas in Venecia, phytopathogenic fungi (57,4%) predominated, followed by animal pathogens (15,2%). This functional view is consistent with the characteristics and conditions of each site and again shows that the communities between Lucero and Venecia differ in both taxonomy and function and are directly related to discharges associated with human activities at each location.

After the treatments and considering the convergence of the effects of these macromycetes on biological systems, the functional analysis revealed the reorganization of bacteria, with an increase in aerobic chemoheterotrophic microorganisms, a function directly related to the degradation of organic contaminants, in all three treatments compared to the control on day 0. In Lucero, *Ganoderma* sp. led the increase, followed by *Pleurotus ostreatus* and *Trametes* sp., while in Venecia, *Pleurotus ostreatus* was more effective than *Ganoderma* sp. This increase in heterotrophic capacity constitutes the most direct functional evidence of the bioremediation potential of macrobasidiomycetes in this system. Since chemoheterotrophy is used as an indicator of biodegradation capacity in water, this aligns with research showing that the metabolic activity of heterotrophic microorganisms reflects pollutant loads and the use of organic substrates in affected rivers (Tiquia, 2010).

In Venecia, treatment with *Trametes* sp. increased the abundance of microorganisms with nitrification functions, which were absent under initial conditions and are crucial for the nitrogen cycle in an agricultural context with high nitrogen loads from fertilizers and livestock excretions. This finding aligns with the higher relative survival of *Trametes* sp. in

Venecia and suggests that its sustained metabolic activity was essential for the development of this emerging function, which is important in irrigation waters influenced by agricultural activities. This point is detailed in the work of Camargo and Alonso, who analyze the ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems and discuss consequences such as eutrophication, acidification, and toxicity associated with different forms of nitrogen (Camargo & Alonso, 2006). The degradation of single-carbon compounds (methanolysis) was virtually nonexistent in the Day 0 Control of Lucero (0,00%) and moderate in Venecia (0,84%). However, it increased with fungal treatments, especially *Pleurotus* in Venecia (3,99%), nearly fivefold. This suggests that the fungi favored methylotrophic bacteria, which can break down compounds such as methanol and methylamines. In these waters, such compounds may be associated with the degradation of organic matter and with the transformation of agricultural and industrial derived contaminants (Kolb, 2009).

FUNGalTraits revealed that all treatments caused a shift in the fungal functional profile toward Plant Pathogen-Wood Saprotroph, with parallel reductions in Animal Pathogen (from 16,6% to 1,1–18,1% in Lucero; from 15,2% to 1,3–5,2% in Venecia) and Undefined Saprotroph (from 57,4% to 1,8–37,8% in Lucero). The extent of this fungal functional reorganization was greater in Venecia than in Lucero, which is consistent with the higher resistance of the fungal community in Lucero, dominated by stress-tolerant basidiomycete yeasts and exogenous mycelium perturbation tolerance, similar results with studies where it has been shown that an exogenous fungal inoculation can reorganize the fungal community of a system (Elgueta et al., 2016).

At Lucero, *Trametes* sp. significantly reduced fungi classified as Animal Pathogen from 99,6% (Control D0) to 29,8%, the largest decrease observed in the study. This was primarily due to the displacement of Sporidiobolaceae (*Rhodotorula*) and suppression of *Apiotrichum* (lfc=-2,73, q<0,05). This finding is ecologically important because *Rhodotorula* and *Apiotrichum*, although from different basidiomycetous lineages, are both opportunistic yeasts that affect immunocompromised individuals, such as cancer patients (Flores-Delgado et al., 2024). In Venecia, *Ganoderma* sp. reduced opportunistic fungi from 78,4% (Control D0) to 2,1%, primarily displacing *Rhodotorula* and *Cryptococcus*, which is classified as a critically important fungal pathogen by the WHO (2022). Nonetheless, this treatment increased *Plectosphaerella* (lfc=+3,54) and *Tolypocladium* (lfc=+3,46), fungi with potential phytopathogenic and entomopathogenic properties (Carlucci et al., 2012), respectively, which somewhat diminishes the overall benefit at this location.

Despite the positive effects on organic degradation and the reduction of opportunistic fungi, FAPROTAX revealed that the intracellular parasitism function increased in all treatments compared to the Day 0 Control at both sites, with *Trametes* sp. in Lucero reaching the highest value in the entire study (13,4% vs. 0,007% in Control D0). This increase, identified by *Legionella, Aquicella*, and *Coxiella* (Declerck, 2010) represents the most significant functional limitation of the evaluated treatments and provides evidence that disturbance of the microbial ecosystem by exogenous mycelium can create niches favorable for opportunistic intracellular pathogens, even when reducing other risk groups. The constant dynamics of microbial community structure are directly related to the system's nutritional and physicochemical conditions, which can be altered by exogenous organisms. This reduces

community resistance and favors the colonization, proliferation, or dominance of pathogenic organisms (Idbella et al., 2023).

By integrating the results of FAPROTAX and FUNGalTraits, the functional balance of the treatments shows a consistent increase in organic degradation capacity by microorganisms with chemoheterotrophic, methylotrophic, and predatory functions. It also reveals a partial reduction in opportunistic fungal guilds, accompanied by a simultaneous increase in intracellular bacterial parasites across all treatments. This pattern suggests that the evaluated macromycetes act as modifiers of the microbial ecosystem with mixed functional effects, and that their application as agents for health-related mycoremediation requires complementary strategies to mitigate the ecological niche they create for intracellular pathogens, Inference shared in Shahi and colleagues' study, which shows that bioaugmentation with white rot fungi not only contributes to remediation but also modifies the structure of the intrinsic microbial community and the functional genes of the system (Shahi et al., 2016).

Taken together, these findings invite a reconsideration of what "mycoremediation" means in complex microbial ecosystems. The results obtained suggest that macrofungal inoculation did not operate as a targeted antimicrobial treatment, but rather as an ecological disturbance with mixed directional effects: while it was associated with a restructuring of community composition and with increases in diversity, it did not produce a selective removal of pathogenic taxa, whose decline was also observed in the controls. This distinction is conceptually relevant, as it reveals that two objectives often assumed to be equivalent to the reduction of health risk through pathogen removal and the functional restoration of the ecosystem may not be aligned, and that the introduction of a fungal species may even

restructure the native microbiome. Precisely defining the goal of the intervention is therefore as decisive as selecting the species employed. Acknowledging this ambiguity does not weaken the contribution of the present work; rather, it delimits its scope as a first community level characterization of a phenomenon whose mechanistic basis remains to be interrogated and situates the mycoremediation of irrigation waters within the broader conceptual frameworks of restoration ecology and environmental biotechnology.

17. CONCLUSIONS

1. The irrigation waters from the Venecia and Lucero sites in the La Ramada District contain structurally distinct bacterial and fungal communities, with Venecia showing greater bacterial and fungal diversity, while Lucero presented more impoverished communities dominated by stress-tolerant taxa. At both sites, water quality is highly contaminated, harboring pathogenic and opportunistic microbial communities, with Lucero's water unsuitable for agricultural irrigation due to its risks to human health and the environment.

2. The functional annotation revealed that the microbial communities at both sites perform active health risk functions from their baseline state, which are invisible through conventional taxonomic characterization. This confirms that not only are there different taxa at each site, but the communities also play distinct ecological roles. The functional profiles in Venecia were characterized by intracellular parasitic bacteria, chemoheterotrophs, and phytopathogens, whereas in Lucero, saprotrophic microorganisms were predominant, with simultaneous risks to human and animal health. These findings support the need to adopt comprehensive molecular approaches for assessing the health of irrigation water in complex contamination contexts.

3. The three macromycetes altered the structure of the native bacterial and fungal communities. The effects varied by species and location, with *Trametes* sp. being the most effective in Venecia, and *Ganoderma* sp. and *Pleurotus ostreatus* in Lucero for bacteria. Conversely, in fungi, only *Ganoderma* sp. induced significant structural changes in Lucero. The overall survival rate was 49%, with *Pleurotus ostreatus* accounting for

only 11% of survivors, indicating that the contamination conditions at both sites exceeded the inoculated fungi's physiological tolerance thresholds, thereby influencing the extent of observed changes in both communities.

4. The effects of the three macromycetes on the health risk taxa were partial and site-dependent. In bacteria, *Klebsiella* decreased across all treatments compared to Day 0, although this decrease was not exclusively attributable to the fungal treatment. Likewise, the constant increase of *Coxiella* with the three fungi in Venecia and the rise of intracellular parasites with *Trametes* sp. in Lucero, driven by *Legionella* and *Aquicella*, represent noteworthy and attention-worthy results. In fungi, *Trametes* sp. in Lucero and *Ganoderma* sp. in Venecia produced the greatest reductions of opportunistic yeasts in the genera *Rhodotorula, Cryptococcus*, and *Apiotrichum*, confirming that no treatment completely or consistently eliminated the priority risk taxa across sites.

5. The overall survival rate of 49% among the inoculated plugs, with *Pleurotus ostreatus* accounting for only 11% of the survivors and *Trametes* sp. representing 23%, indicates that the contamination conditions at both sites exceeded the physiological tolerance threshold of the inoculated fungi. Furthermore, the magnitude and direction of the observed changes in the microbial communities were influenced by both the metabolic activity of the live mycelium and the effects of necromass released after its death.

6. The three evaluated macromycetes demonstrated a partial, differential, and site-dependent mycoremediation potential on the microbial communities in the irrigation waters of the La Ramada District. *Ganoderma* sp. was the most effective in waters

subjected to industrial and urban discharges with high loads of recalcitrant organic matter, stimulating the greatest capacity for bacterial organic degradation; *Trametes* sp. showed the highest tolerance to contamination conditions and was the only one to promote nitrification in waters influenced by agricultural and domestic discharges from horticultural and livestock sources. On the other hand, *Pleurotus ostreatus*, despite its high mortality, caused the greatest reorganization of the bacterial community in Lucero through its necromass. However, no fungus consistently eliminated priority health-risk taxa, and all increased functions related to intracellular parasites at least one site. The high mycelial mortality at both sites indicates that mycoremediation with macromycetes under non-sterile conditions and high contamination loads results in a transient modification of the microbial ecosystem that is ecologically relevant but insufficient as an autonomous sanitation strategy. Therefore, the implementation of complementary strategies such as immobilization, preconditioning, repeated application, or integration into systems of metabolic cooperation among different bioremediation organisms is necessary to achieve a sustained health impact in agricultural irrigation systems with complex contamination.

18. LIMITATIONS AND PERSPECTIVES

This study is one of the first approaches using amplicon metabarcoding (16S rRNA and ITS2) to assess the potential of macromycetes for mycoremediation of microbial communities in agricultural irrigation waters with complex contamination in Colombia, providing molecular evidence that goes beyond conventional microbiological monitoring approaches.

As an initial step in this field of research, the experimental design within microcosms enabled control of the variables of interest and allowed the identification of causal relationships between fungal treatments and observed community changes, thereby laying the foundation for larger-scale studies. The 49% mycelial survival rate under real, non-sterile, and highly contaminated water conditions should not be viewed as a system failure; rather, it demonstrates the relative resilience of these organisms under extreme physicochemical conditions and delineates a documented tolerance threshold that informs the development of future application strategies.

The implementation of mycelial immobilization systems, solid support bioreactors, or encapsulation matrices represents the next logical step to extend the metabolic activity of fungi beyond the identified mortality threshold, particularly in waters with mixed contaminant loads. Incorporating shotgun metagenomics in subsequent studies would allow confirmation of the actual enzymatic activity of microbial communities and direct quantification of ligninolytic gene expression during treatment. Ultimately, the integration of targeted enzymatic assays, metabolomic analyses, and direct pathogen viability testing will enable discernment of whether the observed effect reflects a biologically active suppression or an ecological displacement, thereby elucidating the mechanistic basis that this first community-level approach cannot resolve. Finally, the differential response pattern

identified in this study among *Ganoderma* sp., *Trametes* sp., and *Pleurotus ostreatus* suggests that multi-species fungal consortia, designed to leverage the metabolic complementarity between these species, could overcome the individual limitations of each fungus and constitute a more robust and sustained mycoremediation strategy for agricultural irrigation systems in complex contamination contexts such as the La Ramada District.

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ANNEXES CHAPTER 1

Venecia sampling site, La Ramada Irrigation District



Supplementary figure 1-1 Venecia sampling site, La Ramada Irrigation District, Cundinamarca, Colombia. (A) Water body covered by aquatic macrophytes, used as the main water source for irrigation of vegetable crops. (B) Adjacent vegetable crop field irrigated with water from the sampling point.

Lucero sampling site, La Ramada Irrigation District



Supplementary figure 1-2 Lucero sampling site, La Ramada Irrigation District, Cundinamarca, Colombia. **(A)** Water body used as the main water source for the irrigation of vegetable crops in an industrial zone (B). Adjacent vegetable crop field irrigated with water from the sampling point.

ANNEXES CHAPTER 3

Bacterial Read count retention per sample across DADA2

Samples	input	filtered	denoisedF	denoisedR	merged	nonchim	percent
1	159377	151430	148306	149131	134605	100756	0,67
2	36660	34672	32169	33101	28151	25380	0,73
3	20892	19786	18568	18900	16225	15204	0,77
4	117791	111377	108179	108977	96178	74177	0,66
5	34904	32901	29764	30494	26030	22046	0,67
6	30223	28408	25558	26290	21371	17268	0,60
7	62853	59511	57986	58459	47182	29989	0,51
8	15204	14443	14076	14167	12998	11096	0,77
9	4747	4495	4323	4338	4088	3917	0,87
11	939	856	773	813	706	706	0,82
12	4579	3959	3766	3812	3358	3111	0,78
13	11120	10421	9986	10129	8781	7940	0,76
14	45791	43652	42535	42815	36767	25465	0,59
15	3583	3127	3020	3023	2813	2726	0,87
16	99602	94008	88443	90283	81202	79447	0,85
17	126914	119794	111789	113983	100495	97011	0,81
18	85352	80561	74638	76237	67455	65719	0,82
19	19747	18537	16836	17259	14625	14265	0,77
20	25161	23606	21434	21920	18284	17401	0,74
21	17517	16429	14664	14980	12250	11840	0,72
22	17576	16572	15469	15665	13782	13551	0,82
23	14294	13440	11688	12140	10235	9827	0,73
-24	40608	38296	35741	36320	31156	29693	0,78
25	21488	20299	19167	19436	17380	15190	0,75
26	29072	27312	25048	25863	21318	20263	0,74
27	64423	60712	56360	57330	48928	44405	0,73
28	4673	4423	3933	4053	3704	3704	0,84
29	5954	5648	5301	5381	4830	4784	0,85
30	4145	3923	3616	3664	3320	3304	0,84
31	16209	15249	13554	14055	12233	12233	0,80
32	20653	19408	17865	18227	15498	15411	0,79
33	14378	13514	12220	12514	10634	10612	0,79
34	23671	21634	20009	20557	17825	16155	0,75
35	28159	26508	24284	24886	21119	19493	0,74
36	66041	61296	57127	58361	49934	44875	0,73
37	49883	47222	42714	44091	36738	33162	0,71
38	36945	34732	32421	33216	28833	25035	0,72
39	110635	104488	96010	98007	82061	70971	0,70

40	41308	38553	35442	36284	30583	27388	0,71
41	13412	12711	12044	12214	10930	10275	0,81
42	19007	17553	15751	16372	13988	12973	0,74
43	1046	930	738	797	639	639	0,69
44	3782	3387	3128	3196	2808	2759	0,81
45	6152	5499	5144	5179	4575	4456	0,81
46	3196	2883	2502	2597	2172	2172	0,75
47	4072	3683	3325	3425	2789	2738	0,74
48	2835	2559	2253	2331	1881	1848	0,72
49	2298	2043	1807	1878	1643	1643	0,80
50	4276	3661	3329	3419	2947	2920	0,80
51	1058	827	547	614	476	476	0,57
52	41301	38960	35888	36991	31177	27578	0,70
53	52625	49596	45801	46617	39074	34428	0,69
54	36636	34547	31699	32291	27588	24614	0,71
55	14656	13854	12885	13185	12061	11803	0,85
56	22785	21663	20624	20892	18983	18215	0,84
57	47707	45268	42931	43427	39698	37397	0,83
58	37593	35777	33241	33985	28393	25454	0,71
59	1241	1133	981	995	864	864	0,76
60	44064	41283	40379	40607	38681	38000	0,92
61	29989	28310	26526	27101	22836	21624	0,76
62	32781	31141	28697	29473	25819	24335	0,78
63	87626	83033	76055	77792	65487	58760	0,71
TOTAL	2043209	1925503	1801057	1834539	1589184	1411491	0,47

Supplementary table 3-1 Read counts per sample at each step of the DADA2 quality filtering pipeline for bacterial 16S rRNA (V4 region) amplicon data. Columns represent: input (raw paired-end reads), filtered (reads retained after quality filtering), denoisedF and denoisedR (denoised forward and reverse reads), merged (successfully merged paired reads), nonchim (reads remaining after chimera removal), and percent (proportion of input reads retained as high-quality sequences). Samples correspond to water from the Venecia and Lucero sampling sites of the La Ramada Irrigation District, Cundinamarca, Colombia, collected across the mycoremediation experimental period (Day 0 and Day 30).

Fungi Read count retention per sample across DADA2

Samples	input	filtered	denoisedF	denoisedR	merged	nonchim	percent
2	73448	63789	61948	62340	53960	27282	0,43
3	120998	109361	108004	107778	94668	61021	0,56
5	97814	86979	85443	85349	73745	48613	0,56
6	76359	65180	63208	63307	55430	28097	0,43
7	36573	27591	27049	26889	22232	10864	0,39
8	94187	80562	79687	79760	65723	45504	0,56
9	87607	79414	78510	78453	67508	49329	0,62
10	131006	125015	124406	124711	113330	92603	0,74
11	88360	84205	83775	83936	73446	61009	0,72
12	134638	128399	127959	128036	116838	82846	0,65
13	118934	113459	113026	112928	100872	79578	0,70
14	88468	84550	84147	84323	76875	52123	0,62
15	114376	108267	107812	108025	99133	62498	0,58
19	74495	24287	21836	23490	19780	16167	0,67
20	94920	30961	30282	30250	26801	21268	0,96
21	66081	21534	21071	21097	18727	15335	0,71
22	71813	52063	50387	50823	45120	17459	0,33
23	60362	2345	1973	2130	1891	1891	0,81
24	40608	16795	16450	16338	13800	6016	0,36
25	123483	109769	109445	109229	101320	100699	0,92
26	74588	21008	18391	20550	17462	15948	0,76
27	174548	114752	114237	113823	104014	101203	0,88
28	92345	23130	21875	22775	21049	20521	0,89
29	117665	29379	29055	29129	27215	26389	0,90
30	81915	20471	20290	20291	19284	18937	0,93
31	94223	41384	39175	40995	37085	31382	0,76
32	120058	52849	52153	52062	47085	39476	0,75
33	83581	36809	36388	36427	33125	28346	0,77
34	71617	56877	55930	56117	47834	29927	0,53
35	82427	24168	21359	23482	19115	9624	0,40
36	127539	67002	65816	65953	56227	33181	0,49
37	31261	7862	7493	7455	6295	2607	0,33
38	54085	30715	26387	29934	22630	11819	0,38
39	108747	49008	47657	47652	38919	19518	0,40
40	75500	203	85	59	59	59	0,29
41	107650	91148	90714	90738	86629	69104	0,76
42	59461	36181	36027	35629	17529	15768	0,44
43	104877	79550	79029	78873	57919	41938	0,52

44	88217	77705	77114	76938	72184	56413	0,73
45	246039	200541	198122	198859	169621	119754	0,60
46	120625	101142	100828	100918	95134	84428	0,83
47	153699	129034	127743	128582	117325	97801	0,76
48	107001	89859	89293	89616	76470	49581	0,55
49	101670	83581	83117	83015	77864	50913	0,61
50	115872	98270	97817	97724	91649	84539	0,86
51	72839	51842	51665	51575	50834	49415	0,95
52	95411	87285	87000	86801	81279	81112	0,93
53	121572	111147	110750	110646	102446	99088	0,89
54	84635	77324	77037	77140	71458	70502	0,91
55	86573	78257	77897	77995	74435	47000	0,60
56	65012	60136	59657	59097	38622	13327	0,22
57	193148	176358	175235	175077	146201	76532	0,43
58	51520	45581	45207	45115	42025	34736	0,76
59	108257	93531	93188	92749	79998	49790	0,53
61	80452	72771	72110	72436	67014	47226	0,65
62	32102	19714	18828	19020	18427	11906	0,60
63	143415	117987	116776	117332	100511	66003	0,56
Total	5524676	3969086	3917863	3931771	3474171	2586015	0,63

Supplementary table 3-2 Read counts per sample at each step of the DADA2 quality filtering pipeline for fungi (ITS2), amplicon data. Columns represent: *input* (raw paired-end reads), *filtered* (reads retained after quality filtering), *denoisedF* and *denoisedR* (denoised forward and reverse reads), *merged* (successfully merged paired reads), *nonchim* (reads remaining after chimera removal), and *percent* (proportion of input reads retained as high-quality sequences). Samples correspond to water from the Venecia and Lucero sampling sites of the La Ramada Irrigation District, Cundinamarca, Colombia, collected across the mycoremediation experimental period (Day 0 and Day 30).

Dominant bacterial families and relative abundance of Enterobacteriaceae

	Treatment	Total reads	1st dominant family (%)	2nd dominant family (%)	3rd dominant family (%)	Enterobacteriaceae (%)
Lucero	Control Day 0	84 778	*Moraxellaceae* (37,46)	*Pseudomonadaceae* (15,45)	*Exiguobacteraceae* (11,52)	8,69
Lucero	Control Day 30	6 776	*Diplorickettsiaceae* (10,82)	*Moraxellaceae* (10,67)	*Caulobacteraceae* (9,00)	1,08
Lucero	*Trametes* sp.	7 892	*Diplorickettsiaceae* (24,05)	*Legionellaceae* (9,02)	*Rhodobacteraceae* (6,20)	0,51
Lucero	*Ganoderma*	67 446	*Moraxellaceae* (16,52)	*Diplorickettsiaceae* (10,42)	*Comamonadaceae* (7,85)	0,16
Lucero	*Pleurotus ostratus*	104 820	*Pseudomonadaceae* (16,42)	*Moraxellaceae* (10,11)	*Xanthobacteraceae* (9,92)	0,23
Venecia	Control Day 0	240 151	*Exiguobacteraceae* (21,71)	*Moraxellaceae* (15,83)	*Pseudomonadaceae* (13,80)	2,56
Venecia	Control Day 30	34 378	*Micrococcaceae* (24,30)	*Comamonadaceae* (9,07)	*Rhodobacteraceae* (8,87)	0
Venecia	*Trametes* sp.	228 874	*Pseudomonadaceae* (9,98)	*Micrococcaceae* (7,23)	*Rhodobacteraceae* (5,71)	0,09
Venecia	*Ganoderma*	36 663	*Xanthomonadaceae* (9,8)	*Moraxellaceae* (7,00)	*Micrococcaceae* (4,34)	0
Venecia	*Pleurotus ostreatus*	116 014	*Pseudomonadaceae* (12,19)	*Comamonadaceae* (11,35)	*Micrococcaceae* (10,34)	0

Supplementary table 3-3 Dominant bacterial families and relative abundance of Enterobacteriaceae across fungal treatments at Venecia and Lucero sampling sites. Values in parentheses represent the relative abundance (%) of each family within the total bacterial community per treatment. Total reads correspond to high-quality sequences retained after DADA2 quality filtering. *Trametes* = *Trametes* sp.; Ganoderma = Ganoderma sp.; *Pleurotus* = *Pleurotus* ostreatus. Enterobacteriaceae values of 0 indicate the family was not detected among the 20 most abundant families in that treatment group.

Dominant fungal families and

Site	Treatment	Total reads	Dominant family (%)	2nd family (%)	3rd family (%)
Lucero	Control Day 0	747 340	*Sporidiobolaceae* (99,2)	*Trichosporonaceae* (0,4)	*Microbotryomycetes* (0,2)
Lucero	Control Day 30	286 567	*Trichosporonaceae* (93,9)	*Ganodermataceae* (5,6)	*Sporidiobolaceae* (0,3)
Lucero	*Ganoderma* sp.	35 988	*Trichosporonaceae* (81,7)	*Ganodermataceae* (16,3)	*Sporidiobolaceae* (1,1)
Lucero	*Pleurotus ostratus*	79 046	*Trichosporonaceae* (87,0)	*Sporidiobolaceae* (11,9)	*Microbotryomycetes* (0,5)
Lucero	*Trametes* sp.	192 412	*Ganodermataceae* (69,3)	*Trichosporonaceae* (26,6)	*Sporidiobolaceae* (3,4)
Venecia	Control Day 0	29 668	*Sporidiobolaceae* (60,0)	*Rhynchogastremaceae* (11,9)	*Cryptococcaceae* (4,1)
Venecia	Control Day 30	34 594	*Ganodermataceae* (99,4)	*Rhynchogastremaceae* (0,2)	*Tremellomycetes* (0,1)
Venecia	*Ganoderma* sp.	76 132	*Ganodermataceae* (61,5)	*Doassansiaceae* (36,2)	*Rhynchogastremaceae* (1,2)
Venecia	*Pleurotus ostratus*	2 800	*Trichosporonaceae* (41,1)	*Rhynchogastremaceae* (6,7)	*Cystobasidiaceae* (3,5)
Venecia	*Trametes* sp.	—	—	—	—

Supplementary table 3-4 Dominant fungal families and total reads by site and fungal treatment. Values in parentheses represent the relative abundance (%) of each family within the total fungal community per treatment group. Total reads correspond to high-quality sequences retained after DADA2 quality filtering. *Trametes* sp. samples from Venecia were excluded due to insufficient sequencing depth (—). *Ganoderma* = *Ganoderma* sp.; *Pleurotus ostreatus* = *Pleurotus ostreatus; Trametes* = *Trametes* sp.

ANNEXES CHAPTER 4

Bacterial FAPROTAX functional annotation

Ecological function	L-C0	L-C30	L-Tra	L-Gan	L-Ple	V-C0	V-C30	V-Tra	V-Gan	V-Ple
Chemoheterotrophy	0,36	2,88	2,34	**7,31**	2,81	7,77	4,75	7,32	7,07	**9,15**
Aerobic chemoheterotrophy	0,35	1,4	1,12	**5,28**	1,73	**5,97**	2,95	4,2	3,68	1,93
Methylotrophy	0	1	0,68	1,4	0,09	0,84	1,04	0,13	0,58	**3,99**
Methanol oxidation	0	1	0,68	1,4	0,09	0,82	1,04	0,12	0,58	**3,99**
Nitrification	0	0	0	0	0,003	0	0,27	**0,39**	0,18	0,05
Aerobic ammonia oxidation	0	0	0	0	0,003	0	0,27	**0,39**	0,18	0,05
Intracellular parasites	0,007	6,78	**13,4**	7,1	3,11	0,003	0,31	1,45	1,89	0,85
Predatory or exoparasitic	0	0,79	1,16	0,18	1,46	0	0,69	1,31	**3,09**	2,99
Fermentation	0,01	0	0,05	0	0,02	0,03	0,17	0,11	0,03	0,04
Hydrocarbon degradation	0	0	0	0	0	0,02	0	0,007	0	0
Sulfate respiration	0	0	0	0	0	0,02	0	0,006	0,03	0

Supplementary table 4-1 Dominant fungal families and total reads by site and fungal treatment. Values in parentheses represent the relative abundance (%) of each family within the total fungal community per treatment group. Total reads correspond to high-quality sequences retained after DADA2 quality filtering. *Trametes* sp. samples from Venecia were excluded due to insufficient sequencing depth (—). Ganoderma = Ganoderma sp.; *Pleurotus ostreatus = Pleurotus ostreatus; Trametes = Trametes* sp.

Fungal FUNGalTraits functional annotation

LUCERO:					
Guild	**C0**	**C30**	***Trametes***	***Ganoderma***	***Pleurotus***
Plant Pathogen-Wood Saprotroph	2,36	**94,83**	**92.82**	67,64	6,33
Undefined Saprotroph	**67,34**	0,98	1,77	4,85	35,85
Animal Pathogen	15,85	1,15	1,09	3,41	**18,6**
Plant Pathogen	5,52	0,81	1,34	**7,53**	**20,58**
Endophyte	1,83	0,16	0,48	3,11	8,3
Fungal Parasite	2,53	1,19	0,19	0	0,27
Ectomycorrhizal	0,77	0,35	0,12	0	0,01
VENECIA:					
Guild	**C0**	**C30**	***Ganoderma***	***Pleurotus***	
Plant Pathogen-Wood Saprotroph	3,98	**85,01**	**79,71**	3,76	
Plant Pathogen	**44,54**	4,26	0,08	20,98	
Animal Pathogen	14,97	0,59	1,3	6,59	
Endophyte	10,23	0,91	0,05	**11,98**	
Endophyte-Plant Pathogen-Wood Saprotroph	4,44	3,44	0	**25,47**	
Fungal Parasite	2,85	0,65	**14,39**	1,98	
Litter Saprotroph-Soil Saprotroph	8,07	1,03	0	1,66	

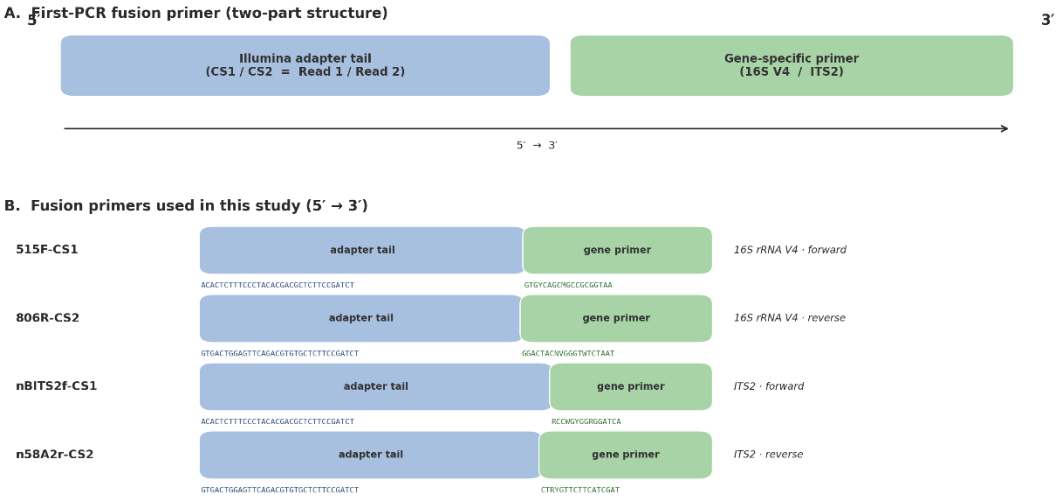
Supplementary table 4-2 Mean relative abundance (%) of fungal guilds inferred by FUNGalTraits annotation for fungal communities at Lucero and Venecia sampling sites across all treatments. Values represent the mean across biological replicates per group. Bold values indicate the highest abundance per guild across all groups within each site. C0 = Control Day 0; C30 = Control Day 30; *Trametes* = *Trametes* sp.; *Ganoderma* = *Ganoderma* sp.; *Pleurotus* = *Pleurotus ostreatus*. *Trametes* sp. data not available for Venecia due to insufficient sequencing depth.

Common and high-risk pathogens found in contaminated water

Type	Microorganism	Frequency in contaminated water	Danger to humans	Main associated disease
Bacterium	*Escherichia coli*	Very high (fecal indicator)	Moderate–high (pathogenic strains)	Gastroenteritis, bloody diarrhea, HUS
Bacterium	*Vibrio cholerae*	Moderate (waters with feces, poor sanitation)	Very high (can be fatal without treatment)	Cholera (severe watery diarrhea, dehydration)
Bacterium	*Salmonella* spp.	High	High	Gastroenteritis, typhoid fever
Bacterium	*Shigella* spp.	High (areas with poor sanitation)	Very high	Shigeliosis (bloody/pus diarrhea, dysentery)
Bacterium	*Campylobacter* spp.	High	High	Gastroenteritis, diarrhea, complications
Bacterium	*Legionella* pneumophila	Moderate (warm waters, systems)	Moderate–high (pulmonary risk)	Legionellosis (severe pneumonia)
Bacterium	*Pseudomonas aeruginosa*	Moderate–high (stagnant waters)	Moderate–high (in immunocompromised)	Respiratory, skin, and urinary infections
Virus	Rotavirus	High (in children, contaminated water)	High (in children <5 years)	Severe gastroenteritis in children
Virus	Norovirus	High	High	Gastroenteritis (diarrhea, vomiting)
Virus	Hepatitis A / E virus	Moderate–high (fecal contamination)	Very high (hepatitis, possible death)	Viral hepatitis
Virus	Enteric adenovirus	Moderate	Moderate	Gastroenteritis, respiratory illness
Parasite	*Giardia lamblia*	High (untreated water)	Moderate–high	Giardiasis (diarrhea, fatigue, malaise)
Parasite	*Cryptosporidium* parvum	Moderate–high	High (in children, elderly, immunocompromised)	Cryptosporidiosis (severe diarrhea)
Parasite	*Entamoeba histolytica*	Moderate (waters with feces)	Very high	Amebiasis (dysentery, possible liver complications)

Supplementary table 5. Common and high-risk pathogens found in contaminated water, grouped by type, with estimated frequency, human health risk, and main associated disease.

Modular structure of the fusion primers used for 16S rRNA (V4) and ITS2



Supplementary Figure 6. Modular structure of the fusion primers used for 16S rRNA (V4) and ITS2 amplicon library preparation. (A) General two-part architecture of a first-PCR fusion primer, comprising a 5′ Illumina adapter tail (CS1/CS2, corresponding to the Read 1/Read 2 sequencing primer sites) fused to a 3′ gene-specific primer. (B) The four fusion primers used in this study, shown 5′→3′ with their exact adapter and gene-specific sequences; block width is proportional to sequence length. Primers were synthesized by IDT Technologies, USA.