MASTER DIPLOMA PIECE

Arta Neziri

2026

UNIVERSITY OF SOPRON

FACULTY OF FORESTRY

MSc in Geobioinformatics

COMPARATIVE METAGENOMIC ANALYSIS OF MICROBIAL COMMUNITIES ASSOCIATED WITH HEALTHY AND DISEASED PINE TREES AND ASSOCIATED SOILS

Arta Neziri

Consultants: **Dr. Géza Király PhD, Dr. Nóra Tünde Lange-Enyedi PhD, Prof. Dr. György Sipos**

Sopron

2026

UNIVERSITY of SOPRON | FACULTY OF FORESTRY
Institute of Forest and Natural Resource Management

9400 Sopron, Bajcsy-Zsilinszky u. 4.
etegi@uni-sopron.hu
+36 99 518 333

MASTER DIPLOMA PIECE TASK STATEMENT

Name of the author of diploma piece: Arta Neziri

Title of diploma piece: Comparative Metagenomic Analysis of Microbial Communities Associated with Healthy and Diseased Pine Trees and Associated Soils

Tasks set for writing the diploma piece:

1. Collection of geospatial data from satellites for the affected conifer forest sites.
2. Retrieval, quality control, and preprocessing of metagenomic and 16S amplicon sequencing datasets, followed by taxonomic assignment of bacterial and fungal communities.
3. Comparative analysis of microbial community composition and diversity between non-symptomatic and symptomatic pine tree and soil samples.
4. Interpretation of the results, and identification of microbial taxa potentially associated with disease progression or plant health.

Consultant: Dr. Géza Király PhD, Dr. Nóra Tünde Lange-Enyedi PhD, Prof. Dr. György Sipos

The length of the essay is not limited. Please prepare the diploma piece following the formal requirements for this type of work, submit 1 hardcopy an electronic version to the university repository in pdf format, identical to the hardcopy by the deadline specified in the study regulations for the academic year 2025/2026.

Sopron, February 26, 2026

Prof. Dr. Lakatos Ferenc
Head of the Institute

Soproni Egyetem Erdőmérnöki Kar SOPRON

Prof. Dr. Kornél Czimber PhD
Responsible person for Geobioinformatics MSc

Prof. Dr. Bálint Heil
Dean

Soproni Egyetem Erdőmérnöki Kar Dékán SOPRON

STATEMENT

I, **Arta Neziri**, the undersigned, (Neptune Code: **TQJ0RV**) signing this statement, declare that the titled

Comparative Metagenomic Analysis of Microbial Communities Associated with Healthy and Diseased Pine Trees and Associated Soils

Thesis

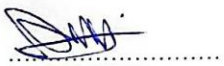
is **my independent work**, during the preparation of the dissertation I abided *the rules of the copyright law LXXVI of 1999* and the regulations of the university concerning the preparation of the thesis, with regard to references and quotations.[9]

I also declare that during the preparation of the dissertation **I did not mislead** the consultant or the instructor who gave the on working independently.

By signing this statement, I acknowledge that if it can be proved that the thesis **was not written by myself** or there is a copyright infringement in relation to my thesis, the University of Sopron **refuses to accept the dissertation and institutes disciplinary proceedings against me.**

The refusal to accept the dissertation and the initiation of disciplinary proceedings shall not affect the additional legal consequences of the infringement (civil law, infringement law, criminal law).

17th April 2026 in Sopron



signature

1999 LXXVI law Article 34 § (1) Any part may be cited from a disclosed work by the source and naming the author indicated as such.
Such citation shall be true to the original and its scope shall be justified by the nature and purpose of the borrowing work.
Section 36 (1) details of publicly held lectures and other similar works, as well as political speeches for information purposes to the extent justified by the purpose – may be freely used. In the case of such use, the source, together with the author's name, must be indicated unless it proves to be impossible.

ABSTRACT

Forest decline is a complex process involving interactions between environmental stressors, host condition, and associated microbial communities. This study investigated the microbial composition of Scots pine trees exhibiting decline symptoms in a forest near Sopron, Hungary, and compared them with neighbouring non-symptomatic individuals. In addition, remote sensing data based on Normalised Difference Vegetation Index (NDVI) were used to assess vegetation dynamics within the study area.

Microbial communities were analysed using both shotgun metagenomic and 16S rRNA amplicon sequencing approaches. The results showed that community composition was primarily structured by tissue type rather than tree health status. Soil samples exhibited the highest diversity, while needle and trunk-associated communities were more constrained. Symptomatic samples showed a decrease in taxon richness, although overall community composition remained relatively stable. Specific taxa, such as members of the Acidobacteria phylum, were reduced in symptomatic samples, while some opportunistic and low-abundance taxa showed slight increases.

Remote sensing analysis revealed a clear reduction in NDVI values around 2021, indicating decreased vegetation vigour. This pattern was consistent across multiple datasets and supported by spatial and temporal analyses.

The combined results suggest that tree decline in the study area is associated with subtle microbial shifts and measurable changes in vegetation condition. These findings highlight the potential of integrating microbiome analysis with remote sensing approaches to better understand forest health dynamics.

Table of Contents

1. Introduction

Forests constitute one of the most crucial ecosystems on our planet, with a significant impact on the quality of life of millions of species, including that of our own - *Homo sapiens*. Being one of our planet's largest carbon sinks, forests represent a powerful shield against the constantly increasing threats of global warming. Studies show that if anthropogenic impact were not a factor in the forests' health, they could store 226.00 billion tons of carbon more than they currently do (Mo et al., 2023). Apart from being key players in carbon sequestration and harbouring a large proportion of Earth's biodiversity, forests also serve as the main source of income for approximately 1.6 billion people worldwide (Cherinet & Lemi, 2023). Therefore, ignoring threats to forests could prove fatal not only in the biological and ecological systems, but also in the environmental and economic aspects of our lives.

Unfortunately, forests continue to be subject to stress and disease caused by various pathogens, pests, and anthropogenic disturbances. One such example is pine wilt disease (PWD) - an ongoing threat to pine trees across several countries in Europe, Africa, Asia, and North America (Proença et al., 2017). Although the primary cause of the disease has been attributed to the pinewood nematode (PWN), *Bursaphelenchus xylophilus* (Proença et al., 2017), its underlying mechanism is thought to be much more complex than a simple cause-effect relationship. An array of interactions involving the genetic makeup of both the causative agents and the affected trees and their soils, as well as their microbiome composition, may play a part in the disease's progression or stagnation (Hou et al., 2025). Moreover, insects which may serve as disease-transmitting vectors add further intricacy to this multiplex system. External factors such as natural disasters, wildfires, climate change, and the human factor may also contribute to the outcome of the disease in a synergistic fashion (Lee & Kim, 2025; Zou et al., 2024; Liu et al., 2022).

In recent years, the prevalence of coniferous forests experiencing pine tree decline has also increased in regions of Hungary. This study focuses particularly on a forested region near the Hungarian city of Sopron. Here the local pine species - Scots pine (*Pinus sylvestris* L.) - has been visibly experiencing decline, with symptoms similar to those of PWD. These trees may serve as hosts to numerous microorganisms, which can be both beneficial for the plant

and detrimental. An imbalance in the composition of the microbial communities may promote plant disease (Halifu et al., 2026). Therefore, the emphasis is put on analysing the microbiomes in the affected trees and the associated soils.

Metagenomic sequencing and analysis approaches help shed light on the microbial genomes present in the affected host trees. On a landscape or regional scale, remote sensing techniques are useful in detecting and monitoring forest condition using satellite- and aircraft-generated data, providing insight into the forest health at the canopy level. As the cause and mechanism behind the Scots pine decline in the Sopron forests is not yet fully understood, we perform a comparative metagenomic analysis of the microbial communities present in symptomatic and non-symptomatic trees and soils, and we utilise satellite imagery to examine canopy stress in the region near Sopron.

We aim to identify the microbial species that may be associated with either plant health or disease-promoting activity, and to determine whether a correlation exists between the microbial communities and the vegetation health indicators derived from remote sensing.

2. Literature Review

2.1. Forest ecosystems' structure and function

2.1.1. Definition and distribution

According to the Food and Agriculture Organization of the United Nations (FAO, 2020), forests are terrestrial zones greater than or equal to an area of 0.5 hectares, predominantly composed of tree species capable of reaching a minimum height of 5 metres, and a canopy (crown) cover of at least 10% of the total land area. Furthermore, forests are vegetated regions that are not in use for agricultural or urban-related purposes. However, forest definitions may vary between countries. For example, in Hungary, as defined in Act No. XXXVII of 2009 on forests, on the protection and management of forests, forests are defined more strictly, generally requiring a minimum area of 0.5 hectares, tree heights exceeding 2 metres, canopy cover of at least 50%, and a minimum average width of 20 metres. (Tobisch & Kottek, 2013).

Based on their geographical location (latitude) and climate zone, forests can be broadly classified into distinct types, with the most widespread ones being tropical, subtropical, temperate, and boreal forests (*Figure 1*). Additional criteria such as the dominant species may further subcategorise these ecosystems into rainforests, deciduous forests, coniferous forests, and dry forests. *P. sylvestris* - the focal species of this study - is characteristic of the boreal and temperate coniferous forests.

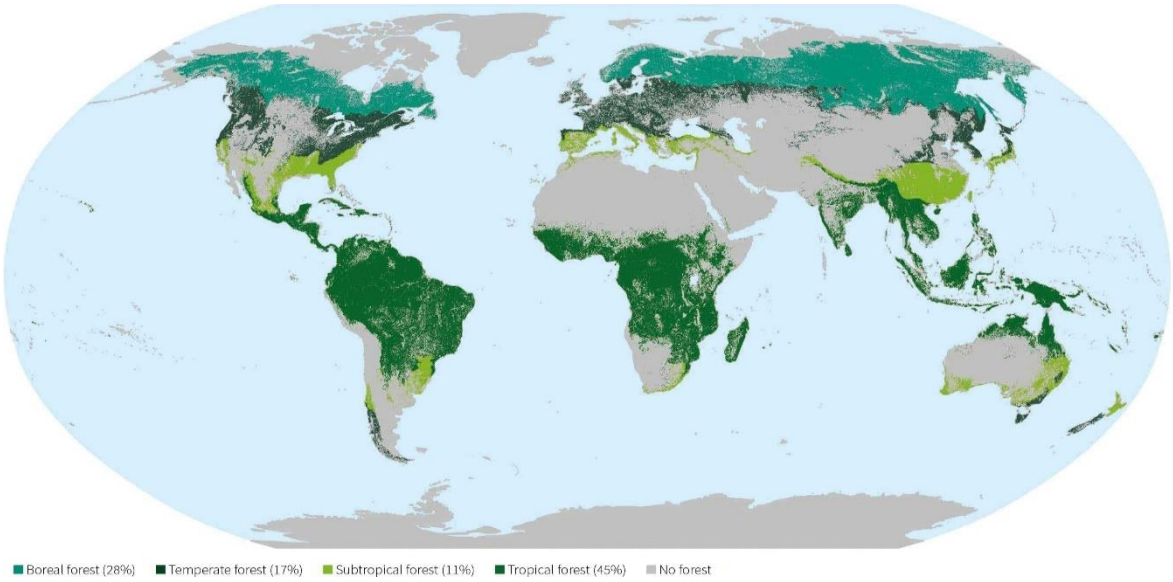


Figure 1: Global distribution of forest types by climatic domain (Source: FAO, 2025)

2.1.2. Layer composition

A unique feature of all forest types is their **vertical stratification** – a special division of forests into layers corresponding to different levels of height, and consequently, different species composition (Juyal et al., 2022). At the very bottom, farthest from sunlight, lies the **forest floor**, sheltering diverse communities of insects, fungi, bacteria, and decomposing organic matter. Still with restricted access to sunlight, the next layer is the **herb layer**, consisting of shade-tolerant soft-stemmed plant species, such as grasses, ferns, and mosses. A moderate amount of light reaches the **shrub layer** and the **understory layer**, which are made up of low-set woody plants and immature small trees, respectively. The **canopy layer** sits higher up, resembling a roof-like structure made up of tree crowns readily exposed to sunlight. It is worth noting that not all layers are present in all forest types; some forest types may also have an **emergent layer**, with trees usually reaching a height of 50-70 metres from the ground, surpassing the canopy (Thiel et al., 2021). The presence of these distinct layers enables millions of species across different taxa to find suitable habitats in forest regions, making them biodiversity hotspots. In this study, modern sequencing technologies and remote sensing approaches are integrated to investigate both the forest floor and canopy layers within the Sopron forest study site.

2.1.3. Function and importance

Forest ecosystems represent intricate natural systems made up of all the interacting biotic (living) and abiotic (non-living) components within a forest (Lee et al., 2025). Members of diverse biological groups, including animals, plants, bacteria, fungi, and archaea, interact directly and indirectly with one another as well as with abiotic factors like water, air, soil, temperature, and chemical compounds. The collective balance of these interactions maintains overall ecological stability in the Earth's ecosystems through processes like carbon sequestration, oxygen production, and climate regulation (Aerts & Honnay, 2011). At the biological level, carbon sequestration occurs when carbon dioxide is converted to biomass through photosynthesis in chlorophyll-rich plants (Brockerhoff et al., 2017). Forests absorb carbon dioxide and store it in plant biomass and soils, exceeding the amount they release through processes like respiration and decomposition. As a result, they are considered carbon sinks – a key feature in the mitigation of climate change, which is largely driven by heat-trapping carbon dioxide in the atmosphere (Kabir et al., 2023).

Another essential process forests participate in is nutrient cycling. Nutrient cycling refers to the movement or circulation of nutrients between organisms, the soil, and the atmosphere. The importance of this process lies in the reuse of key nutrients including nitrogen, phosphorus, and potassium, and in the minimisation of their loss (Balbinot et al., 2024). Forests provide suitable conditions for efficient nutrient cycling, as they readily allow movement of nutrients from the soil to the growing plants, and then with the help of microorganisms, from the dead decomposing plants back to the soil. The efficiency of this process ensures that the soil in the forests is fertile and promotes plant health (Shi et al., 2024).

Aside from ecological and biological impacts, forests constitute a major part of the global economy, particularly in developing countries in the continent of Africa, where forest-dependent industries are a major source of employment. For instance, in Ethiopia, one of the largest sources of income is the sale of forest-generated products like resin, gum, charcoal, medicinal plants, forest foods, and construction materials (Cherinet & Lemi, 2023).

2.2. Pine forest ecology and distribution

Based on their reproductive anatomy, all seed-bearing plants can be divided into one of the two major groups: angiosperms or gymnosperms, which exhibit different characteristics. In the broad sense, angiosperms are characterised by the presence of flowers and enclosed seeds, whereas gymnosperms are flowerless, with exposed or naked seeds (Yang et al., 2024). These differences are reflected in the ability of each group to thrive in distinct environmental conditions. For instance, many gymnosperms thrive in cold, dry, nutrient-poor environments (Zhang et al., 2017). Conifer trees are amongst the most abundant gymnosperm species, with predominance in the boreal and temperate zones, as well as high elevations in subtropical regions (Yang et al., 2024; Aiba, 2016). Their cone-bearing reproductive anatomy and their evergreen needle-resembling leaf structure make them ideally fit for the environments in those zones. Pine trees constitute one of the largest genera of coniferous trees within the family of Pinaceae – *Pinus*, with more than 120 species worldwide (Kovach et al., 2010). While their native range mainly encompasses North America, Europe and Asia, there are also pine plantations distributed throughout the Southern Hemisphere (*Figure 2*). Europe hosts a mix of native and introduced species of varying abundance. Particularly in Hungary, the most common species are Scots pine (*Pinus sylvestris* L.) which is native, and black pine (*Pinus nigra* J. F. Arnold) which is mostly introduced via plantation (Móricz et al., 2018). Despite being ecologically resilient and well-adapted to their environments, these trees face growing threats to pests, pathogens, and environmental stressors.

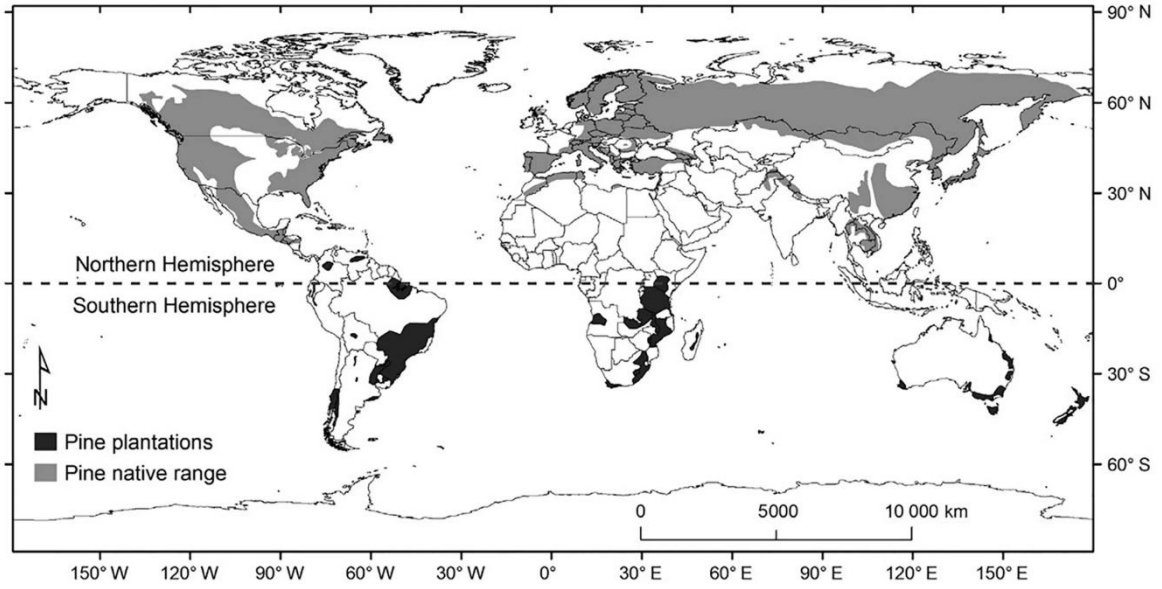


Figure 2: Global distribution of pine native range and plantations (Source: Lantschner et al., 2017; accessed via Hoeksema et al., 2020)

2.3. Pine forest decline and emerging threats

Although reports from the Global Forest Resources Assessment (FAO, 2025) show the general deforestation rate has started to globally slow down, there is still a pine forest decline trend affecting forests at a global scale (Pimentel et al., 2025). The most frequently encountered culprits behind this decline are pathogens like pinewood nematodes (*Bursaphelenchus xylophilus*), invasive seed bugs like *Leptoglossus occidentalis*, bark beetles, and several pathogenic bacteria and fungi (more detail in section 2.4.3)(Luchi et al., 2020). Abiotic stress manifested through climate change, wildfires, drought, storms, and anthropogenic activities like urbanisation and logging further exacerbate the negative impacts on forest health (Knutzen et al., 2025; Gea-Izquierdo et al., 2019). For instance, a forest exposed to a natural disaster may be more susceptible to pathogen invasion. Additionally, forests subject to anthropogenic land alteration may consequently suffer reduced nutrient cycling and carbon sequestration efficiencies, contributing, over time, to global warming, which in turn would bring about pathogen-favourable conditions like drought and wildfires. When the damaging impact caused by these factors is greater than the resilience capacity of the forests, decline occurs (Vásquez-Grandón et al., 2018). Therefore, considering all these factors as a whole, rather than individually, is crucial in establishing

forest-protective methods (Pimentel et al., 2025). Unfortunately, several pine species continue to be exposed to these stressors, with some examples provided in the next subsections.

2.3.1. Pine wilt disease

One of the most well-known pine diseases to date is pine wilt disease (PWD), which was first observed in the Japanese city of Nagasaki, in 1905, albeit the causal agent was revealed only 66 years later, in 1971 (Mamiya, 1983; Zhu et al., 2012). The affected trees exhibit visible symptoms including brownish or reddish needles, and stem shrinkage.

B. xylophilus, also known as the pine wood nematode, is a parasitic roundworm species native to North America. Notably, its activity is not linked with high levels of pathogenicity in its native range. However, when introduced to other countries, it causes the lethal pine wilt disease. Although many pine species are vulnerable to its invasive mechanism, there are species like stone pine (*P. pinea* L.), which appears to be resistant to this nematode's harm, even when it is present at high densities (European and Mediterranean Plant Protection Organization [EPPO], 2026). This might be related to the efficiency of defence mechanisms of the host species.

P. sylvestris is one of the species severely damaged by this pathogen, which leads to their mortality. The first occurrence of pine wilt disease in Europe was in 1999, in Portugal, subsequently being detected in Spain and Madeira in 2008. Today, strict phytosanitary measures like quarantine regulations, monitoring programs, and immediate removal of affected trees are used to prevent the spread of the disease (EPPO, 2026).

2.3.2. Dothistroma needle blight

Another disease frequently affecting pine ecosystems is Dothistroma needle blight, caused by two pathogenic fungal species: *Dothistroma septosporum* (Doroguine) Morelet and *D. pini* Hulbary (Mullett et al., 2021). These pathogens infect the needles of numerous pine species, inducing a type of cell death known as foliar necrosis. The infected trees experience reduced growth, premature needle cast, and in some cases, mortality. While *D. septosporum*

is globally distributed, present throughout a wide range of pine forests and plantations, *D. pini* is more spatially restricted, with distribution in parts of North America and Europe.

Although the exact date of the first incidence of this disease is not known, dendrochronological evidence suggests its presence in Canada as early as the 19th century (Mullett et al., 2021). Later, in the middle of the 20th century, the disease resulted in one of the largest outbreaks in pine plantations in the Southern Hemisphere, resulting in significant financial losses. Since then, the disease has been showing an increase in prevalence in the Northern Hemisphere, primarily affecting Canada, the United Kingdom, and France. In contrast to *B. xylophilus*, which is considered a Quarantine Pest (see previous section), *D. septosporum* and *D. pini* are considered Regulated Non-Quarantine Pests in Europe (Mullett et al., 2021).

2.3.3. Pine decline in Hungary

Located in Central Europe, Hungary has a varied landscape that supports high biodiversity. A major part of the country is constituted by the Carpathian Basin which is an ecologically rich lowland surrounded by mountains on its sides (Matzarakis & Gulyás, 2006). Being in the temperate climatic zone and characterised by a continental climate, Hungary is home to diverse plant species occurring in forests and grasslands. Scots pine (*P. sylvestris*) is one of the native pine species in the region; however, it is already relict in some areas such as Fenyőfő (Horváth et al., 2022). This is believed to result from the combined consequences of elevated temperatures, aridity, and pests like bark beetles. Black pine (*P. nigra)* is a relatively drought-tolerant pine species in Hungary that was introduced in the 19th century. It was proposed as a good substitute for the drought-sensitive Scots pine. However, later studies conducted in *P. nigra* show that it is experiencing growth decline caused by severe drought and a subsequent pathogenic fungal infection (Móricz et al., 2018). In a forest condition assessment by the International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests (ICP Forests), in 2024, it was found that fungal infection was present in 50% of all the black pines that were sampled and assessed (Michel et al., 2024). This observation further emphasises the need to explore ways to mitigate and prevent this tree damage.

2.4. Role of microorganisms in plant health and disease

When studying plant health and disease diagnostics, it is crucial to acknowledge the fact that plants are not isolated entities; instead, they are hosts to taxonomically diverse microorganisms, including bacteria, archaea, fungi, viruses, nematodes, and protists. Together with these microorganisms, the plant forms a so-called **holobiont** – a collective system of intricately interacting species (Vandenkoornhuyse et al., 2015). The collection of all the present microorganisms in a specific environment, such as plant tissue or a compartment, is called a **microbiome**.

2.4.1. Microbiome structure

There are three main environments in a plant where these microorganisms may reside: the rhizosphere, the phyllosphere, and the endosphere (Santoyo, 2025).

The **rhizosphere** is associated with the roots of the plant and constitutes the soil that is in direct contact with them. This root-associated compartment is further divided into the following subregions: endorhizosphere, rhizoplane, ectorhizosphere, and bulk soil (Gouda et al., 2018). The **endorhizosphere** contains the microbes residing inside the root tissues. **Rhizoplane** refers to the outer surface of the roots. The **ectorhizosphere** is the soil section immediately surrounding the roots, and **bulk soil** is the soil that is not in direct contact with the roots. The rhizosphere region is rich in microbial species that usually promote plant growth through key processes, such as nitrogen fixation, nutrient solubilisation, and phytohormone production, which will be discussed in more detail in the next subsection.

The **phyllosphere** corresponds to all the superficial areas of the plant that are above the ground, including leaf surface, stem surface, and reproductive structures such as flowers and fruits (De Mandal & Jeon, 2023). Although it is less suitable as a habitat for microorganisms than the rhizosphere, due to exposure to abiotic factors like sunlight, storms, drought, and wind, it still contains a significant amount of microbes. The microbes in this region are often involved in protection of the plant from the aforementioned abiotic stressors, including UV light and desiccation (De Mandal & Jeon, 2023). These microbes are also thought to have a protective role against plant pathogens, serving as a line of defence, or biocontrol against

plant diseases. Therefore, phyllosphere microbiome composition is an essential area of research in plant disease diagnostic and treatment studies.

Lastly, the **endosphere** represents all the regions beyond the surface – the inner parts of the plant. It can be further classified into root endosphere, stem endosphere, leaf endosphere, and fruit endosphere (Zhang et al., 2023). The inhabitants of the endosphere are known as **endophytes**, which are organisms living inside the plant. The diversity of microorganisms living in the endosphere is lower than in the other regions, as they are directly subject to plants' immune system, therefore they are selectively filtered (Zhang et al., 2023). The microorganisms in this region are also related to crucial processes like plant growth and protection. In this study we focus on the rhizosphere (bulk soil) and endosphere (needle and trunk tissue), through metagenomic and 16S rRNA amplicon sequencing analyses.

Finally, the microbial composition of a plant is not random, but rather shaped by evolutionary and phylogenetic constraints (Trivedi et al., 2022). In other words, the complex interactions between the host plant, the surrounding environment, and the microbes themselves determine which microorganisms persist in the plant and which do not. Moreover, the microbiome in all of these plant compartments is important in the well-being of a plant, and their efficiency is based on the balance and composition of the microbial species. Disturbance of microbial composition in plants – also known as **dysbiosis** – is often related to plant disease and reduced fitness (Arnault et al., 2023). Furthermore, although some microorganisms are beneficial and crucial for the plants, there are also pathogenic ones whose presence may cause harm; the next sections provide some examples of each group.

2.4.2. Beneficial microorganisms and their functions

For a plant to be healthy, several processes must be running smoothly. Those processes include nutrient and water uptake from the soil, photosynthesis in the leaves, and protection and tolerance against biotic and abiotic stressors. A successful outcome of all these processes depends on the complex interactions between the host plant and its microbiome, as well as the environmental conditions. These interactions are often mutualistic in nature, i.e. all organisms involved benefit from the relationship (Tharanath et al., 2024). In this case, plants benefit from facilitated water acquisition and protection from pathogens and biotic or abiotic

damage, whereas the microorganisms have a nutrient-rich habitat in the plant. Some microbiome species are always present in the same plant species, across different samples – these are referred to as the **core microbiome** (Grzyb & Szulc, 2024). As their functions are essential for the plant, they are consistently present in it. For instance, members of Alpha-proteobacteria, belonging to the order of Rhizobiales, and genus of *Sphingomonas*, are indicated to be part of the core microbiota for pine species (Addison et al., 2023). From the fungal taxa, members of the *Phaeotheca spp.* (class of Dothideomycetes), and *Phaeococcomyces spp.* (class of Eurotiomycetes), were shown to be abundant in *Pinus radiata* (Addison et al., 2023). Some species may have an enormous impact even at low abundance – these are called **keystone species** (Grzyb & Szulc, 2024). For example, members of the *Desulfosporosinus* genus exert a crucial function in sulfate reduction in certain wetland soils, while constituting only 0.06% of the total microbiome community (Banerjee et al., 2018). Some taxa are intensively involved with many of the surrounding species, being at the centre of these interactions – these are called **hub taxa** (Grzyb & Szulc, 2024). Usually, the most dominant taxa in plant core microbiota are bacteria and fungi.

Enhanced nutrient uptake is one of the most important processes in which microorganisms are key players for the plant. Examples include nitrogen fixation, phosphate solubilisation, and mycorrhizal activity (Richardson et al., 2009).

Nitrogen fixation is a process that converts nitrogen gas (N_2) into ammonia (NH_3) (Barakat et al., 2016). This conversion is vital for plants, as plants can only utilise ammonium (NH_4^+) and nitrate (NO_3^-), which are derived from nitrogen fixation and subsequent transformations in the soil, for plant growth and development. The microorganisms carrying out this conversion can be both symbiotic and free-living species in the soil. Examples of such organisms include members of the *Rhizobium* and *Azotobacter* genera, which inhabit the root nodules (primarily in legumes) and the rhizosphere soil, respectively (Aasfar et al., 2021).

Mycorrhizae are a special group of fungi, found in the root regions, where they mediate nutrient and water exchange between the roots and the soil, and in return they get the carbohydrates from the host plant (Kuo et al., 2014). They can be arbuscular mycorrhizae

(AM), with structures inside the roots, or ectomycorrhizal (ECM), which have a sheath around the roots. Members of the phylum Glomeromycota are representatives of the AM group, whereas most common ectomycorrhizal fungi are Basidiomycota and Ascomycota (Kuo et al., 2014). The latter ones are especially important in pine species, which they help grow in nutrient-poor and challenging soil environments.

Phosphate is another essential nutrient for plant growth, involved in processes like energy transfer (in the form of adenosine triphosphate), nucleic acid synthesis, as well as root, flower, and seed formation and development (Bakki et al., 2024). However, it is often found in insoluble form in the soil. Phosphate solubilisation, similar to nitrogen fixation, is carried out by root- and soil-habiting microorganisms, which release phosphatase enzymes and organic acid compounds capable of dissolving mineral phosphate compounds into plant-available phosphorus ions. Key genera in this process include *Pseudomonas* and *Bacillus* (Bakki et al., 2024).

Hormonal regulation is also essential in plant growth, and microbes are key players in the production of many of these hormones, including phytohormones, auxins, gibberellins, cytokinins, and many more (Teiba et al., 2023). All of these hormones are involved in growth-regulating processes, essential for plant health, and many of them are produced by microbial communities which are commonly referred to as plant growth-promoting microorganisms (PGPM). Examples include members of the bacterial genera: *Azospirillum*, *Azotobacter*, *Pseudomonas*, *Bacillus*, *Microbacterium*, *Rhizobium*, and *Mycobacterium*. *Trichoderma* is also one of the most well-known fungal genera involved in phytohormonal plant growth promotion (Teiba et al., 2023).

Protection from unwanted pathogens is also critical to maintain a healthy state for plants. The beneficial microbes aid in this by competing with the pathogenic bacteria for the same resources, by producing pathogen-specific antimicrobial compounds, and by stimulating the host plant immune responses (Fuller et al., 2023). These actions combined constitute **biocontrol**, and this is an active area of research in plant pathology. *Pseudomonas*, *Bacillus*, *Burkholderia*, and *Streptomyces* are amongst the bacterial genera frequently associated with

these features, whereas *Trichoderma* and *Penicillium* represent the fungal genera contributing to these protective actions (Fuller et al., 2023).

Despite the immense benefits of the beneficial microbes, there are pathogenic ones which may often disrupt these well-organised complex interactions, compromising the plant's health. Unveiling their mechanisms could help in developing effective biocontrol strategies against them.

2.4.3. Pathogenic microorganisms and their effects

Some microorganisms can be pathogenic to plants, impeding normal growth, causing sickness, and consequently the death of the plant. However, this disease outcome depends on three factors: the virulence of the given pathogen microbe, the vulnerability of the host plant, and the suitability of the environmental conditions. These three factors in literature are often referred to as the **plant disease triangle** (Klopfenstein et al., 2009).

The environmental conditions are important in determining the outcome of the infection. Certain pathogens require certain environmental conditions to survive, thrive, and subsequently infect a plant (Klopfenstein et al., 2009). Moreover, unfavourable environmental conditions to the plant, such as drought, storms, or anthropogenically caused damage, may weaken the plant defence system, making them more sensitive and vulnerable to pathogen infections, opening ways to disease and decline.

In the previous subsection, bacteria and fungi were amongst the most dominant taxa in plant-beneficial microorganisms. A similar pattern is observed here as well, however, with viruses, nematodes and protists following in importance (Agrios, 2009).

Plant-pathogenic fungi are known to cause a range of diseases, like root-rot, cankers, and general forest decline. The usual taxa behind these diseases are *Armillaria* species, *Botryosphaeria*, *Diplodia*, *Phytophthora* spp., *Cronartium ribicola*, and many others (Sturrock et al., 2011). Amongst the pathogenic bacteria, several taxa are well known as plant pathogens, including species from the genera *Pseudomonas*, *Ralstonia*, *Agrobacterium*, *Xanthomonas*, *Erwinia*, *Xylella*, *Dickeya*, and *Pectobacterium* (Adioumani

et al., 2022). Pathogenic viral infections are less common in forest ecosystems, compared to fungal and bacterial ones. Cherry leaf roll virus and tobacco rattle virus are amongst the most encountered ones in woody plants in forests (Rumbou et al., 2021).

Some members of the phylum Nematoda are amongst the most abundant plant pathogens; they are parasites which cause disease and death in plants by feeding on the plant tissues. For instance, *B. xylophilus*, the pine wood nematode, which mainly disrupts the water circulation system in plants, more specifically, in the plant's vascular tissues, xylem (Umebayashi & Fukuda, 2025). They may also reside in parenchymal cells of the plant and the resin canals, impeding proper water transport and wilting the plant (Umebayashi & Fukuda, 2025).

As there are plant-associated microorganisms that can be both health-promoting and disease-inducing, understanding the microbial communities in a plant, and identifying their associations with plant health or disease, is crucial in finding disease-preventative methods and suitable treatments. Advanced sequencing technologies are powerful tools that allow us to make this identification at the genetic level.

2.5. Metagenomic approaches for studying microbial communities

Traditionally, studying microorganisms was done through culturing and growing them on media, in laboratory conditions. This method is still in use today; however, it has a few caveats. Many microorganisms are fastidious, meaning they have specific requirements when it comes to the media they can grow in, and it is not feasible to meet their growth requirements in vitro. Additionally, they are often outgrown by fast-growing microbes. These limitations are overcome by metagenomics technologies, which can extract DNA and sequence it in situ, without the need for culturing. The DNA sequenced here is a collection of the genetic material found in the sample taken, and it can correspond to several different species and organisms (Pérez-Cobas et al., 2020).

Metagenomics relies on next-generation sequencing (NGS) methods which are high-throughput and allow for millions of DNA fragments to be sequenced at once. The most common platforms include Illumina sequencing, Oxford Nanopore Technologies, and

PacBio (Macip et al., 2025). Illumina provides short reads of high accuracy, whereas the other two provide longer reads, suitable for studying whole genomes.

Two well-known metagenomics sequencing approaches include amplicon sequencing and shotgun sequencing. In amplicon sequencing, only a certain region of interest is sequenced. This region is determined by the primers used, and usually it is selected so that it comes from conserved and highly variable regions, that allow for universal primer use and species identification, respectively. For instance, the 16S rRNA gene in bacteria is a common marker for this purpose, as it has both conserved regions which are present in all bacteria, and highly variable regions which differ from species to species (Macip et al., 2025). Similarly, in fungi, internal transcribed spacer (ITS) regions serve the same purpose, as highly variable regions that are flanked by regions that are conserved throughout all fungi members (Pérez-Cobas et al., 2020). In shotgun sequencing, the whole genetic material in the sample is sequenced, therefore giving us more information regarding the species and their genetic composition (Pérez-Cobas et al., 2020). While 16S rRNA gene sequencing tells us which species or genera are present, shotgun sequencing tells us which genes and associated functions they have. Both methods are important in analysing microbial communities in plants, especially when identifying microbial communities in healthy and diseased plants.

The raw data obtained from the sequencing process are then processed before being used for analysis. The processing steps include quality control and, if necessary, trimming, to remove low-quality, adapters and erroneous parts. In cases of amplicon sequencing, after filtering, similar sequences are grouped or clustered together into operational taxonomic units (**OTUs**) or amplicon sequence variants (**ASVs**) (Chiarello et al., 2022). Then the processed data are compared against databases to assign taxonomic classification.

An important measure obtained through this part is the **relative abundance**, expressed in percentage (%), showing the percentage of sequences belonging to a certain taxon in a certain sample. This uncovers which taxa are more dominant in a sample. Two other crucial measures are alpha and beta diversity.

Alpha diversity corresponds to the diversity of the species found within one sample, and it sheds light on two parameters: richness, and evenness, which are measured through, for

example, the **Shannon index** (Kers & Saccenti, 2022). The Shannon index describes how many species are present, and how evenly they are distributed in the sample. Another measure of alpha diversity is also the **Simpson index**, which reveals which species are dominant, if there are any, by measuring the probability that two random individuals in each sample belong to the same species (Kers & Saccenti, 2022). Alpha diversity helps determine the complexity of the microbial community in a sample.

Beta diversity, on the other hand, shows how diverse the microbial communities between two samples are (Kers & Saccenti, 2022). For instance, it could help us compare the microbial communities in a non-symptomatic and a symptomatic tree sample. Examples of beta diversity measures include **Bray–Curtis dissimilarity** and **UniFrac distance** (Kers & Saccenti, 2022). The former measures the difference of the species and their abundance between two samples, whereas the latter measures the difference of microbial communities between two samples taking into account their evolutionary relationships.

All these approaches are helpful in plant health and disease studies, particularly in studying the aetiology of a disease, as they allow for detailed identification and comparison of the microbiomes of the pathogen-affected and unaffected samples.

2.6. Remote sensing for forest health monitoring

Remote sensing involves obtaining information about our planet from a distance – without direct contact, usually using satellites, drones or aircraft (Lechner et al., 2020). It is particularly advantageous as it allows us to monitor our planet at a broad scale, without physically disturbing the environment. Moreover, it can provide us with information for the same location at different times, allowing us to track changes as a function of time. Early detection of change is also possible with remote sensing – which is highly useful in forest health and management studies (Pontius et al., 2020). For instance, physiological changes in stressed trees are detected by sensors before they appear to be symptomatic to the human eye.

Among the available approaches, optical satellite data are widely used for assessing vegetation condition. In this study, data from Copernicus Programme satellites, specifically

Sentinel-2 and Sentinel-3, are utilised. These platforms provide consistent, high-frequency observations suitable for analysing temporal changes in vegetation.

Optical sensors rely on electromagnetic radiation, whose source is often the Sun. More precisely, the sunlight that reaches the vegetation is partially absorbed by the chlorophyll pigments in plants for photosynthesis. As plants do not absorb at all wavelengths of light, they reflect some of it – this reflected light is detectable by sensors such as satellites, drones, and aircraft. The wavelengths of absorbed, reflected, and emitted radiation by plants are unique and they comprise the spectral signature of vegetation areas, and as such can be used to determine vegetative zones. Plants absorb red (0.600-0.700 μm) and blue light (0.400-0.500 μm), while reflecting the green (0.500-0.600 μm) and near infrared light (NIR) (0.700-1.300 μm) – hence the green colour in their leaves (NIR is not visible to the human eye) (*Figure 3*). As the primary factor controlling leaf reflectance in the NIR wavelength range is the cellular structure of the plant (the mesophyll), NIR reflectance is significant as it provides insight into internal leaf structure.

To mathematically measure the vegetation state, there are vegetation indices which help determine the greenness and health of a certain vegetated area (Lechner et al., 2020). The most commonly used index is the normalised difference vegetation index (NDVI) which is obtained by dividing the difference between the reflected NIR and the reflected red light (R) with their sum (Boiarskii & Hasegawa, 2019), as shown by the formula (1):

$$\boldsymbol{NDVI = (NIR - R)/(NIR + R)} \quad \textbf{(1)}$$

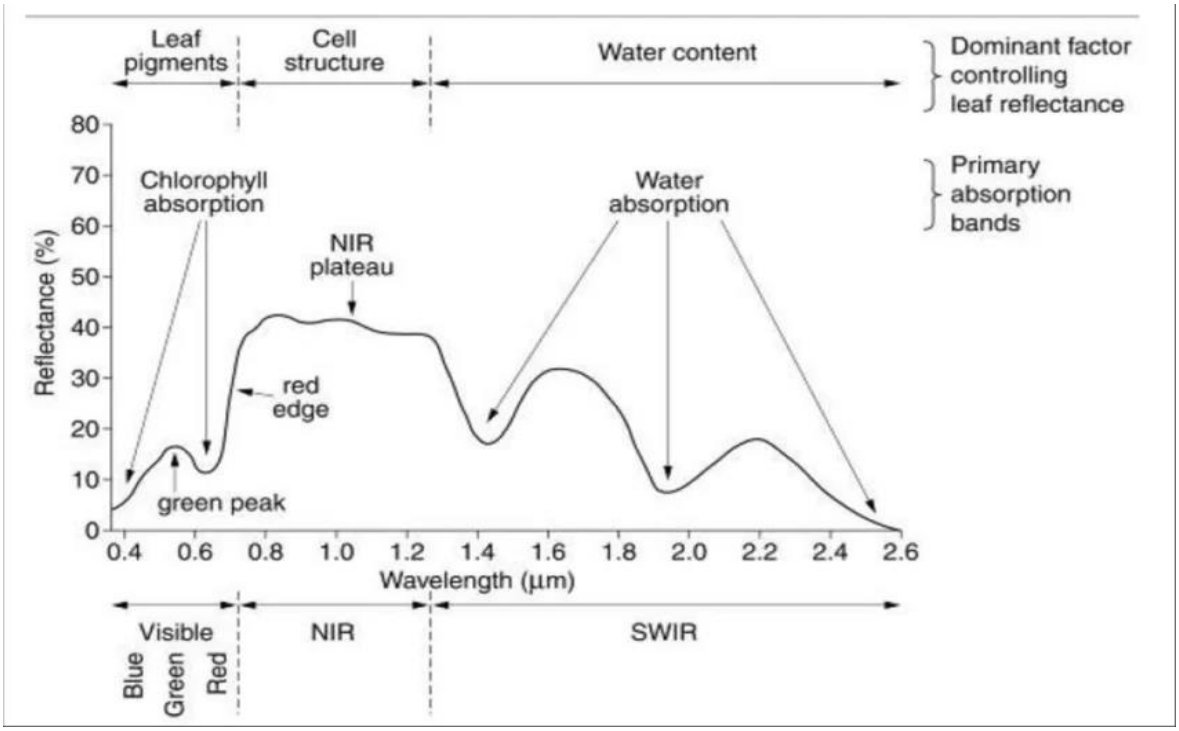


Figure 3: The typical vegetation spectral reflectance curve (Source: Keyworth et al., 2009)

In forestry, remote sensing is crucial to obtain data for vegetated areas subject to stress, drought, or disease. Damaged and drying plants are associated with lower NDVI values (Keyworth et al., 2009). Other indices used as stress markers include but are not limited to enhanced vegetation index (EVI), greenness index, moisture stress index (MSI), and modified soil adjusted vegetation index (MSAVI). Determining which index is more apt for a specific study depends on the sensitivity of the sensor being used (the detectable wavelengths) and the environmental conditions of the site of interest. For instance, an area with scarce vegetation would better be studied using soil-adjusted indices, which diminish background-generated reflectance (Pontius et al., 2020).

Temporal analysis of NDVI further enhances its utility, allowing the identification of anomalous years or periods of reduced vegetation performance. By comparing NDVI values across multiple years, it is possible to distinguish between normal seasonal variability and abnormal declines linked to environmental stress or disease events.

Although remote sensing data on its own cannot directly determine the cause of disease, as it primarily captures canopy-level characteristics, it provides valuable complementary information to sequencing and bioinformatics analyses.

In this study, NDVI derived from Sentinel-2 and Sentinel-3 data is used to assess spatial and temporal patterns of vegetation condition in Scots pine stands near Sopron. These remote sensing observations are integrated with microbiome data to explore potential relationships between canopy-level stress and microbial community composition.

3. Materials and Methods

3.1. Study area and sample collection

The pine tree samples were collected in a forest located in the Sopron-Fertőmellék District, in Hungary (47°39'29.7"N 16°33'21.5"E), on August 25th, 2021 (*Figure 4*). Some of the pine trees in this location were visibly affected by a disease, with some of them being already dead. The affected trees were exhibiting symptoms such as yellowing and wilting (*Figure 5*). We took samples from one non-symptomatic *P. sylvestris* tree (A) and its neighbouring symptomatic tree (B). Samples were collected from the soil (1), needle (2) tissues, and trunk tissues (3) into sterile containers, for both the symptomatic trees and their non-symptomatic neighbouring trees.

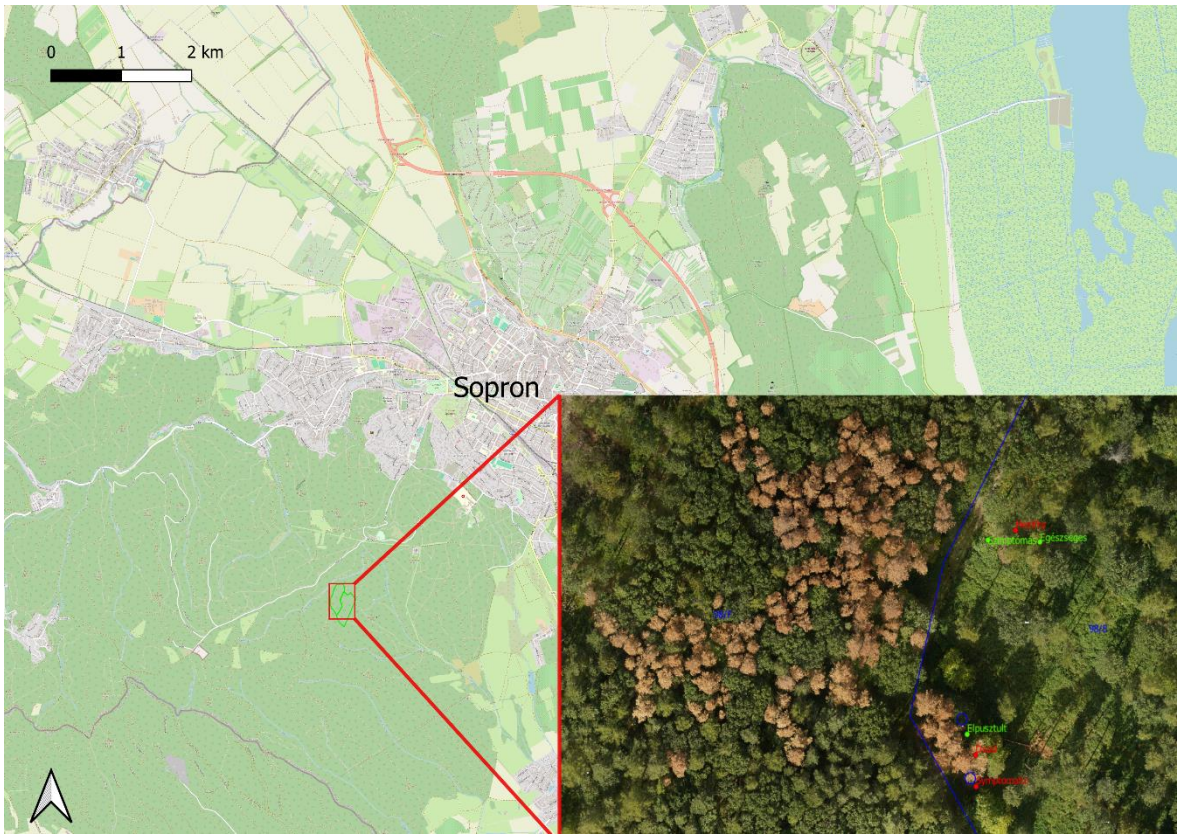


Figure 4: The location of the study site near Sopron

Figure 5: Non-symptomatic (A), symptomatic (B), and dead (C) pine trees in Sopron

3.2. DNA extraction and isolation

Sterile scalpels were used to scrape sample material from the tissues of interest. The samples were then immediately stored under 4 °C, until further processing. Subsequently, sterile mortar and pestle were used to homogenise 0.25 g of each sample into a powdery consistency, under liquid nitrogen.

DNA extraction and isolation was performed using the DNeasy® PowerSoil® Pro Kit from QIAGEN, following the manufacturer's protocol. This kit is efficient in extracting DNA material from environmental samples that are rich in PCR inhibitors. Upon extraction, DNA was then stored at -20 °C, to prevent unwanted degradation.

Subsequent processes including PCR, quality checking and sequencing were performed in the Hungarian Centre for Genomics and Bioinformatics, Szentágothai Research Centre, University of Pécs.

3.3. DNA sequencing and bioinformatics processing

Sequencing was performed at two levels, using shotgun metagenomic sequencing and 16S rRNA amplicon sequencing. Shotgun metagenomic sequencing was performed on trunk (A3, B3) tissues of *P. sylvestris* to characterise both eukaryotic and prokaryotic communities, whereas 16S rRNA amplicon sequencing was done on trunk (A3, B3), needle (A2, B2) and soil (A1, B1) samples to exclusively target the bacterial taxa across them.

3.3.1. Shotgun Metagenomic Sequencing

Shotgun sequencing was performed on the symptomatic and non-symptomatic trunk samples using the Illumina NovaSeq 6000 platform (San Diego, CA, United States) with paired-end reads of 150 nucleotides. NEB Next Ultra II DNA Library Preparation kit (Ipswich, MA, United States) was used for library preparation. FastQC (Andrews, 2010) and Trimmomatic (Bolger et al., 2014) were used to quality check, adapter removal and length filtering. Cleaned reads were aligned to the NCBI (Nt) database (Sayers et al., 2022) using the KMA aligner (Clausen et al., 2018) for taxonomic classification, followed by community profiling with CCMetagen (Marcelino et al., 2020). The resulting datasets were then classified into read count tables for both the eukaryotic and prokaryotic taxa, at different taxonomic levels, including phylum, family, genus, and species.

3.3.2. PCR for the Amplicon Data

The 16S rRNA gene region was amplified from soil (A1, B1), trunk (A3, B3) and needle (A2, B2) samples to assess the bacterial community composition. New England Biolabs (NEB) LongAmp™ Taq 2× Master Mix (Ipswich, MA, United States) was used for amplification, together with the universal bacterial primers 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') and 1492R (5'-TAC GGY TAC CTT GTT ACG ACT T-3').

PCR amplification was carried out with an initial denaturation step at 94 °C for 30 seconds, followed by 25 cycles of denaturation at 94 °C for 30 seconds, annealing at 61 °C for 30 seconds, and extension at 65 °C for 120 seconds. A final extension step was performed at 65 °C for 10 minutes, followed by a hold at 4 °C.

3.3.3. Amplicon Sequencing (16S rRNA sequencing)

The Oxford Nanopore MinION platform was used to perform 16S rRNA amplicon sequencing on soil (A1, B1) , trunk (A3, B3) and needle (A2, B2) samples from symptomatic and non-symptomatic trees, using a single-end long-read approach. Bioinformatic processing of the amplicon data was conducted externally.

The amplicon library was prepared using the Native Barcoding DNA Kit (SQK-LSK-109) (Oxford Nanopore Technologies, Oxford, United Kingdom), and sequencing was performed on the MinION Mk1B device. The resulting datasets were summarised into read count tables at the class level, covering six sample types:

• Non-symptomatic soil (A1)

• Non-symptomatic needles (A2)

• Non-symptomatic trunk (A3)

• Symptomatic soil (B1)

• Symptomatic needles (B2)

• Symptomatic trunk (B3).

3.4. Data Analysis and Statistics

3.4.1. Shotgun metagenomics data

Relative taxonomic abundance was calculated for both eukaryotic and prokaryotic datasets in non-symptomatic and symptomatic samples. The relative abundance of each taxon was determined by dividing the number of reads assigned to a given taxon by the total number of reads in the respective sample, multiplied by 100. Calculations were performed separately at different taxonomic levels, including phylum, family, genus and species.

All analyses and visualisations were carried out in R (version 4.5.1) (R Core Team, 2025). Circular and stacked plots were generated using the packages *tidyverse* (Wickham et al., 2019), *circlize* (Gu et al., 2014), *patchwork* (Pedersen, 2025), *RColorBrewer* (Neuwirth, 2022), *tibble* (Müller & Wickham, 2025), *stringr (Wickham, 2025b)*, and *forcats* (Wickham, 2025a), while alpha diversity analyses were performed using *tidyverse*, vegan (Oksanen et al., 2026), and *patchwork*.

3.4.2. Amplicon 16S data

Amplicon data analysis and visualisation were performed in R (version 4.5.1), using the packages *tidyverse*, *vegan*, *pheatmap* (Kolde, 2025), and *RColorBrewer*.

3.5. Remote sensing analysis

Remote sensing analysis was conducted to assess spatial and temporal variation in vegetation condition within the study area using Google Earth Engine.

Sentinel-2 surface reflectance imagery (COPERNICUS/S2_SR_HARMONIZED) was used to derive NDVI values for the study area (Drusch et al., 2012). Images were filtered by spatial extent and cloud cover (<20-30%, depending on the workflow). Cloud and shadow effects were reduced using quality masking approaches, including the Scene Classification Layer (SCL) and QA60 band. NDVI was calculated from the near-infrared (B8) and red (B4) bands.

Forest compartment boundaries were obtained from erdoterkep.nebih.gov.hu. The study area was defined based on forest compartments 98E and 98F, and further divided into affected and non-affected zones. The non-affected area was generated by excluding the affected region from the overall study area geometry (98E and 98F combined). This allowed comparison of vegetation condition across different spatial units.

To analyse vegetation dynamics, NDVI time series were generated for the full year 2021 across all regions. In addition, mean NDVI values for August 2021 were calculated for each region, representing peak vegetation conditions. These values were used to compare differences between compartments and between affected and non-affected areas.

Spatial patterns of vegetation condition were assessed by generating NDVI maps for August 2021. An NDVI anomaly map was also produced by subtracting the mean NDVI of a baseline period (2016-2020) from the August 2021 NDVI values.

To investigate temporal trends, a smoothed NDVI time series was generated for the affected area using a ±15-day moving window, reducing short-term variability. Furthermore, annual NDVI trends were derived using median composites for the main vegetation period (May-September) for each year between 2017 and 2026.

To complement the Sentinel-2 analysis, Sentinel-3 OLCI data (COPERNICUS/S3/OLCI) were used to assess interannual NDVI variability (Drusch et al., 2012). NDVI was calculated using radiance bands, and values outside the valid range (0-1) were excluded. Seasonal composites were generated for the peak vegetation period (July-August), using the 75th percentile to reduce the influence of outliers. Only years with at least five observations were included. Interannual variability was expressed as Z-score anomalies.

The Sentinel-3 results were compared with outputs obtained from the TEMRE platform (Somogyi et al., 2018).

NDVI raster outputs (GeoTIFF format) were exported from Google Earth Engine and further processed in RStudio. In R, raster data were converted into data frames and visualised using ggplot2. Final maps were produced by overlaying NDVI layers with compartment boundaries, and map elements such as scale bars, north arrows, and labels were added. Additional visual assessment of vegetation changes was conducted using historical imagery from Google Earth Pro (version 7.3.7.1155).

4. Results

4.1. Shotgun metagenomic results

After quality filtering and processing, a total of approximately 450,069 reads were obtained for the non-symptomatic samples and 471,298 reads for the symptomatic samples in the shotgun metagenomic dataset, indicating comparable and sufficient sequencing depth for downstream taxonomic analysis.

The shotgun metagenomic data revealed a slight but consistent shift in microbial community composition between non-symptomatic and symptomatic samples in both the prokaryotic and eukaryotic datasets (*Figure 6*). In the prokaryotic community, the dominant phyla across both conditions were Proteobacteria, Bacteroidetes, Actinobacteria, Firmicutes, Synergistetes, and Acidobacteria (*Figure 6A*). Low-abundance phyla (<1%) were grouped into the "Others" category. A noticeable difference was observed for Acidobacteria, which exhibited higher relative abundance in non-symptomatic samples compared to symptomatic samples.

For the eukaryotic dataset, after quality filtering, the metagenomic dataset comprised 2,010,039 reads for the non-symptomatic samples and 2,252,768 reads for the symptomatic samples. The majority of reads were assigned to host-derived sequences (Streptophyta), accounting for over 99% of the total reads in both samples.

After removal of host-derived sequences (Streptophyta) and normalisation, Ascomycota and Basidiomycota represented the dominant fungal phyla in both conditions (*Figure 6B*). In addition, Oomycota, Mucoromycota, and Ciliophora showed higher relative abundance in symptomatic samples, whereas Apicomplexa was detected exclusively in symptomatic samples, albeit at low abundance.

At the genus level, both datasets displayed broadly similar community structures between conditions, with several condition-associated shifts. In the prokaryotic dataset, *Bacteroides* was the most abundant genus (~35%), followed by *Klebsiella* (~15%), *Staphylococcus* (~10-13%), and *Cronobacter* (~6-7%) (*Figure 6C*). When comparing conditions, *Klebsiella*, *Staphylococcus*, *Cronobacter*, and *Acinetobacter* showed slight increases in symptomatic

samples, whereas *Bacteroides* and *Halomonas* showed slight decreases. A pronounced reduction was observed for unclassified Burkholderiales in symptomatic samples (~2.2% to ~0.6%). The “Others” category, representing low-abundance (<0.5%) genera, was slightly higher in non-symptomatic samples.

In the eukaryotic dataset, Adineta (~60%) and Rotaria (~19%) dominated in both symptomatic and non-symptomatic samples (*Figure 6D*). The remaining community consisted of lower-abundance taxa, including *Brachionus* (~4%). The most notable shifts included a substantial increase in *Brugia* in symptomatic samples and a marked decrease in *Armillaria*. *Malassezia* also showed increased relative abundance in symptomatic samples. Several taxa were detected exclusively in symptomatic samples, although some of these occurred at low relative abundances (<0.5%) and were not visually prominent in the plots. These included Obtectomera, *Stylonychia*, Sordariomyceta, and Apicomplexa, indicating the presence of condition-specific low-abundance eukaryotic lineages.

It should be noted that Caulimoviridae was detected in the dataset; however, as a viral taxon, it was excluded prior to downstream analyses and visualisation, due to the study focus on prokaryotic and eukaryotic communities.

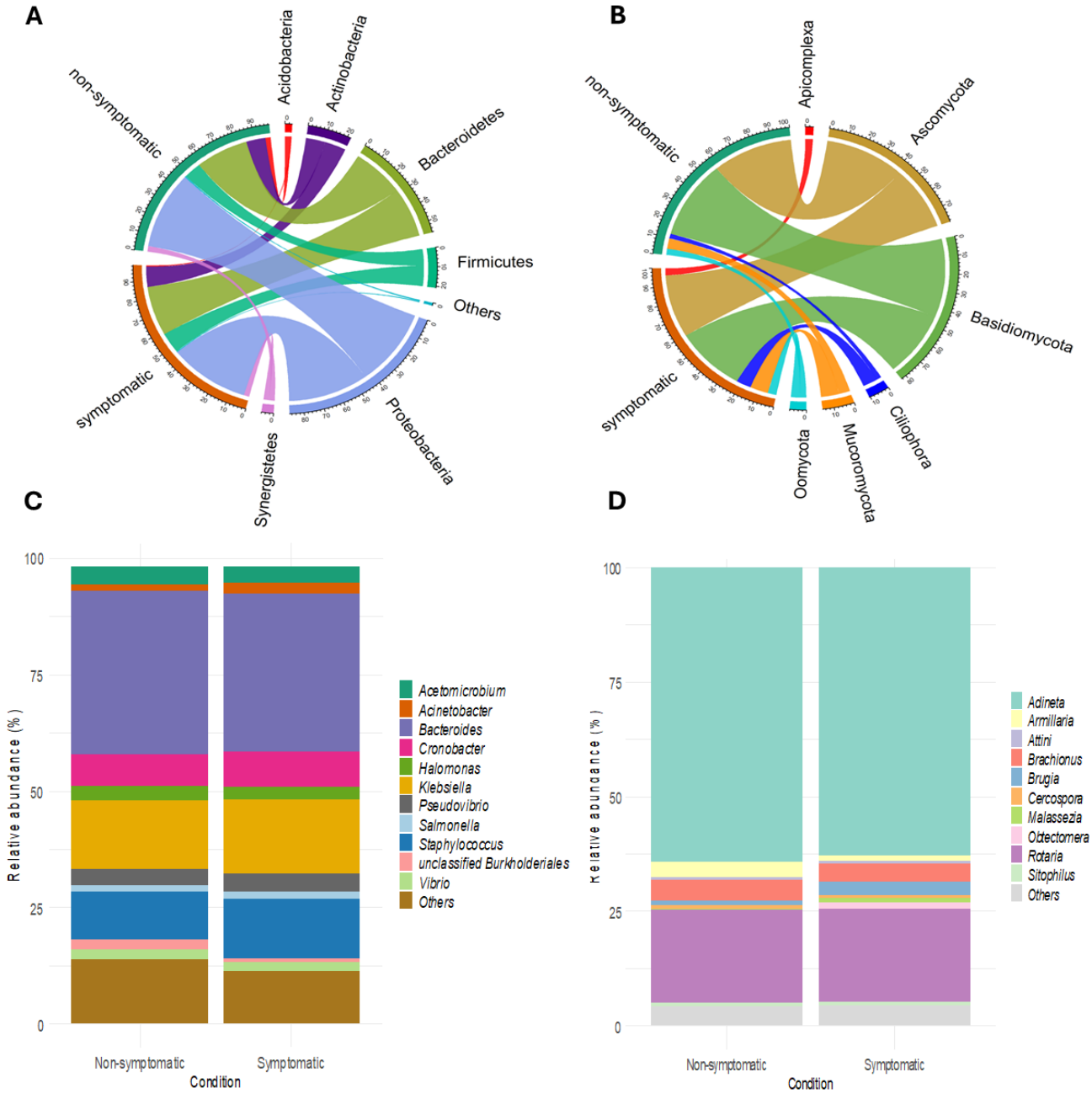


Figure 6: Taxonomic composition of shotgun metagenomic datasets from symptomatic and non-symptomatic Pinus sylvestris trunk samples. (A) Relative abundance of prokaryotic phyla. (B) Relative abundance of eukaryotic phyla, excluding host (Pinus sylvestris) sequences. (C-D) Stacked bar plots showing the relative abundance of the most dominant prokaryotic and eukaryotic genera, respectively, across symptomatic and non-symptomatic samples. The "Others" category represents low-abundance genera (mean relative abundance <0.5%).

Alpha diversity of metagenomic prokaryotic communities was assessed using Shannon diversity (H'), Simpson diversity (1-D), and species richness (S) (*Table 1*). Shannon diversity showed a slight decrease in symptomatic samples (H' = 2.40) compared to non-symptomatic samples (H' = 2.52). In contrast, Simpson diversity remained constant between conditions (1-D = 0.833 for both). Species richness was reduced in symptomatic samples (S = 39) relative to non-symptomatic samples (S = 53), indicating a lower number of observed taxa in infected samples.

In case of eukaryotic diversity, Shannon index showed a slight increase in symptomatic samples (H' = 1.47) compared to non-symptomatic samples (H' = 1.40) (*Table 1*). Similarly, Simpson diversity showed a minor increase in symptomatic samples (1-D = 0.607 vs 0.590). Species richness remained unchanged between conditions (S = 20 for both).

Community	Condition	Shannon Index (H')	Simpson Index (1-D)	Taxon Richness (S)
Prokaryotic	Non-symptomatic	2.52	0.833	53
Prokaryotic	Symptomatic	2.40	0.833	39
Eukaryotic	Non-symptomatic	1.40	0.590	20
Eukaryotic	Symptomatic	1.47	0.607	20

Table 1: Alpha diversity indices for the shotgun metagenomic prokaryotic and eukaryotic datasets

4.2. Amplicon sequencing results

Analysis at the class level for the amplicon dataset revealed that prokaryotic community composition varied across tissue types and subtly health conditions (*Figure 7*). Across all samples, the community was dominated by Gammaproteobacteria (33-36% depending on tissue), Actinobacteria (9-13%), Alphaproteobacteria (7-11%), Bacilli (5-8%), and Clostridia (3-6%).

Clear differences were observed between tissues. Soil samples showed a more diverse composition, with relatively higher contributions from classes such as Alphaproteobacteria (15-18%), Acidobacteriia (1-4%; included in the "Other" category in the overall plot due to low global abundance), and Negativicutes (10-15%). In contrast, needle samples were more

strongly dominated by Gammaproteobacteria (35-40%) and Actinobacteria (10-12%). Trunk samples exhibited a composition similar to the needles, with slight differences; Alphaproteobacteria were slightly more abundant (7-8%), and Gammaproteobacteria slightly less abundant (30-35%).

When comparing non-symptomatic and symptomatic samples, the prokaryotic class composition remained fairly consistent between the two conditions in all tissue types. Slight decreasing trends were observed for Actinobacteria and Alphaproteobacteria, particularly in trunk samples. In the soil samples, however, Actinobacteria showed a slight decrease in the symptomatic sample, whereas Alphaproteobacteria and Gammaproteobacteria experienced a slight increase. Unclassified taxa with unknown class were not included in this analysis.

Overall, the differences between tissues appeared more pronounced than those between health conditions, although infection was associated with subtle shifts in the relative abundance of several dominant prokaryotic classes.

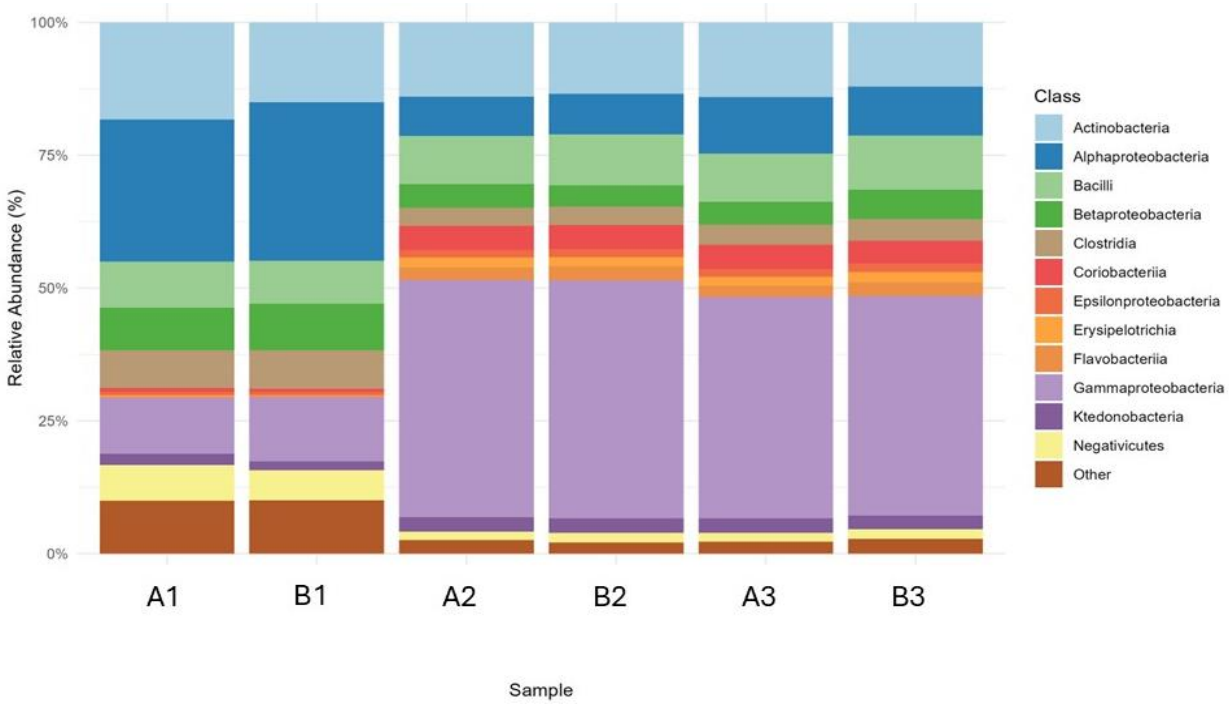


Figure 7: Prokaryotic community composition in 16S rRNA amplicon sequencing datasets at class level, for non-symptomatic and symptomatic soil (A1, B1), needle (A2, B2), and trunk (A3, B3) samples. The "Other" category represents low-abundance bacterial classes (mean relative abundance <1%).

The heatmap revealed variation in class-level relative abundance across both tissue types and health conditions (*Figure 8*). Several bacterial classes exhibited noticeable differences between non-symptomatic and symptomatic samples, as indicated by contrasting colour intensities. These differences were particularly evident in soil samples, where multiple classes showed higher relative abundance in the non-symptomatic condition compared to the symptomatic condition.

In contrast, needle and trunk samples displayed more similar abundance patterns between non-symptomatic and symptomatic conditions, with fewer pronounced differences across bacterial and archaeal classes.

The heatmap provides a more detailed view of the community by highlighting variation in less abundant bacterial and archaeal classes that were not clearly visible in the stacked bar plots. For instance, Nitrospira, Thermoprotei, and Halobacteria show a clear decrease in the symptomatic soil sample compared to the non-symptomatic one. Thermotogae and Anaerolineae also exhibit a moderate decrease in symptomatic soil and needle tissues compared to their non-symptomatic counterparts.

Methanococci and Armatimonadia, on the other hand, show an increase in symptomatic soil samples compared to non-symptomatic samples. Several additional classes also display decreases in symptomatic samples, particularly in needle and trunk tissues, although these changes are generally less pronounced.

Hierarchical clustering of bacterial classes grouped taxa with similar abundance patterns across samples, highlighting sets of classes that responded similarly across tissues and conditions.

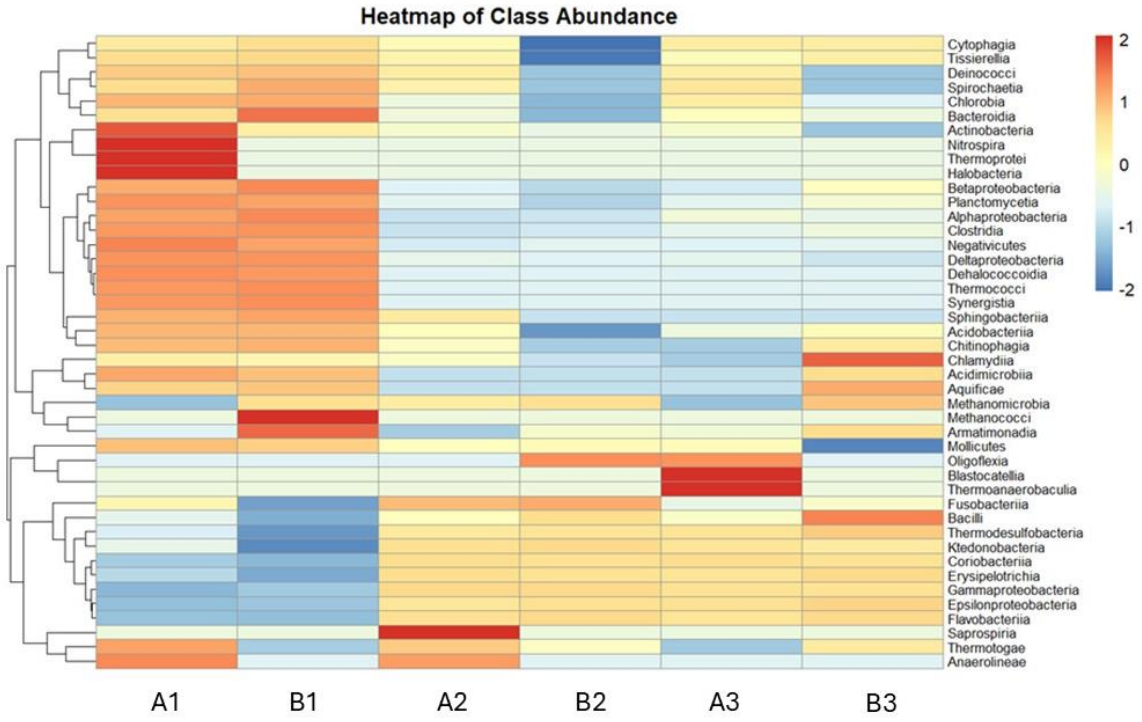


Figure 8: Heatmap of bacterial and archaeal class-level abundance in 16S rRNA amplicon datasets. Hierarchical clustering of non-symptomatic (A1-A3) and symptomatic (B1-B3) samples based on relative abundance patterns of bacterial classes across different tissue types (needles (A2/B2), soil (A1/B1), and trunk (A3/B3)).

Differential abundance at bacterial class level was assessed using log2 fold change values comparing symptomatic and non-symptomatic samples across three tissues (needles, soil, and trunk). Positive values indicate higher abundance in symptomatic samples, while negative values indicate lower abundance relative to non-symptomatic controls (*Figure 9)*.

Bacterial and archaeal classes showed heterogeneous responses to symptom development, with both increases and decreases in relative abundance depending on taxonomic group and tissue type. In some cases, no change in abundance was observed between conditions. Generally, the magnitude of log2 fold change values varied across taxa, potentially indicating uneven restructuring of the microbial community across conditions.

When comparing tissue types, needle-associated communities displayed the widest range of log2 fold change values, suggesting more pronounced differences between symptomatic and

non-symptomatic samples. Soil samples exhibited moderate shifts in abundance across taxa, while trunk-associated communities showed comparatively more selective changes.

For example, Acidobacteriia showed the largest decrease in needle samples, reaching values below -3, while displaying an increase in trunk samples. The largest decrease in soil samples was observed for Nitrospira, which showed no change in the other two tissue types. Chitinophagia and Armatimonadia showed the largest increases in trunk and soil samples, respectively.

As the dataset consists of aggregated count data without biological replication per condition, statistical significance testing was not performed. Therefore, results are interpreted in a descriptive manner based on log2 fold change patterns.

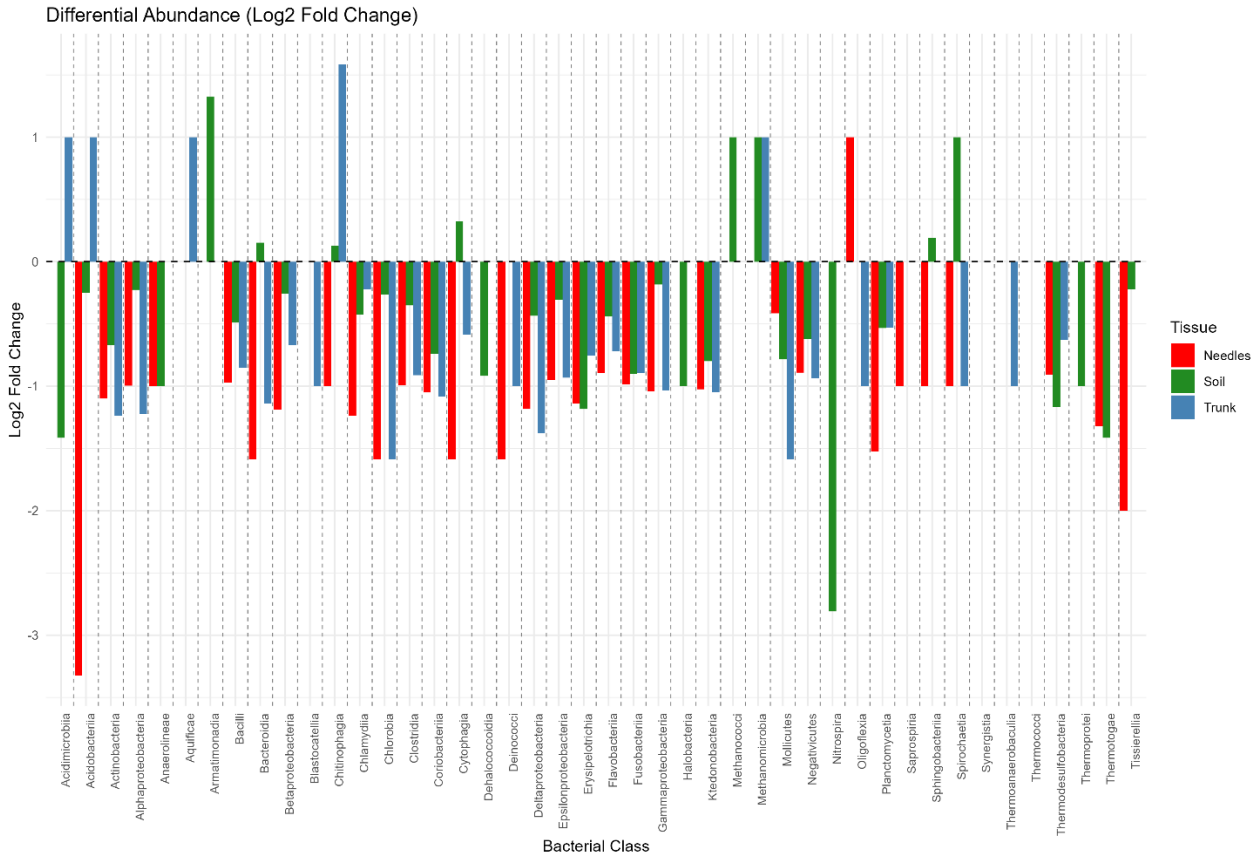


Figure 9: Differential abundance analysis of bacterial and archaeal classes in the 16S rRNA amplicon dataset. Log2 fold change of bacterial class abundance between non-symptomatic and symptomatic samples across needle, soil, and trunk tissues, identifying taxa enriched or depleted under disease conditions.

Alpha diversity was also assessed for the amplicon dataset, by calculating species richness (S), Shannon diversity index (H'), and Simpson diversity index (1-D) for symptomatic and non-symptomatic samples across needles, soil, and trunk (*Table 2*). The differences between the non-symptomatic and symptomatic samples were visible, although not drastic.

Species richness was highest in soil samples, followed by needles and trunk. Non-symptomatic samples showed slightly higher richness compared to symptomatic samples in all tissues, with the most pronounced difference observed in needle-associated communities (32 vs 24 taxa).

Shannon diversity values showed only minor variation between conditions, with soil communities exhibiting the highest diversity overall (H'≈2.31 in non-symptomatic samples). Only slight decreases were observed in soil and needle samples under symptomatic conditions, while trunk samples showed a marginal increase. Simpson diversity index values remained relatively stable across all samples (0.75-0.86), indicating that community dominance structure was largely unaffected by symptom development.

As the dataset is based on aggregated count data without biological replication per condition, statistical testing was not performed. Therefore, observed differences in alpha diversity are interpreted descriptively.

Tissue	Condition	Shannon Index (H')	Simpson Index (1-D)	Taxon Richness (S)
Needles	Non-symptomatic	1.97	0.76	32
Needles	Symptomatic	1.96	0.759	24
Soil	Non-symptomatic	2.31	0.858	38
Soil	Symptomatic	2.27	0.849	36
Trunk	Non-symptomatic	2.01	0.779	30
Trunk	Symptomatic	2.06	0.787	28

Table 2: Alpha diversity for the prokaryotic amplicon dataset across different tissues and conditions

4.3. Remote sensing analysis results

The NDVI analysis revealed both spatial and temporal variation in vegetation condition within the study area. The results are presented in Figures 10-17 and include spatial patterns, comparisons between the two compartments of interest (98E and 98F), the affected and non-affected areas, and temporal trends over multiple years.

The spatial distribution of NDVI in August 2021 (*Figure 10*) shows noticeable differences between areas within compartments 98E and 98F. Lower NDVI values appear in certain parts of the study area, indicating reduced vegetation greenness. This is further highlighted by the NDVI anomaly map, where negative values are visible in these same areas, showing a decrease compared to the baseline period (2016-2020).

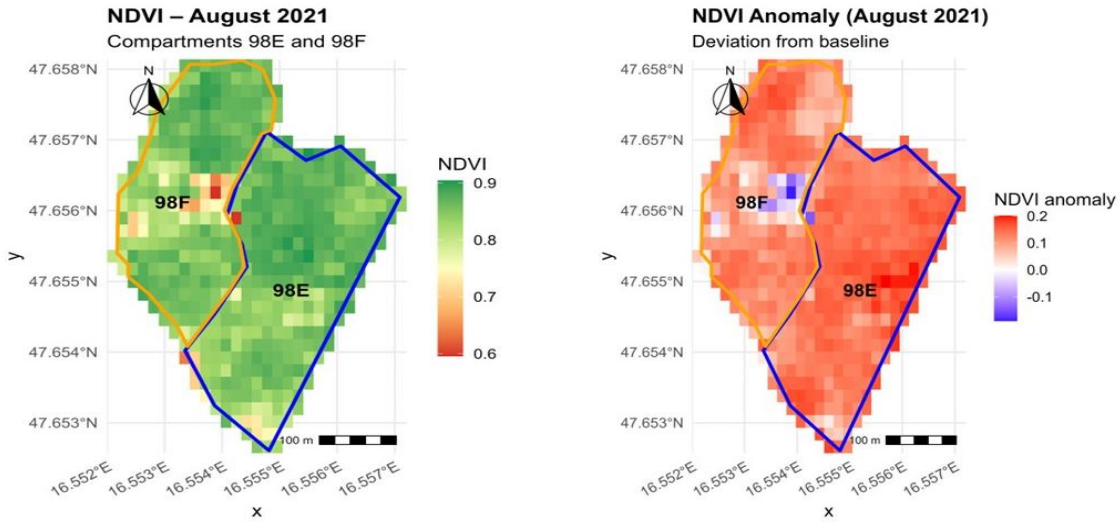


Figure 10: Spatial distribution of NDVI (left) and NDVI anomaly (right) for August 2021 in compartments 98E and 98F. The anomaly map represents the difference between August 2021 NDVI and the mean NDVI of the baseline period (2016–2020), highlighting areas of reduced vegetation condition.

A comparison of mean NDVI values for the month of August in 2021 (*Figure 11*) shows clear differences between the studied regions. The affected area expectedly has the lowest NDVI values, while the non-affected area and the two compartments show higher values. It is also shown that the 98F compartment has lower NDVI compared to the 98E compartment, in agreement with the visual presentation in *Figure 13*.

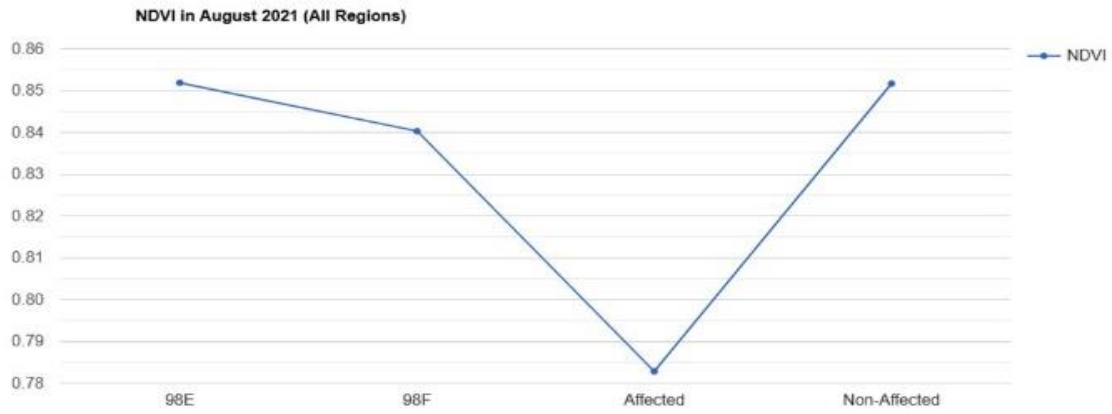


Figure 11: Comparison of mean NDVI values for August 2021 across compartments 98E, 98F, affected area, and non-affected area. Lower NDVI values are observed in the affected area, indicating reduced vegetation vigour

The seasonal NDVI dynamics for 2021 (*Figure 12*) follow a similar pattern across all regions. NDVI values increase during the spring months, reach a peak in summer, and then decrease towards autumn. During peak season, the affected area shows visibly lower NDVI values compared to the non-affected area and the two compartments.

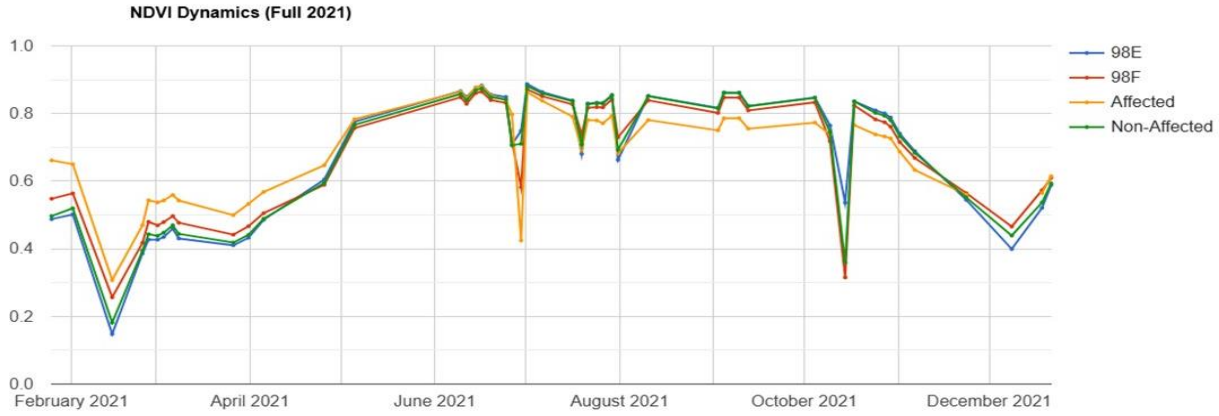


Figure 12: Seasonal NDVI dynamics for 2021 across compartments 98E, 98F, affected, and non-affected areas, based on Sentinel-2 observations, showing similar temporal patterns with consistently lower NDVI values in the affected area.

The smoothed NDVI time series for the affected area (*Figure 13*) shows clear seasonal fluctuations over the study period from 2017 to 2026. The pattern resembles regular

vegetation growth cycles, but there is a noticeable reduction in NDVI around the peak season of 2021 compared to the other years.

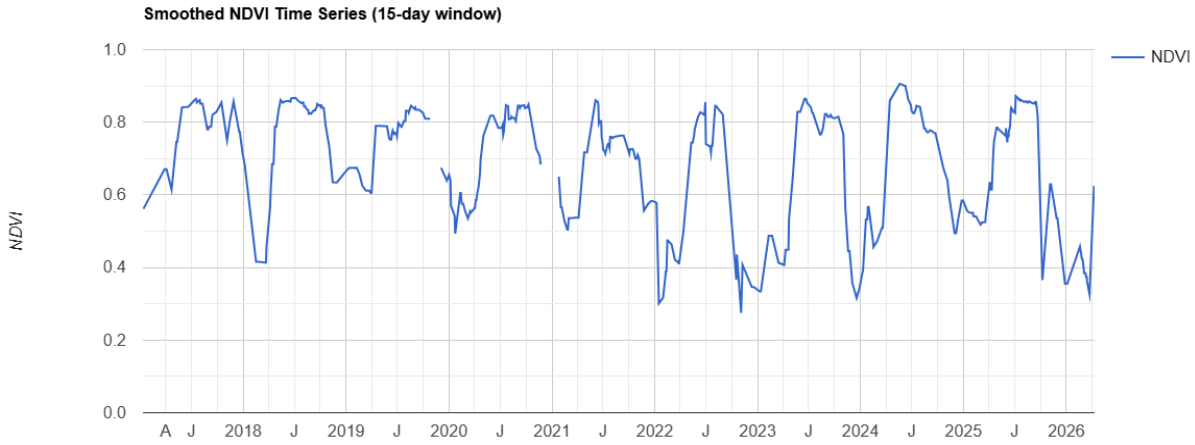


Figure 13: Smoothed NDVI time series based on a ±15-day temporal averaging window for the affected area from 2017 to beginning of 2026, illustrating seasonal vegetation dynamics.

The NDVI decrease characterising 2021 is much more visible in the next plot, which shows the annual NDVI trend derived from Sentinel-2 data (*Figure 14*). The lowest NDVI value occurs in 2021, while other years show relatively higher values, indicating that vegetation condition was reduced during that year.

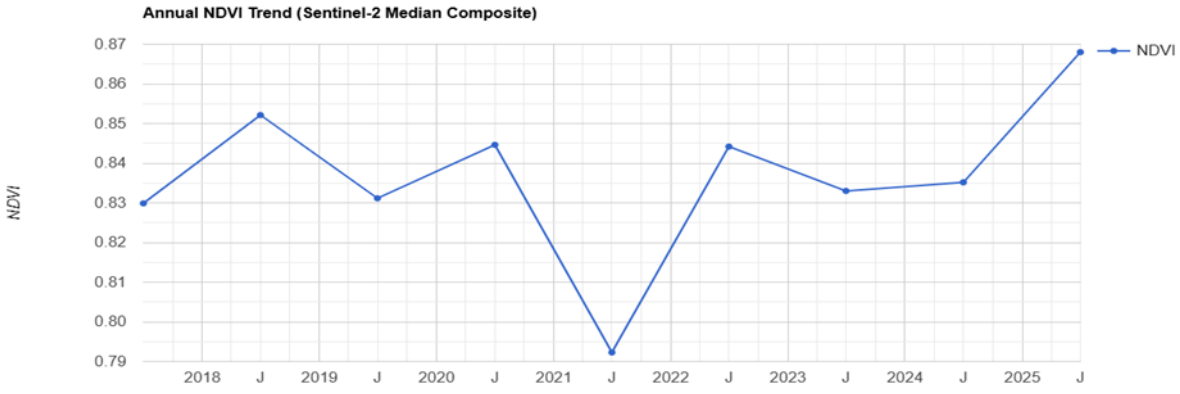


Figure 14: Annual NDVI trend derived from Sentinel-2 median composites (2018-2026), showing interannual variability in vegetation condition, with the lowest NDVI values observed in 2021.

Similar patterns are observed in the Sentinel-3 derived NDVI anomalies (*Figure 15*), where negative Z-score values are present around 2021, indicating below-average vegetation conditions. The results obtained from the Hungarian TEMRE platform (*Figure 16*) show a comparable trend, with reduced NDVI values during the same period. The consistency between these datasets suggests that the observed patterns are reliable.

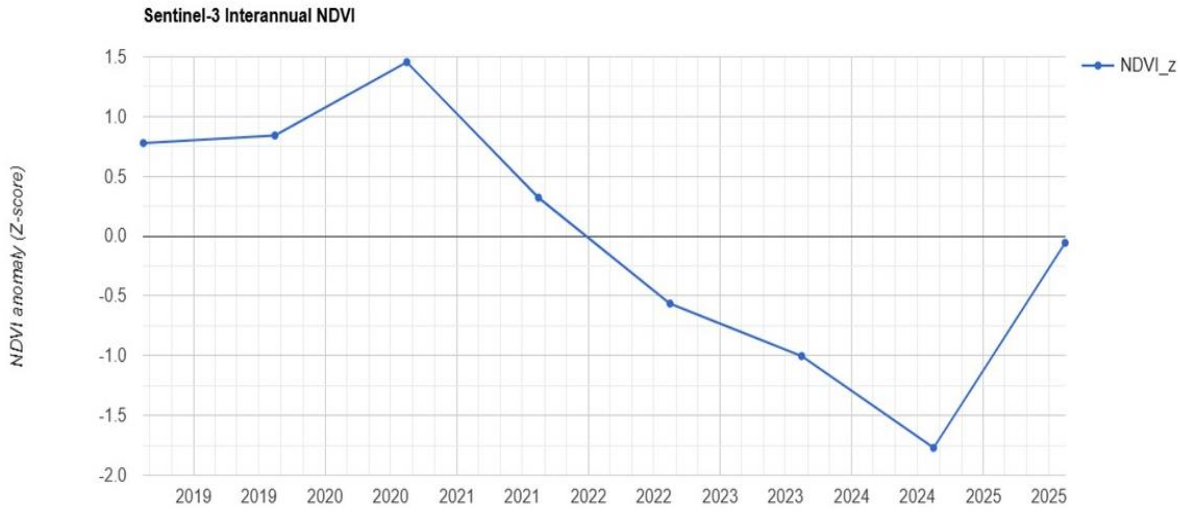


Figure 15: Interannual NDVI anomalies expressed as Z-scores derived from Sentinel-3 data (2019-2026). Negative values indicate below-average vegetation condition, while positive values indicate above-average conditions

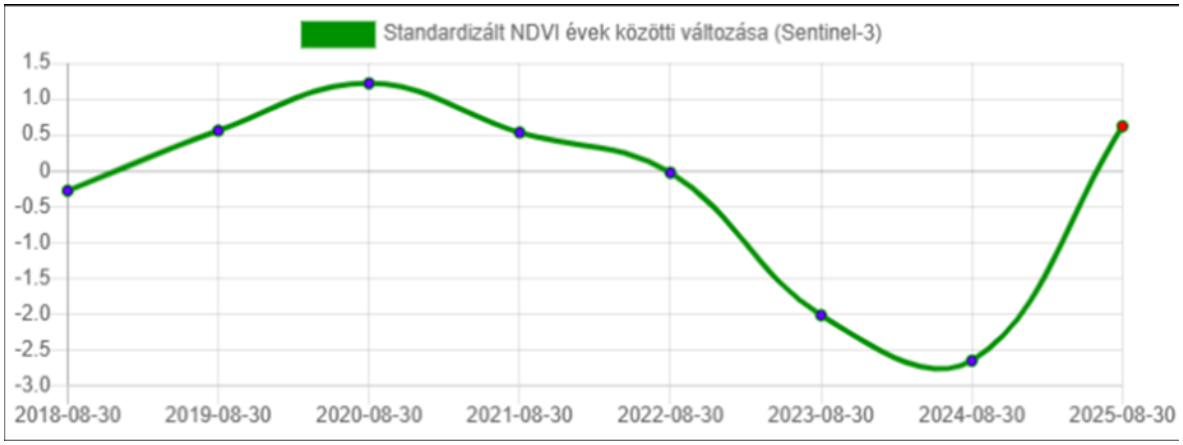


Figure 16: Interannual NDVI anomalies derived from Sentinel-3 data (2019-2026) obtained from TEMRE platform.

The change in vegetation condition within the study area is also visualised using historical imagery from Google Earth Pro (*Figure 17*). The image from 2020 shows relatively dense and uniformly green canopy cover, whereas the 2021 image reveals distinct areas of discoloured canopy, with orange-brown tones visible within the compartment. By June 2022, these areas appear more open, suggesting that trees in the affected zones had been removed.

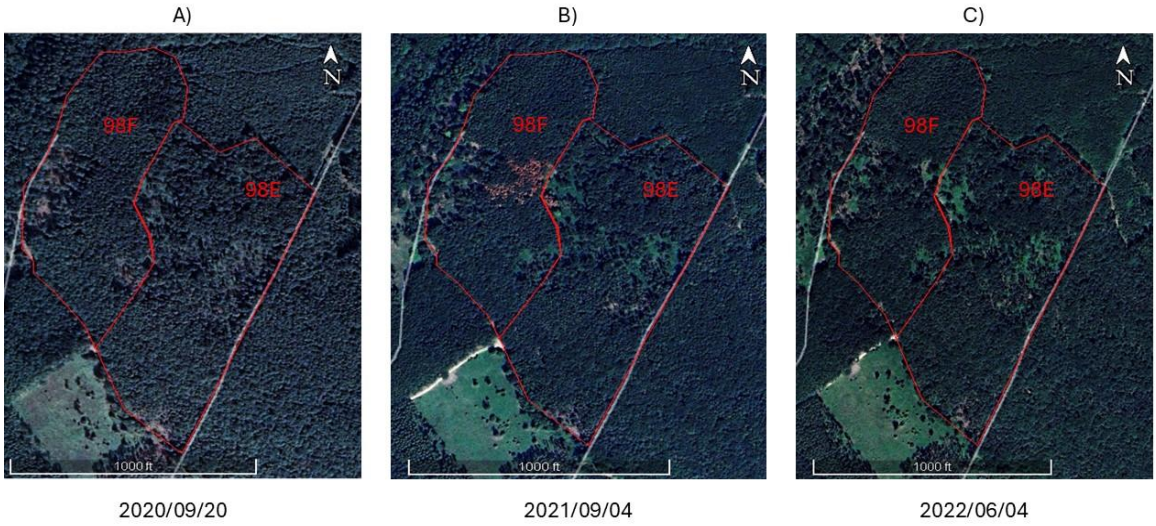


Figure 17: Historical satellite imagery of the study area showing vegetation conditions at three time points: September 20th 2020 (A), September 4th 2021 (B), and June 4th 2022 (C). Compartment boundaries (98E and 98F)are overlaid to provide spatial context.

5. Discussion

In this study, we investigated the microbial composition associated with Scots pine trees exhibiting signs of decline in a forest near Sopron, Hungary. We compared non-symptomatic trees with neighbouring symptomatic individuals in order to explore associations between plant health status and microbial community structure. In addition, we integrated remote sensing data to assess vegetation dynamics within the same study area through NDVI-based time-series analysis.

The study focused on bacterial, archaeal, and fungal communities in order to characterise both prokaryotic and eukaryotic components of the pine-associated microbiome. In particular, we aimed to identify taxa that differ in relative abundance between symptomatic and non-symptomatic conditions, thereby providing insights into how microbial community composition shifts in relation to host health status.

This approach may help identify microbial taxa potentially associated with disease or stress conditions, which could serve as candidates for future biocontrol strategies. Furthermore, the inclusion of remote sensing data enables the assessment of vegetation condition at an ecosystem scale over extended temporal periods, providing broader context for the observed microbial patterns.

Our shotgun metagenomic analysis indicates that microbial communities in both the prokaryotic and eukaryotic datasets remained relatively stable, although slight shifts were observed under diseased conditions. In the prokaryotic dataset, this shift is reflected in a decrease in species richness from 53 to 39 (*Table 1*). Acidobacteria are visibly reduced in symptomatic trunk samples (*Figure 6A*), which is in agreement with previous studies reporting their enrichment in non-symptomatic pine trees compared to diseased ones (Lasa et al., 2024). Previous studies have also shown that members of the Acidobacteria phylum may have plant growth-promoting properties, possibly through auxin production pathways (Kielak et al., 2016).

At the genus level, *Bacteroides* were the most abundant in both conditions, followed by *Klebsiella* and *Staphylococcus* (*Figure 6C*). The dominance of *Bacteroides* could be

explained by their ability to ferment and digest complex carbohydrates in the plant (Wexler, 2007), and as structurally-complex plant compartments such as pine trunks likely favour taxa with such metabolic capabilities. Although they remain dominant under both conditions, their abundance is shown to be slightly reduced in the symptomatic samples. This could possibly hint at their role in plant well-being.

Klebsiella, on the other hand, is also thought to be associated with plant growth-promoting processes, such as nitrogen fixation, phytohormone production, and phosphorus and potassium solubilisation (Duran-Bedolla et al., 2021). However, there are certain strains that may rise to become opportunistic and pathogenic under disease conditions, such as *Klebsiella variicola*. This could explain the minor increase of this genus in the symptomatic samples compared to the non-symptomatic ones.

Staphylococcus, *Cronobacter*, and *Acinetobacter* also exhibit a slight increase in the symptomatic samples, suggesting possible correlation with disease promotion and opportunistic behaviour. Lastly, members of the order of Burkholderiales were shown to exhibit marked decrease in symptomatic samples, which could suggest a potential role in plant stability and baseline functioning. It is known that members of this order are involved in plant protection from unwanted pathogens by competing with the pathogenic bacteria for the same resources, by producing pathogen-specific antimicrobial compounds, and by stimulating the host plant immune responses (Fuller et al., 2023).

The “Others” category, representing low-abundance (<0.5%) genera, was slightly higher in non-symptomatic samples. From this result, it could be implied that decline symptoms may be associated with the absence of certain species which may be beneficial at low abundance.

Alpha diversity analysis also shows visible decrease in taxon richness in the symptomatic samples for the prokaryotic dataset (*Table 1*). This outcome is generally consistent with dysbiosis patterns often observed in disturbed or diseased systems, which may involve reduced diversity and community restructuring.

In the eukaryotic dataset, 99% of the sequences belonged to the host phyla (Streptophyta) which was removed prior to further analysis. From the fungal phyla, Ascomycota and

Basidiomycota were the dominant groups in both the non-symptomatic and symptomatic samples. This result is also supported by previous studies, which show these two phyla as the dominant fungal taxa in pine species, especially enriched in samples from diseased pines, such as those affected by pine wilt disease (Vicente et al., 2021). Both phyla are known to consist of members of different relationship types, such as saprophytes, opportunists, and decomposers, and a shift in their abundance and composition is generally expected under disease conditions. Being ectomycorrhizal fungi, Basidiomycota and Ascomycota help pine species grow in nutrient-poor and challenging soil environments (Kuo et al., 2014).

Oomycota, Mucoromycota, Ciliophora are three taxonomic groups which appear to increase in symptomatic samples, implying possible association with disease promotion. Apicomplexa are exclusively found in the symptomatic condition, suggesting potential disease-associated activity. Although Apicomplexa are primarily known as obligate parasites of animals, their presence in plant-associated datasets may reflect indirect ecological interactions, such as changes in microfaunal communities or the accumulation of environmental DNA in stressed tissues.

Adineta (~60%) and Rotaria (~19%) dominated in both symptomatic and non-symptomatic samples (*Figure 6D*), indicating a strong and stable representation of rotifer-associated lineages across conditions.

The most notable shifts included a substantial increase in *Brugia* in symptomatic samples and a marked decrease in *Armillaria*. The increase of *Brugia*, which is a roundworm genus primarily associated with animal parasitism (Evans et al., 2024), may reflect indirect ecological effects, such as changes in host-associated microfaunal communities or the presence of environmental DNA, rather than a direct interaction with the plant host.

Malassezia also showed increased relative abundance in symptomatic samples, which may indicate a shift toward opportunistic or stress-associated fungal communities, as this genus is commonly linked to host-associated and lipid-rich environments.

Several taxa were detected exclusively in symptomatic samples, although some occurred at low relative abundances (<0.5%) and were not visually prominent in the plots. These

included Obtectomera (a higher-level insect clade), Stylonychia (ciliate protozoa), members of Sordariomycetes (fungal class), and Apicomplexa, indicating the presence of condition-specific low-abundance eukaryotic lineages. The detection of these taxa likely reflects broader changes in the microeukaryotic community structure associated with symptomatic conditions, rather than direct pathogenic interactions with the host.

Alpha diversity results also show a slight increase in Simpson and Shannon indices, although taxon richness remains unchanged (*Table 1*). This may suggest that opportunistic taxa arise in the eukaryotic dataset under symptomatic conditions, while beneficial organisms are reduced.

The 16S rRNA amplicon analysis confirmed that tissue type represented the primary driver of bacterial community structure, while health status induced only subtle compositional shifts. Soil communities exhibited the highest diversity and the greatest taxonomic heterogeneity, consistent with their role as a reservoir of microbial diversity, whereas needle and trunk-associated communities were more constrained and dominated by a smaller set of bacterial classes. These results are in accordance with data from published literature, as trunk and needle compartments tend to be less suitable as habitats for microorganisms than the soil and roots, due to exposure to abiotic factors like sunlight, storms, drought, and wind (see section 2.4.1).

Although symptomatic and non-symptomatic samples largely shared similar community profiles, minor but consistent shifts in relative abundance were observed, suggesting early-stage microbial restructuring associated with tree decline rather than a complete community turnover.

The reduction of Acidobacteriia in symptomatic tissues, particularly in needles and trunk samples, aligns with previous observations linking this phylum to stable, oligotrophic, and plant-beneficial environments. Their decrease may therefore reflect a loss of beneficial microbial support functions under declining tree health.

Similarly, changes in Nitrospira abundance suggest potential alterations in nitrogen cycling processes within symptomatic trees, which may indicate a disruption of core nutrient

transformation pathways associated with microbial imbalance. The lack of change in other tissues supports the idea that their ecological role is primarily soil-associated.

Differential abundance analysis also emphasises the increase of Chitinophagia and Armatimonadia, which may reflect a shift toward taxa involved in decomposition and utilization of complex organic matter (del Rio et al., 2010). This could be linked to increased availability of host-derived substrates, such as decaying tissue or stress-related exudates, particularly in trunk and soil environments. Such shifts may indicate a transition toward a more heterotrophy-driven microbial community under symptomatic conditions.

When compared with the shotgun metagenomic dataset, the amplicon-based results support the overall observation that microbial community composition is more strongly structured by tissue type than by health status. Both approaches indicate relatively stable core bacterial communities, with disease-related changes manifesting as subtle shifts in specific taxa rather than large-scale compositional turnover. However, the amplicon dataset provides a more conservative view of bacterial diversity, whereas the shotgun approach revealed additional variation in less abundant and eukaryotic lineages, suggesting that microbial responses to tree decline may be more pronounced outside of the dominant bacterial fraction.

The slight reduction in alpha diversity in symptomatic samples, particularly in needle-associated communities, suggests a modest loss of microbial complexity under declining tree health. However, the overall stability of Simpson diversity indicates that dominant taxa remain largely unchanged, implying that disease-associated effects are currently subtle and may represent an early stage of microbiome destabilisation.

The NDVI-based remote sensing analysis provides complementary ecosystem-scale evidence of vegetation decline within the study area. Across multiple datasets and temporal resolutions, a consistent reduction in vegetation greenness was observed around the year 2021, indicating a period of reduced canopy vitality. This pattern was evident both in spatial anomaly maps and in temporal NDVI trajectories, where lower-than-average values were consistently detected during peak growing season. The spatial heterogeneity observed between compartments 98E and 98F further suggests that vegetation decline was not uniform

across the landscape, but rather spatially structured, with certain sections of the forest exhibiting more pronounced reductions in greenness.

The temporal NDVI trends further support this observation, as the affected area exhibited a clear deviation from its long-term seasonal pattern, with a noticeable dip in vegetation condition during 2021. This anomaly was consistently detected across Sentinel-2, Sentinel-3, and the TEMRE dataset, indicating that the observed decline is robust and not dependent on a single data source. In addition, historical satellite imagery confirms structural changes in canopy cover between 2020 and 2022, suggesting that the NDVI decline is associated with visible changes in forest structure, potentially including canopy thinning or tree removal in affected areas.

When compared with the microbial datasets, the NDVI-derived vegetation decline aligns temporally with the observed shifts in microbial community composition. Although microbial changes were generally subtle and dominated by tissue type effects, the presence of consistent compositional shifts in both bacterial and eukaryotic communities suggests that microbial restructuring may be associated with broader ecosystem-level stress signals. In particular, the slight reduction in microbial diversity and the changes observed in key bacterial taxa coincide with the period of reduced vegetation vigour identified in 2021, suggesting a potential link between host condition and associated microbiome dynamics.

However, it is important to note that the observed microbial changes do not indicate a drastic community collapse, but rather a gradual shift in relative abundance patterns. This suggests that the microbiome may respond to host decline in a buffered or delayed manner, potentially reflecting early-stage ecosystem destabilisation rather than advanced disease progression.

Taken together, the NDVI and microbiome analyses provide complementary perspectives on forest health at different scales. While remote sensing captures structural and physiological changes in vegetation at the ecosystem level, microbial sequencing reveals more fine-scale shifts in host-associated communities. The agreement between these datasets in identifying a period of reduced vegetation condition around 2021 supports the interpretation that the study area experienced a measurable stress event during this period. At the same time, the relatively modest microbial shifts suggest that the associated

microbiome is still largely stable, indicating that the system may be in an intermediate stage of disturbance rather than full ecological collapse.

Despite the insights provided by this study, several limitations should be acknowledged. Firstly, the microbial datasets were based on aggregated samples without biological replication for each condition, which limits the ability to perform statistical testing and restricts interpretation to descriptive patterns. Secondly, the use of DNA-based sequencing does not distinguish between active and inactive microorganisms, meaning that some detected taxa may not be functionally relevant within the studied environment. In addition, while the integration of remote sensing data provides valuable ecosystem-scale context, it does not allow direct identification of the specific drivers underlying the observed vegetation decline. Therefore, the relationships identified between microbial composition and vegetation condition should be interpreted as associations rather than causal links.

Future studies should aim to incorporate biological replication and higher temporal resolution sampling to better capture the dynamics of microbial community change during tree decline. The inclusion of functional approaches, such as metatranscriptomics or metabolomics, would provide deeper insight into the active roles of microbial taxa and their contribution to host health. Furthermore, integrating environmental variables such as soil chemistry, moisture, and climate data could help disentangle the abiotic factors driving both vegetation decline and microbiome shifts. Long-term monitoring combining remote sensing and microbial analysis would be particularly valuable for understanding the progression from early-stage stress to advanced ecosystem disturbance.

This study demonstrates that Scots pine decline is associated with measurable but relatively subtle shifts in both microbial community composition and vegetation condition. While remote sensing data clearly indicate a period of reduced canopy vitality around 2021, microbial communities appear to respond more gradually, maintaining a largely stable core structure with only minor compositional changes. This suggests that microbiome alteration may represent an early or intermediate stage of ecosystem response to stress, rather than a direct or immediate driver of decline. The combined use of high-resolution sequencing and remote sensing approaches provides a more comprehensive understanding of forest health,

highlighting the importance of integrating multiple scales of analysis when investigating complex ecological processes.

6. Conclusions

This study aimed to explore whether tree decline in Scots pine observed near Sopron is reflected in associated microbial communities and whether these changes correspond to broader patterns in vegetation condition. The results indicate that, while measurable differences between symptomatic and non-symptomatic trees are present, these differences remain relatively subtle and do not suggest a major disruption of the microbiome.

A key outcome of this work is that microbial community structure appears to be largely maintained even under declining host conditions, with variation driven more strongly by tissue type than by health status. This suggests that, at the stage captured in this study, tree decline is not accompanied by a complete loss of microbial stability, but rather by gradual and selective shifts within an otherwise resilient system.

At the same time, the remote sensing analysis provides clear evidence that the study area experienced a period of reduced vegetation condition, particularly around 2021. The consistency of this signal across multiple datasets indicates that the observed decline is not an isolated observation, but part of a broader ecosystem-level change. When considered together, these findings suggest that microbial responses may lag behind, or respond more gradually to, changes in host condition that are already detectable at the canopy level.

From a practical perspective, this highlights the value of combining different approaches when assessing forest health. Remote sensing allows for the detection of large-scale changes in vegetation condition, while microbiome analysis provides insight into underlying biological processes that are not directly visible. The integration of these methods may therefore improve early detection and monitoring strategies, particularly in systems where decline develops progressively rather than abruptly.

However, the study also has several limitations that need to be considered. The lack of biological replication and the descriptive nature of the analysis limit the strength of the conclusions that can be drawn. In addition, the focus on a single study area means that the results may not be directly transferable to other forest systems or environmental conditions.

Future work should aim to expand both the spatial and temporal scope of sampling, include replicated datasets, and incorporate functional analyses to better understand the ecological roles of the detected microbial taxa. Further research is also needed to clarify whether the observed microbial shifts are a cause, consequence, or simply a correlate of tree decline.

To conclude, the findings of this study suggest that forest decline may be characterised by gradual, multi-scale changes rather than abrupt transitions. While vegetation-level signals of stress can be clearly detected, associated microbial communities appear to respond in a more buffered and incremental manner, reflecting the complexity of plant-microbe-environment interactions.

7. Summary

This thesis examined the relationship between microbial community composition and tree health in Scots pine, with a focus on trees showing symptoms of decline. Non-symptomatic and symptomatic trees were compared using both shotgun metagenomic and 16S rRNA amplicon sequencing approaches. In addition, remote sensing data based on NDVI were used to assess vegetation condition within the study area over time.

The results show that microbial community composition is primarily influenced by tissue type, with soil samples exhibiting the highest diversity, followed by needles and trunk tissues. Differences between symptomatic and non-symptomatic samples were present but generally small, indicating that microbial communities remain relatively stable even under declining tree health. A decrease in taxon richness was observed in symptomatic samples, suggesting a modest level of disturbance, while dominant taxa remained largely unchanged.

Certain microbial groups showed consistent patterns, such as a reduction in Acidobacteria-related taxa in symptomatic samples, which may indicate the loss of beneficial or stability-associated microorganisms. At the same time, some opportunistic or low-abundance taxa appeared more frequently in symptomatic conditions, suggesting gradual community shifts rather than abrupt changes.

The remote sensing analysis revealed a clear reduction in vegetation greenness around the year 2021, as indicated by decreased NDVI values across multiple datasets. This pattern was consistent across spatial and temporal analyses and was supported by historical satellite imagery, which showed visible changes in canopy condition.

When considered together, the results suggest that tree decline in the study area is associated with both reduced vegetation vigour and subtle changes in microbial community structure. While the microbial shifts are not drastic, they may reflect early-stage responses to stress.

The study highlights the value of combining microbiome analysis with remote sensing approaches to assess forest health at different scales. However, further research is needed to

better understand the functional implications of these microbial changes and to establish stronger links between microbial composition and tree condition.

Acknowledgements

I would like to express my utmost gratitude to my supervisors Dr. Nóra Tünde Lange-Enyedi, Dr. Géza Király, and Dr. György Sipos for their unreserved support throughout this work – it was a privilege to be under their guidance which has an incomparable contribution to this thesis. I would like to further thank Nóra and Dr. Boris Indic for their continuous assistance throughout the programme - their proactive support was always reassuring and I'm grateful I had the opportunity to learn from their knowledge. I am especially thankful to Dr. Simang Champramary for his immense help concerning the bioinformatics analyses. Lastly, I would like to express my heartfelt thanks to my family, especially my parents, for their ceaseless support in every aspect of my life.

References

Aasfar, A., Bargaz, A., Yaakoubi, K., Hilali, A., Bennis, I., Zeroual, Y., & Meftah Kadmiri, I. (2021). Nitrogen Fixing Azotobacter Species as Potential Soil Biological Enhancers for Crop Nutrition and Yield Stability. *Frontiers in Microbiology*, *12*, 628379. https://doi.org/10.3389/fmicb.2021.628379

Addison, S., Armstrong, C., Wigley, K., Hartley, R., & Wakelin, S. (2023). What matters most? Assessment of within-canopy factors influencing the needle microbiome of the model conifer, Pinus radiata. *Environmental Microbiome 2023 18:1*, *18*(1), 45-. https://doi.org/10.1186/s40793-023-00507-8

Adioumani, A. E., Kakou-Ngazoa, S., Trebissou, J. N. D., Martin, T., Addablah, A., Koudou, A., Kouassi, K. S., Sina, M. K., Coulibaly, N., Aoussi, S., & Dosso, M. (2022). Isolation and identification of phytopathogenic bacteria in vegetable crops in West Africa. *African Journal of Microbiology Research*, *2022*(4), 167–177. https://doi.org/10.5897/AJMR2021.9610

Aerts, R., & Honnay, O. (2011). Forest restoration, biodiversity and ecosystem functioning. *BMC Ecology 2011 11:1*, *11*(1), 29-. https://doi.org/10.1186/1472-6785-11-29

Agrios, G. N. (2009). Plant Pathogens and Disease: General Introduction. *Encyclopedia of Microbiology*, *Third Edition*, 613–646. https://doi.org/10.1016/B978-012373944-5.00344-8

Aiba, S. (2016). *Vegetation Zonation and Conifer Dominance Along Latitudinal and Altitudinal Gradients in Humid Regions of the Western Pacific*. 89–114. https://doi.org/10.1007/978-4-431-55954-2_5

Andrews, S. (2010). *Babraham Bioinformatics - FastQC A Quality Control tool for High Throughput Sequence Data*. https://www.bioinformatics.babraham.ac.uk/projects/fastqc/

Arnault, G., Mony, C., & Vandenkoornhuyse, P. (2023). Plant microbiota dysbiosis and the Anna Karenina Principle. *Trends in Plant Science*, *28*(1), 18–30. https://doi.org/10.1016/j.tplants.2022.08.012

Bakki, M., Banane, B., Marhane, O., Esmaeel, Q., Hatimi, A., Barka, E. A., Azim, K., & Bouizgarne, B. (2024). Phosphate solubilizing Pseudomonas and Bacillus combined with rock phosphates promoting tomato growth and reducing bacterial canker disease. *Frontiers in Microbiology*, *15*, 1289466. https://doi.org/10.3389/fmicb.2024.1289466

Balbinot, C. L., Marques, R., Tonello, K. C., Pasquetti Berguetti, Á. L., & Larsen, J. G. (2024). Recent insights in soil nutrient cycling: perspectives from Pinus and Eucalyptus forest studies around the world. *IForest - Biogeosciences and Forestry*, *17*(6), 394. https://doi.org/10.3832/ifor4530-017

Banerjee, S., Schlaeppi, K., & van der Heijden, M. G. A. (2018). Keystone taxa as drivers of microbiome structure and functioning. *Nature Reviews Microbiology 2018 16:9*, *16*(9), 567–576. https://doi.org/10.1038/s41579-018-0024-1

Barakat, M., Cheviron, B., & Angulo-Jaramillo, R. (2016). Influence of the irrigation technique and strategies on the nitrogen cycle and budget: A review. *Agricultural Water Management*, *178*, 225–238. https://doi.org/10.1016/j.agwat.2016.09.027

Boiarskii, B., & Hasegawa, H. (2019). Comparison of NDVI and NDRE Indices to Detect Differences in Vegetation and Chlorophyll Content. *JOURNAL OF MECHANICS OF CONTINUA AND MATHEMATICAL SCIENCES*, *spl1*(4). https://doi.org/10.26782/jmcms.spl.4/2019.11.00003

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, *30*(15), 2114–2120. https://doi.org/10.1093/BIOINFORMATICS/BTU170

Brockerhoff, E. G., Barbaro, L., Castagneyrol, B., Forrester, D. I., Gardiner, B., González-Olabarria, J. R., Lyver, P. O. B., Meurisse, N., Oxbrough, A., Taki, H.,

Thompson, I. D., van der Plas, F., & Jactel, H. (2017). Forest biodiversity, ecosystem functioning and the provision of ecosystem services. *Biodiversity and Conservation 2017 26:13*, *26*(13), 3005–3035. https://doi.org/10.1007/s10531-017-1453-2

Cherinet, A., & Lemi, T. (2023). The Role of Forest Ecosystems for Carbon Sequestration and Poverty Alleviation in Ethiopia. *International Journal of Forestry Research*, *2023*(1), 3838404. https://doi.org/10.1155/2023/3838404

Chiarello, M., McCauley, M., Villéger, S., & Jackson, C. R. (2022). Ranking the biases: The choice of OTUs vs. ASVs in 16S rRNA amplicon data analysis has stronger effects on diversity measures than rarefaction and OTU identity threshold. *PLOS ONE*, *17*(2), e0264443. https://doi.org/10.1371/journal.pone.0264443

Clausen, P. T. L. C., Aarestrup, F. M., & Lund, O. (2018). Rapid and precise alignment of raw reads against redundant databases with KMA. *BMC Bioinformatics 2018 19:1*, *19*(1), 307-. https://doi.org/10.1186/S12859-018-2336-6

De Mandal, S., & Jeon, J. (2023). Phyllosphere Microbiome in Plant Health and Disease. *Plants*, *12*(19), 3481. https://doi.org/10.3390/plants12193481

del Rio, T. G., Abt, B., Spring, S., Lapidus, A., Nolan, M., Tice, H., Copeland, A., Cheng, J. F., Chen, F., Bruce, D., Goodwin, L., Pitluck, S., Ivanova, N., Mavromatis, K., Mikhailova, N., Pati, A., Chen, A., Palaniappan, K., Land, M., … Lucas, S. (2010). Complete genome sequence of Chitinophaga pinensis type strain (UQM 2034T). *Standards in Genomic Sciences 2010 2:1*, *2*(1), 87–95. https://doi.org/10.4056/SIGS.661199

Drusch, M., Del Bello, U., Carlier, S., Colin, O., Fernandez, V., Gascon, F., Hoersch, B., Isola, C., Laberinti, P., Martimort, P., Meygret, A., Spoto, F., Sy, O., Marchese, F., & Bargellini, P. (2012). Sentinel-2: ESA's Optical High-Resolution Mission for GMES Operational Services. *Remote Sensing of Environment*, *120*, 25–36. https://doi.org/10.1016/j.rse.2011.11.026

Duran-Bedolla, J., Garza-Ramos, U., Rodríguez-Medina, N., Aguilar Vera, A., & Barrios-Camacho, H. (2021). Exploring the environmental traits and applications of Klebsiella variicola. *Brazilian Journal of Microbiology*, *52*(4), 2233. https://doi.org/10.1007/S42770-021-00630-Z

European and Mediterranean Plant Protection Organization (EPPO). (2026). *Bursaphelenchus xylophilus (BURSXY)[Datasheet]| EPPO Global Database*. https://gd.eppo.int/taxon/BURSXY/datasheet

Evans, C. C., Pilotte, N., & Moorhead, A. R. (2024). Current Status of the Diagnosis of Brugia spp. Infections. *Pathogens 2024, Vol. 13*, *13*(9). https://doi.org/10.3390/PATHOGENS13090714

FAO. (2020). *FRA 2020: Global forest resources assessment – Terms and definitions*. https://openknowledge.fao.org/server/api/core/bitstreams/531a9e1b-596d-4b07-b9fd-3103fb4d0e72/content

FAO. (2025). Global Forest Resources Assessment 2025. *Global Forest Resources Assessment 2025*. https://doi.org/10.4060/cd6709en

Fuller, E., Germaine, K. J., & Rathore, D. S. (2023). The Good, the Bad, and the Useable Microbes within the Common Alder (Alnus glutinosa) Microbiome—Potential Bio-Agents to Combat Alder Dieback. *Microorganisms 2023, Vol. 11, 11*(9). https://doi.org/10.3390/microorganisms11092187

Gea-Izquierdo, G., Férriz, M., García-Garrido, S., Aguín, O., Elvira-Recuenco, M., Hernandez-Escribano, L., Martin-Benito, D., & Raposo, R. (2019). Synergistic abiotic and biotic stressors explain widespread decline of Pinus pinaster in a mixed forest. *Science of The Total Environment*, *685*, 963–975. https://doi.org/10.1016/j.scitotenv.2019.05.378

Gouda, S., Kerry, R. G., Das, G., Paramithiotis, S., Shin, H. S., & Patra, J. K. (2018). Revitalization of plant growth promoting rhizobacteria for sustainable

development in agriculture. *Microbiological Research*, *206*, 131–140. https://doi.org/10.1016/j.micres.2017.08.016

Grzyb, T., & Szulc, J. (2024). Deciphering Molecular Mechanisms and Diversity of Plant Holobiont Bacteria: Microhabitats, Community Ecology, and Nutrient Acquisition. *International Journal of Molecular Sciences 2024, Vol. 25, 25*(24). https://doi.org/10.3390/ijms252413601

Gu, Z., Gu, L., Eils, R., Schlesner, M., & Brors, B. (2014). circlize implements and enhances circular visualization in R. *Bioinformatics*. https://doi.org/10.1093/bioinformatics/btu393

Halifu, S., Zhang, S., Liu, G., Yang, L., & Deng, X. (2026). Changes in the Microbial Communities of Picea schrenkiana Needles Following Lirula macrospora Infection. *Plants*, *15*(3), 449. https://doi.org/10.3390/plants15030449

Hoeksema, J. D., Averill, C., Bhatnagar, J. M., Brzostek, E., Buscardo, E., Chen, K. H., Liao, H. L., Nagy, L., Policelli, N., Ridgeway, J., Rojas, J. A., & Vilgalys, R. (2020). Ectomycorrhizal Plant-Fungal Co-invasions as Natural Experiments for Connecting Plant and Fungal Traits to Their Ecosystem Consequences. *Frontiers in Forests and Global Change*, *3*, 539127. https://doi.org/10.3389/ffgc.2020.00084

Horváth, A., Lakatos, F., Szűcs, P., Patocskai, Z., Végh, P., Winkler, D., Bidló, A., & Gálos, B. (2022). Climate Change Induced Tree Mortality in a Relict Scots Pine (Pinus sylvestris L.) Forest. *Acta Silvatica & Lignaria Hungarica*, *18*(1), 25–40. https://doi.org/10.37045/aslh-2022-0002

Hou, Z., Wang, M., Xu, H., Wang, M., & Hannula, S. E. (2025). Differential effects of pine wilt disease on root endosphere, rhizosphere, and soil microbiome of Korean white pine. *Microbiology Spectrum*, *13*(4). https://doi.org/10.1128/spectrum.02326-24

Juyal, P., Dhondiyal, P., & Padalia, K. (2022). *PLANT ECOLOGY* (Padalia K., S.N. Ojha, Juyal P., Dhondiyal P., & Joshi P., Eds.; 2022nd ed.). Uttarakhand Open University, Haldwani, Nainital-263139.

Kabir, M., Habiba, U. E., Khan, W., Shah, A., Rahim, S., Rios-Escalante, P. R. D. los, Farooqi, Z.-U.-R., Ali, L., Shafiq, M., Habiba, U. E., Khan, W., Shah, A., Rahim, S., Rios-Escalante, P. R. D. los, Farooqi, Z.-U.-R., Ali, L., & Shafiq, M. (2023). Climate change due to increasing concentration of carbon dioxide and its impacts on environment in 21st century; a mini review. *Journal of King Saud University – Science*, *35*(5), 102693. https://doi.org/10.1016/j.jksus.2023.102693

Kers, J. G., & Saccenti, E. (2022). The Power of Microbiome Studies: Some Considerations on Which Alpha and Beta Metrics to Use and How to Report Results. *Frontiers in Microbiology*, *12*, 796025. https://doi.org/10.3389/fmicb.2021.796025

Keyworth, S., Jarman, M., & Medcalf, K. (2009). *Assessing the Extent and Severity of Erosion on the Upland Organic Soils of Scotland using Earth Observation A GIFTSS Implementation Test FINAL Report*.

Kielak, A. M., Cipriano, M. A. P., & Kuramae, E. E. (2016). Acidobacteria strains from subdivision 1 act as plant growth-promoting bacteria. *Archives of Microbiology*, *198*(10), 987. https://doi.org/10.1007/S00203-016-1260-2

Klopfenstein, N. B., Kim, M.-S., Hanna, J. W., Richardson, B. A., & Lundquist, J. E. (2009). Approaches to predicting potential impacts of climate change on forest disease: an example with Armillaria root disease. *Res. Pap. RMRS-RP-76. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 10 p.*, *076*. https://doi.org/10.2737/RMRS-RP-76

Knutzen, F., Averbeck, P., Barrasso, C., Bouwer, L. M., Gardiner, B., Grünzweig, J. M., Hänel, S., Haustein, K., Johannessen, M. R., Kollet, S., Müller, M. M., Pietikäinen, J. P., Pietras-Couffignal, K., Pinto, J. G., Rechid, D., Rousi, E., Russo,

A., Suarez-Gutierrez, L., Veit, S., … Gliksman, D. (2025). Impacts on and damage to European forests from the 2018-2022 heat and drought events. *Natural Hazards and Earth System Sciences*, *25*(1), 77–117. https://doi.org/10.5194/nhess-25-77-2025

Kolde, R. (2025). *pheatmap: Pretty Heatmaps*. https://doi.org/10.32614/CRAN.package.pheatmap

Kovach, A., Wegrzyn, J. L., Parra, G., Holt, C., Bruening, G. E., Loopstra, C. A., Hartigan, J., Yandell, M., Langley, C. H., Korf, I., & Neale, D. B. (2010). The Pinus taeda genome is characterized by diverse and highly diverged repetitive sequences. *BMC Genomics*, *11*(1). https://doi.org/10.1186/1471-2164-11-420

Kuo, A., Kohler, A., Martin, F. M., & Grigoriev, I. V. (2014). Expanding genomics of mycorrhizal symbiosis. *Frontiers in Microbiology*, *5*(NOV), 99209. https://doi.org/10.3389/fmicb.2014.00582

Lantschner, M. V., Atkinson, T. H., Corley, J. C., & Liebhold, A. M. (2017). Predicting North American Scolytinae invasions in the Southern Hemisphere: *Ecological Applications*, *27*(1), 66–77. https://doi.org/10.1002/eap.1451

Lasa, A. V., Fernández-González, A. J., Villadas, P. J., Mercado-Blanco, J., Pérez-Luque, A. J., & Fernández-López, M. (2024). Mediterranean pine forest decline: A matter of root-associated microbiota and climate change. *Science of The Total Environment*, *926*, 171858. https://doi.org/10.1016/J.SCITOTENV.2024.171858

Lechner, A. M., Foody, G. M., & Boyd, D. S. (2020). Applications in Remote Sensing to Forest Ecology and Management. *One Earth*, *2*(5), 405–412. https://doi.org/10.1016/j.oneear.2020.05.001

Lee, M. K., Chun, J. H., & Lee, C. B. (2025). Relative importance of biotic, abiotic and stand age factors in influencing ecosystem multifunctionality across forest stand types in South Korea. *Frontiers in Forests and Global Change*, *8*, 1675167. https://doi.org/10.3389/ffgc.2025.1675167

Lee, T., & Kim, J. (2025). Rapid spread and high prevalence of the pine wilt disease around wildfire areas. *Trees, Forests and People*, *20*(2), 100805. https://doi.org/10.1016/j.tfp.2025.100805

Liu, Y., Huang, J., Wu, J., & Zong, S. (2022). Natural Factors Play a Dominant Role in the Short-Distance Transmission of Pine Wilt Disease. *SSRN Electronic Journal*. https://doi.org/10.2139/ssrn.4160807

Luchi, N., Ioos, R., & Santini, A. (2020). Fast and reliable molecular methods to detect fungal pathogens in woody plants. *Applied Microbiology and Biotechnology 2020 104:6*, *104*(6), 2453–2468. https://doi.org/10.1007/s00253-020-10395-4

Macip, G., Soler-Comas, A., Palomeque, A., Motos, A., Llonch, B., Canseco-Ribas, J., Bueno-Freire, L., Calabretta, D., Kiarostami, K., Cabrera, R., Ferrer, M., Torres, A., & Fernandez-Barat, L. (2025). Comparative analysis of illumina and oxford nanopore sequencing platforms for 16S rRNA profiling of respiratory microbial communities. *Scientific Reports 2025 15:1*, *15*(1), 33688-. https://doi.org/10.1038/s41598-025-18768-3

Mamiya, Y. (1983). Pathology of the Pine Wilt Disease Caused by Bursaphelenchus xylophilus . *Annual Review of Phytopathology*, *21*(1), 201–220. https://doi.org/10.1146/annurev.py.21.090183.001221

Marcelino, V. R., Clausen, P. T. L. C., Buchmann, J. P., Wille, M., Iredell, J. R., Meyer, W., Lund, O., Sorrell, T. C., & Holmes, E. C. (2020). CCMetagen: comprehensive and accurate identification of eukaryotes and prokaryotes in metagenomic data. *Genome Biology*, *21*(1). https://doi.org/10.1186/S13059-020-02014-2

Matzarakis, A., & Gulyás, Á. (2006). A Contribution to the Thermal Bioclimate of Hungary-mapping of the Physiologically Equivalent TEMPERATURE58. *Urbanclimate.Net*. https://www.academia.edu/22149630/A_Contribution_to_the_Thermal_Bioclima

te_of_Hungary_mapping_of_the_Physiologically_Equivalent_TEMPERATURE 58

Michel, A., Haggenmüller, K., Kirchner, T., Prescher, A.-K., Schwärzel, K., & Wohlgemuth, L. (2024). *Forest Condition in Europe The 2024 Assessment ICP Forests Technical Report under the UNECE Convention on Long-range Transboundary Air Pollution (Air Convention) FOREST CONDITION IN EUROPE: The 2024 Assessment*. https://doi.org/10.3220/ICPTR1732702585000

Mo, L., Zohner, C. M., Reich, P. B., Liang, J., de Miguel, S., Nabuurs, G. J., Renner, S. S., van den Hoogen, J., Araza, A., Herold, M., Mirzagholi, L., Ma, H., Averill, C., Phillips, O. L., Gamarra, J. G. P., Hordijk, I., Routh, D., Abegg, M., Adou Yao, Y. C., … Crowther, T. W. (2023). Integrated global assessment of the natural forest carbon potential. *Nature 2023 624:7990*, *624*(7990), 92–101. https://doi.org/10.1038/s41586-023-06723-z

Móricz, N., Garamszegi, B., Rasztovits, E., Bidló, A., Horváth, A., Jagicza, A., Illés, G., Vekerdy, Z., Somogyi, Z., & Gálos, B. (2018). Recent Drought-Induced Vitality Decline of Black Pine (Pinus nigra Arn.) in South-West Hungary—Is This Drought-Resistant Species under Threat by Climate Change? *Forests 2018, Vol. 9, 9*(7). https://doi.org/10.3390/f9070414

Müller, K., & Wickham, H. (2025). *tibble: Simple Data Frames*. https://doi.org/10.32614/CRAN.package.tibble

Mullett, M. S., Drenkhan, R., Adamson, K., Boroń, P., Lenart-Boroń, A., Barnes, I., Tomšovský, M., Jánošíková, Z., Adamčíková, K., Ondrušková, E., Queloz, V., Piškur, B., Musolin, D. L., Davydenko, K., Georgieva, M., Schmitz, S., Kačergius, A., Ghelardini, L., Orlović, J. K., … Konečný, A. (2021). Worldwide Genetic Structure Elucidates the Eurasian Origin and Invasion Pathways of Dothistroma septosporum, Causal Agent of Dothistroma Needle Blight. *Journal of Fungi 2021, Vol. 7, 7*(2), 1–28. https://doi.org/10.3390/jof7020111

Neuwirth, E. (2022). *RColorBrewer: ColorBrewer Palettes*. https://doi.org/10.32614/CRAN.package.RColorBrewer

Oksanen, J., Simpson, G. L., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Solymos, P., Stevens, M. H. H., Szoecs, E., & others. (2026). vegan: Community Ecology Package. *R Package Version 2.7-3*. https://doi.org/10.32614/CRAN.package.vegan

Pedersen, T. L. (2025). *patchwork: The Composer of Plots*. https://doi.org/10.32614/CRAN.package.patchwork

Pérez-Cobas, A. E., Gomez-Valero, L., & Buchrieser, C. (2020). Metagenomic approaches in microbial ecology: an update on whole-genome and marker gene sequencing analyses. *Microbial Genomics*, *6*(8), mgen000409. https://doi.org/10.1099/mgen.0.000409

Pimentel, C. S., Pires, D., Campôa, J., Branco, J., Marques, R., Mota, M. M., Calvão, T., Pimentel, C. S., Campôa, J., Pires, D., Branco, · J, Mota, · M M, Marques, · R, & Calvão, · T. (2025). Multiple factors associated with forest decline in the context of control measures for the pinewood nematode. *European Journal of Plant Pathology 2025 174:1*, *174*(1), 189–204. https://doi.org/10.1007/s10658-025-03122-0

Pontius, J., Schaberg, P., & Hanavan, R. P. (2020). Remote sensing for early, detailed, and accurate detection of forest disturbance and decline for protection of biodiversity. *Remote Sensing of Plant Biodiversity*, 121–154. https://doi.org/10.1007/978-3-030-33157-3_6

Proença, D. N., Grass, G., & Morais, P. V. (2017). Understanding pine wilt disease: roles of the pine endophytic bacteria and of the bacteria carried by the disease-causing pinewood nematode. *MicrobiologyOpen*, *6*(2), e00415. https://doi.org/10.1002/mbo3.415

R Core Team. (2025). *R: A Language and Environment for Statistical Computing*. https://www.R-project.org/

Richardson, A. E., Barea, J. M., McNeill, A. M., & Prigent-Combaret, C. (2009). Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. *Plant and Soil 2009 321:1*, *321*(1), 305–339. https://doi.org/10.1007/s11104-009-9895-2

Rumbou, A., Vainio, E. J., & Büttner, C. (2021). Towards the Forest Virome: High-Throughput Sequencing Drastically Expands Our Understanding on Virosphere in Temperate Forest Ecosystems. *Microorganisms*, *9*(8), 1730. https://doi.org/10.3390/microorganisms9081730

Santoyo, G. (2025). Advances in the Plant Microbiome: Rhizosphere, Endosphere, and Phyllosphere. *Microorganisms 2025, Vol. 13*, *13*(11). https://doi.org/10.3390/microorganisms13112581

Sayers, E. W., Bolton, E. E., Brister, J. R., Canese, K., Chan, J., Comeau, D. C., Connor, R., Funk, K., Kelly, C., Kim, S., Madej, T., Marchler-Bauer, A., Lanczycki, C., Lathrop, S., Lu, Z., Thibaud-Nissen, F., Murphy, T., Phan, L., Skripchenko, Y., … Sherry, S. T. (2022). Database resources of the national center for biotechnology information. *Nucleic Acids Research*, *50*(D1), D20–D26. https://doi.org/10.1093/NAR/GKAB1112

Shi, F., Ren, J., & Zhang, Y. (2024). Updates on Plants, Soil, Microorganisms, and Their Interactions in Forest Ecosystems. *Forests 2025, Vol. 16*, *16*(1). https://doi.org/10.3390/f16010058

Somogyi, Z., Koltay, A., & Móricz, N. (2018). *Forest health monitoring system in Hungary based on MODIS products*. http://erdoterkep.nebih.gov.hu/erdokar/index.htm

Sturrock, R. N., Frankel, S. J., Brown, A. V., Hennon, P. E., Kliejunas, J. T., Lewis, K. J., Worrall, J. J., & Woods, A. J. (2011). Climate change and forest diseases. *Plant Pathology*, *60*(1), 133–149. https://doi.org/10.1111/j.1365-3059.2010.02406.x

Teiba, I. I., El-Bilawy, E. H., Elsheery, N. I., & Rastogi, A. (2023). Microbial Allies in Agriculture: Harnessing Plant Growth-Promoting Microorganisms as Guardians against Biotic and Abiotic Stresses. *Horticulturae 2024, Vol. 10, 10*(1). https://doi.org/10.3390/horticulturae10010012

Tharanath, A. C., Upendra, R. S., & Rajendra, K. (2024). Soil Symphony: A Comprehensive Overview of Plant–Microbe Interactions in Agricultural Systems. *Applied Microbiology 2024, Vol. 4, Pages 1549-1567*, *4*(4), 1549–1567. https://doi.org/10.3390/applmicrobiol4040106

Thiel, S., Tschapka, M., Heymann, E. W., & Heer, K. (2021). Vertical stratification of seed-dispersing vertebrate communities and their interactions with plants in tropical forests. *Biological Reviews*, *96*(2), 454–469. https://doi.org/10.1111/brv.12664

Tobisch, T., & Kottek, P. (2013). *Forestry-related Databases of the Hungarian Forestry Directorate*.

Trivedi, P., Batista, B. D., Bazany, K. E., & Singh, B. K. (2022). Plant–microbiome interactions under a changing world: responses, consequences and perspectives. *New Phytologist*, *234*(6), 1951–1959. https://doi.org/10.1111/nph.18016

Umebayashi, T., & Fukuda, K. (2025). Resin canal leakage underlying xylem dysfunction in pine wilt disease. *BioRxiv*, 2025.06.06.656100. https://doi.org/10.1101/2025.06.06.656100

Vandenkoornhuyse, P., Quaiser, A., Duhamel, M., Le Van, A., & Dufresne, A. (2015). The importance of the microbiome of the plant holobiont. *New Phytologist*, *206*(4), 1196–1206. https://doi.org/10.1111/nph.13312

Vásquez-Grandón, A., Donoso, P. J., & Gerding, V. (2018). Forest Degradation: When Is a Forest Degraded? *Forests 2018, Vol. 9, 9*(11). https://doi.org/10.3390/f9110726

Vicente, C. S. L., Soares, M., Faria, J. M. S., Ramos, A. P., & Inácio, M. L. (2021). Insights into the Role of Fungi in Pine Wilt Disease. *Journal of Fungi 2021, Vol. 7, 7*(9). https://doi.org/10.3390/JOF7090780

Wexler, H. M. (2007). Bacteroides: the Good, the Bad, and the Nitty-Gritty. *Clinical Microbiology Reviews*, *20*(4), 593. https://doi.org/10.1128/CMR.00008-07

Wickham, H. (2025a). *forcats: Tools for Working with Categorical Variables (Factors)*. https://doi.org/10.32614/CRAN.package.forcats

Wickham, H. (2025b). *stringr: Simple, Consistent Wrappers for Common String Operations*. https://doi.org/10.32614/CRAN.package.stringr

Wickham, H., & others. (2019). Welcome to the tidyverse. *Journal of Open Source Software*, *4*(43), 1686. https://doi.org/10.21105/joss.01686

Yang, Y., Yang, Z., & Ferguson, D. K. (2024). The Systematics and Evolution of Gymnosperms with an Emphasis on a Few Problematic Taxa. *Plants 2024, Vol. 13, 13*(16). https://doi.org/10.3390/plants13162196

Zhang, M., Ji, C., Zhu, J., Wang, X., Wang, D., & Han, W. (2017). Comparison of wood physical and mechanical traits between major gymnosperm and angiosperm tree species in China. *Wood Science and Technology 2017 51:6, 51*(6), 1405–1419. https://doi.org/10.1007/s00226-017-0954-1

Zhang, Y., Ding, C. T., Jiang, T., Liu, Y. H., Wu, Y., Zhou, H. W., Zhang, L. S., & Chen, Y. (2023). Community structure and niche differentiation of endosphere bacterial microbiome in Camellia oleifera. *Microbiology Spectrum*, *11*(6), e01335-23. https://doi.org/10.1128/spectrum.01335-23

Zhu, L. hua, Ye, J., Negi, S., Xu, X. ling, Wang, Z. li, & Ji, J. yi. (2012). Pathogenicity of Aseptic Bursaphelenchus xylophilus. *PLoS ONE*, *7*(5), e38095. https://doi.org/10.1371/journal.pone.0038095

Zou, Y., Ge, X., Zong, S., & Newman, J. A. (2024). Climate change may make pine wilt disease more prevalent. *Journal of Applied Ecology*, *61*(12), 3028–3039. https://doi.org/10.1111/1365-2664.14801