University of Zagreb

Faculty of Science
Department of Biology

Lucia Pole

MICROBIAL COMMUNITIES AS POTENTIAL PREDICTORS OF FOREST SOIL HEALTH IN THE MEDITERRANEAN REGION OF CROATIA

DOCTORAL DISSERTATION

Zagreb, 2026

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Supervisors:
Armin Mešić, PhD – Scientific Advisor
Tijana Martinović, PhD – Research Associate

Zagreb, 2026

Sveučilište u Zagrebu

Prirodoslovno-matematički fakultet
Biološki odsjek

Lucia Pole

MIKROBNE ZAJEDNICE KAO POTENCIJALNI POKAZATELJI ZDRAVLJA ŠUMSKOG TLA U MEDITERANSKOJ REGIJI HRVATSKE

DOKTORSKI RAD

Mentori:
dr. sc. Armin Mešić – znanstveni savjetnik
dr. sc. Tijana Martinović – znanstveni suradnik

Zagreb, 2026.

This doctoral thesis was made at the Laboratory for Biological Diversity, Division for Marine and Environmental Research at Ruđer Bošković Institute and Laboratory of Environmental Microbiology at the Institute of Microbiology of the CAS in Prague, under the supervision of Armin Mešić and Tijana Martinović, within the FunMed project (Environmental drivers of fungal community composition in the Mediterranean region of Croatia, HRZZ-IP-2022-10-5219) and ForFungiDNA project (Enhancement of Croatian forest ecosystem services through assessment of fungal diversity based on DNA barcoding, HRZZ-IP-2018-01-1736) funded by the Croatian Science Foundation and by the Public Institution Kamenjak under the project grant Fungal diversity research and monitoring using eDNA metabarcoding. The dissertation was completed as part of the Doctoral Programme in Biology at the University of Zagreb, Faculty of Science, Department of Biology.

About supervisors

Armin Mešić, PhD – Scientific Advisor

Armin Mešić, PhD, graduated in biology (ecology) from the Faculty of Science, University of Zagreb, in 1999. He completed his doctoral studies and obtained his PhD in 2006 at the Faculty of Science, University of Zagreb, in natural sciences, biology (mycology). Since 2000, he has been employed at the Ruđer Bošković Institute (RBI) in Zagreb. Since 2019, he has been head of the Laboratory for Biological Diversity, Department of Marine and Environmental Research. In 2023, he was appointed as Senior Scientist. He is the founder and secretary of the Croatian Mycological Society and curator of the national collection of fungal samples, the Croatian National Fungarium (CNF), hosted at the Ruđer Bošković Institute. His main scientific interests are the taxonomy, ecology, phylogeny, and biodiversity of fungi and other microorganisms (bacteria), using integrative taxonomic and eDNA metabarcoding methods. He has described 24 species and one genus of fungi new to science. He was Principal Investigator in 14 and collaborator in 39 national and international scientific and expert projects. He has published 70 scientific articles in international peer-reviewed journals. He has successfully supervised one undergraduate (BSc), four graduate (MSc), and two doctoral (PhD) students. He is a member of the editorial board of the internationally peer-reviewed journal Sydowia (Austria) and reviewer of over 150 scientific papers for more than 30 international scientific journals. He serves in advisory roles within international expert bodies: as curator for the taxonomy of the phylum Basidiomycota (Fungi) on the Outline of Fungi website, as a member of the international expert consortium "Consortium for Fungi and Fungus-like Classification", and as a member of the expert group preparing the proposal for the IUCN Global Red List of Threatened Fungi Species within the International Union for Conservation of Nature (IUCN), Cambridge, United Kingdom. Since 2017, he has served as a scientific consultant to the Institute for Medical Research and Occupational Health (Zagreb) for the identification of fungi in poisoning cases. He has been a guest lecturer at the Faculty of Agriculture and the Faculty of Science, University of Zagreb. He has also distinguished himself in the promotion and popularisation of science through public lectures and participation in radio and television programmes.

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She currently works at the Institute of Microbiology in Prague and is also affiliated with the Department of Forest Physiology and Genetics at the Slovenian Forestry Institute in Ljubljana, where she has been active since 2011. Since 2023, she has also been an external associate at the Ruđer Bošković Institute in Zagreb. She is a member of several international scientific organisations and has participated in over 30 international conferences. She has contributed as a team member and collaborator to more than 10 research projects, including several LIFE, COST, and Horizon 2020 projects, and has served as the principal investigator on a project funded by the Grant Agency of Charles University. In addition, she has participated as a guest lecturer or teaching assistant in several university courses and has mentored visiting students and hosted researchers.

To date, she has authored 21 publications in peer-reviewed journals. Her research focuses on the environmental drivers of forest soil microbial communities, their role in ecosystem functioning and services, and their responses to natural and anthropogenic disturbances. A central theme of her work is understanding how microbial communities support forest resilience under disturbance pressures. More recently, she has contributed to the development of microbial soil monitoring protocols to improve soil health assessments and climate-smart management of European forests.

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University of Zagreb
Faculty of Science
Department of Biology

Doctoral thesis

Microbial communities as potential predictors of forest soil health in the Mediterranean region of Croatia

Lucia Pole, Ruđer Bošković Institute, Croatia

Soil microbial communities play an important role in ecosystem functioning, including nutrient cycling and organic matter decomposition. However, a comprehensive understanding of soil microbial communities in Mediterranean forest ecosystems remains lacking. This study investigated drivers of soil microbial community diversity and composition in protected Mediterranean forests in Croatia and evaluated microbial communities as predictors of soil health across mainland and island sites. Soil bacterial and fungal communities were characterised using Illumina sequencing (16S rRNA, ITS2), and analyses of soil physical and chemical properties, enzyme activities, and microbial biomass were conducted. Results showed that spatial structuring consistently emerged as the strongest driver of both fungal and bacterial communities, whereas the dominant vegetation effect was pronounced for ectomycorrhizal (EcM) fungi. Island size did not consistently predict diversity, and geographic distance showed an unexpected positive relationship with fungal richness. Almost complete loss of EcM fungi was observed with increasing distance from the mainland, accompanied by increased plant pathogen abundance on more isolated islands. The abiotic soil health index was calculated from soil physical and chemical properties measured in this study and used for soil health prediction. Enzyme activities and bacterial community composition were the strongest predictors, while anthropogenic disturbance likely influenced soil health indirectly by restructuring microbial communities. This study indicates that microbial communities can serve as reliable predictors of soil health, with implications for conservation management and soil monitoring in the studied Mediterranean ecosystems.

PhD thesis contains: 142 pages, 20 figures, 20 tables, 365 references, original in English
Keywords: fungi, bacteria, Mediterranean forest, islands, indicators, Random Forest

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Sveučilište u Zagrebu Doktorski rad
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Biološki odsjek

Mikrobne zajednice kao potencijalni pokazatelji zdravlja šumskog tla u Mediteranskoj regiji Hrvatske

Lucia Pole, Institut Ruđer Bošković, Hrvatska

Mikrobne zajednice u tlu imaju važnu ulogu u funkcioniranju ekosustava, uključujući kruženje hranjivih tvari i razgradnju organske tvari. Međutim, sveobuhvatno poznavanje mikrobnih zajednica tla u mediteranskim šumskim ekosustavima je nedovoljno istraženo. U ovom istraživanju analizirani su različiti čimbenici koji utječu na raznolikost i strukturu mikrobnih zajednica u tlima mediteranskih šuma u Hrvatskoj te njihov potencijal kao pokazatelja zdravlja tla. Zajednice bakterija i gljiva analizirane su sekvenciranjem na Illumina platformi (16S rRNA and ITS2), a tlo različitim fizikalnim i kemijskim svojstvima. Uz to procijenjena je mikrobna enzimatska aktivnost i mikrobna biomasa. Rezultati su pokazali da na strukturu i raznolikost mikrobnih zajednica najviše utječu prostorne promjene, dok je na ektomikorizne gljive prevladavo utjecaj dominatne vegetacije. Utjecaj veličine otoka nije bio značajan za mikrobnu raznolikost, dok je geografska udaljenost među otocima pokazala neočekivan pozitivan utjecaj na bogatstvo vrsta gljiva. Uočen je gotovo potpuni gubitak ektomikroznih gljiva s povećanjem udaljenosti od kopna, istovremeno popraćen s porastom biljnih patogena na izoliranim otocima. Abiotski indeks zdravlja tla izračunat je na temelju izmjerenih fizikalnih i kemijskih svojstava tla u ovom u ovom istraživanju. Mikrobna enzimatska aktivnost i sastav bakterijskih zajednica pokazali su se kao najvažniji pokazatelji zdravlja tla u prediktivnim modelima, dok je ljudsko djelovanje neizravno utjecalo na zdravlje tla kroz restrukturiranje mikrobnih zajednica. Ovo istraživanje ukazuje na to da mikrobne zajednice mogu biti pouzdani pokazatelji zdravlja tla, s implikacijama za gospodarenje i očuvanje istraživanih mediteranskih ekosustava, kao i za monitoring tla.

Rad sadrži: 142 stranice, 20 slika, 20 tablica, 365 literaturnih navoda, jezik izvornika engleski
Kjučne riječi: gljive, bakterije, mediteranske šume, otoci, indikatori, Random Forest

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1. Introduction

The Mediterranean biogeographic region is recognised as one of the global biodiversity hotspots, with accelerating pressures from climate change, land-use transformation, and increased anthropogenic disturbance. Soil is among the most complex and biodiverse ecosystems, where microorganisms (fungi and bacteria) mediate nutrient cycling and organic matter decomposition, sustaining terrestrial ecosystem functioning. Despite their ecological importance, soil microbial communities remain incompletely understood, particularly in Mediterranean forest ecosystems, where a combination of climatic stress, land-use legacies, resource scarcity, and exceptional biodiversity amplifies ecological complexity. Soils are the foundation of forest ecosystems; thus, their degradation threatens essential ecosystem services.

In this dissertation, soil microbial communities from Mediterranean forests within the protected areas of Donji Kamenjak and Brijuni National Park (Istria, Croatia) were analysed. First, environmental factors that shape soil microbial community diversity and composition were investigated. Second, the influence of intrinsic island characteristics, together with tourism pressure on microbial communities across an island-mainland gradient was examined. Thirdly, and most significantly, it was evaluated whether soil microbial communities could serve as reliable and independent predictors of soil health, quantified using the abiotic soil health index. Together, this constitutes ecologically relevant research, from understanding what structures soil microbial communities to understanding how these microbial communities can inform us about the health of the ecosystem they inhabit.

2. Literature overview

2.1. Mediterranean region

The Mediterranean region or Mediterranean Basin is characterised by mosaic ecosystems with exceptional biodiversity, endemic species, and a millennia-long history of anthropogenic influence (Coll et al., 2012; Lejeusne et al., 2010). Due to its location at the crossroads of three continents, Africa, Asia, and Europe, the Mediterranean region is renowned for its significant contributions to the global economy and trade. Its coast supports a high density of inhabitants and is one of the most popular tourist destinations in the world (Coll et al., 2010). Traditional classification defines the Mediterranean climate as a mid-latitude temperate climate with a dry summer season, which can be either warm or hot, with cool and humid winters, as present around the majority of the Mediterranean Sea. Precipitation across the region is unevenly distributed; scarce and irregular in many southern areas, where water availability is already a problem (Coll et al., 2010; Köppen, 1900).

The Mediterranean region represents one of the 34 global hotspots of biological diversity, with the most diverse biome in Europe (Barredo et al., 2016; Myers et al., 2000). Almost half of the animals and plants, as well as more than half of the habitats specified in the European Union Habitat Directive (Council of the European Communities, 1992), occur in the Mediterranean region. However, models project an 11-15% decline in these valuable habitats by the end of this century, mainly due to severe desertification (Anav & Mariotti, 2011; Barredo et al., 2016). Various scientific studies concur that in the 21st century, the Mediterranean region will experience a decrease in rainfall and widespread warming over most of its areas (Gualdi et al., 2013; Planton et al., 2012; Ulbrich et al., 2013). These aspects of climate change pose a high risk to the environment and society, through negative impacts on human health (Diffenbaugh et al., 2007), plants (Gordo & Sanz, 2010), agriculture (Bindi & Olesen, 2011; Tanasijevic et al., 2014) and the marine ecosystem (Lejeusne et al., 2010; Rivetti et al., 2014). Consequently, according to current observations and future projections (Urdiales-Flores et al., 2023; Zittis et al., 2019), the Mediterranean region is one of the most vulnerable regions highly responsive to global climate change, with warming projected to continue at a rate exceeding the global avarage (Lionello & Scarascia, 2018).

Besides facing climate change challenges, the Mediterranean region is under constant increasing threat from direct and indirect human pressures. Specifically, during the past decades, tourism, urban sprawl, agricultural expansion, and afforestation have strongly shaped these landscapes (Malavasi et al., 2016). A long history of land-use in the Mediterranean region has created valuable cultural landscapes, conversly, human activities have caused significant degradation of soil and water resources (Karamesouti et al., 2015; Tieskens et al., 2017). Owing to its unique cultural and environmental characteristics, along with long land-use history, the Mediterranean region is characterised by a diversity of land systems of varying intensities and levels of functionality (Malek et al., 2018). Mosaic landscapes have been of great value for the region, wherein various activities, including livestock grazing, agricultural production, and forestry, co-occur, and many of which are associated with biodiversity values (Lindner et al., 2014).

2.2. Mediterranean forest ecosystems

Forests provide several ecosystem services, such as carbon storage and climate regulation through carbon exchange between soil and atmosphere. Specifically, Mediterranean forests sequester approximately 5 billion tonnes of carbon, representing 1.6% of global forest carbon (FAO, 2015). In 2020, forests and other wooded lands covered ~123.5 million ha in the Mediterranean region, ~99.2 million ha were covered by forest, and 24 million ha were covered by other wooded lands (de Dato et al., 2025). Mediterranean forests are characterised by a pronounced precipitation deficit during warm summers and nutrient-poor soils, resulting in stress conditions for vegetation (Gauquelin, 2012; Gauquelin et al., 2018). Forest management is typically multifunctional, providing non-wood products (such as cork, pine nuts, resin, mushrooms, and berries), protection from land degradation, water preservation, recreation, and climate mitigation through carbon storage. Regional and local complexity of Mediterranean forest ecosystems arises from both natural and anthropogenic influences (high variation in hydroclimate, morphology, geography, and historical land-use), all of which contribute to an exceptional biodiversity both above- and belowground (Gauquelin et al., 2018; Gravuer et al., 2020; Lefèvre & Fady, 2016). However, these ecosystems have been under constant threat from accelerated climate change, land-use, wildfires, and the introduction and expansion of invasive species (Gauquelin et al., 2018; Underwood et al., 2009). Climate change (warming, increased aridity, frequent and prolonged droughts) negatively affects forests through alterations in vegetation physiology and phenology, affects forest composition, and contributes to forest dieback (Bellard et al., 2012; Carnicer et al., 2011; Gentilesca et al., 2017; Ogaya & Peñuelas, 2021). Land-use change exerts both positive and negative, direct and indirect impacts, depending on the specific area. Land-use change increases the probabilities of soil erosion and, due to landscape homogenisation, increases the frequency of wildfires (García-Ruiz et al., 2020; Karamesouti et al., 2015; Queiroz et al., 2014; Stoate et al., 2009). Over a decade (2011 – 2023), wildfires burned on average ~600,000 ha of land annually, presenting a severe threat to Mediterranean forests and ecosystem services (Baudena et al., 2023). Cattle browsing and grazing, when conducted in a controlled, organised manner, can help reduce fire risk. However, they can also contribute to soil erosion and degradation (Schoenbaum et al., 2018; Teruel-Coll et al., 2019).

2.2.1. Vegetation and soil of Mediterranean forest ecosystems

Assessments of vegetation diversity are of critical importance for revealing changes in habitats and ecosystems functioning due to pronounced anthropogenic or natural drivers taking place in Mediterranean forests (Chiarucci et al., 2017). Mediterranean vegetation is dominated by woody plants with specific and conservative traits, including slow growth and high sclerophylly associated with water and nutrient deficits (Sardans & Peñuelas, 2013). In addition, the development of deep and extensive root systems is defining characteristic of Mediterranean vegetation, allowing the uptake of water from deep soil layers during periods of drought (Baldocchi & Xu, 2007; Hernandez-Santana et al., 2008; Padilla & Pugnaire, 2007). This adaptation enables the occupation of different soil layers and reflects the large capacity of coexisting species to minimise competition for soil resources, with a positive effect on the notable cover and species diversity of vegetation in the region (Mattia et al., 2005; Mereu et al., 2009).

Typical Mediterranean forest vegetation comprises deciduous and evergreen broadleaved trees, with the *Quercus* genus as the most representative*,* with over 20 species (e.g., *Q. cerris*, *Q. ilex*, *Q. pubescens*, *Q. suber*). *Quercus* species are distributed throughout the Mediterranean region owing to their adaptability to climate variability. Furthermore, Mediterranean vegetation is characterised by conifers, particularly *Pinus* species (e.g., *P. halepensis*, *P. nigra*, *P. pinaster*, *P. pinea*) (Noce et al., 2017; Noce & Santini, 2018). Pronounced human activities, through forest management, have a great influence on Mediterranean landscapes, contributing to forest degradation and the development of shrublands (e.g., garrigue with *Cistus* spp.) (Blondel, 2006; Ljubičić et al., 2020; Médail et al., 2019; Peñuelas & Sardans, 2021). Vegetation taxa, each with distinct ecological roles and successional stages, collectively contribute to the conservation and functioning of Mediterranean ecosystems. *Cistus* spp. as shrubs play a key role in the formation of characteristic maquis or garrigue and are considered pioneer species capable of colonising early successional habitats, especially in areas affected by overgrazing, forest fires, or other environmental disturbances. On the other hand, they are often involved in the spread of forest fires as they synthesise oils and resins that are highly flammable (Kielar et al., 2025; Zalegh et al., 2021). Aleppo pine (*P. halepensis*), particularly well adapted to the fire regimes and water deficit in the Mediterranean region, with rapid colonisation after fire due to its serotinous cones (Elvira et al., 2025), plays a crucial role in afforestation (Novak et al., 2016), and in promoting microhabitats favourable to perennial grasses (Maestre et al., 2003). Holm oak (*Q. ilex*) is a keystone species supporting biodiversity and ecosystem services, primarily found in western Mediterranean countries (Suicmez & Avci, 2023). Its ability to resprout after disturbances such as fires, grazing, or drought has allowed its population to persist over time despite extensive human activities (Barbeta & Peñuelas, 2016). Climatogenic vegetation of native Holm oak forests along the Croatian coast is mostly present in its degraded forms (maquis, garrigue) or has been replaced by other taxa more tolerant of harsh climatic conditions, such as Aleppo pine. Other species, such as *Carpinus* sp., *Ostrya* sp., *Acer* sp., *Alnus* sp., *Ulmus* sp., *Fraxinus sp*., *Populus* sp., and many species in the Rosaceae family, contribute to the formation of typical deciduous mixed Mediterranean forests of very high vegetation diversity (Noce & Santini, 2018).

Mediterranean forests have a wide variety of soils that are generally leached and of medium to low fertility, due to water and nutrient deficits, with nitrogen and phosphorus as the most frequent limiting nutrients. Therefore, soil moisture and nutrient supply, and their accessibility to vegetation, strongly influence Mediterranean forest ecosystems. Main observed characteristics of Mediterranean soils are: 1) erosion linked to the sparse soil cover and high impact of human activities; 2) low rate of organic matter in soils, especially in arid areas; 3) rubification driven by iron oxidation under specific Mediterranean climatic conditions causing the red colour of some Mediterranean soils and 4) discontinuous functioning in litter decomposition and the activity of soil microorganisms involved in key processes, due to drought season (Gauquelin et al., 2018).

Regarding soil properties, several physical properties cannot be significantly modified or changed (e.g., texture). Generally, Mediterranean soils have a higher clay content than those in temperate regions, and their pH ranges from slightly acidic to moderately alkaline. While nitrogen and phosphorus are frequent limiting nutrients in Mediterranean forests (Sardans & Peñuelas, 2013), Mediterranean soils typically contain adequate levels of elemental Ca and Mg. Conversely, deficiencies of elemental Fe, Cu, Mn, Zn, and B are frequent in some cultivated and calcareous soils. Salinisation is a major problem in Mediterranean soils, which is occasionally accompanied by specific toxicity issues due to poor irrigation and low precipitation. However, the principles for good management of irrigation have been known for decades, yet salinisation continues to be a major issue in Mediterranean soils (Daliakopoulos et al., 2016; Du et al., 2020; Sardans & Peñuelas, 2013; Torrent, 2004).

The relationship between vegetation and soil is bidirectional, as described by the plant-soil feedback concept: vegetation not only responds to existing soil properties but also actively modifies them through direct and indirect processes (Mariotte et al., 2018). This interaction can produce either positive (soil changes promote plant growth and health) or negative (plants are harmed by altered soil properties) outcomes. These dynamics influence soil biota, leading to shifts in pH, organic matter, enzyme activities, and nutrient availability. In turn, these changes reshape the composition and functioning of soil microbial communities (Brunel et al., 2020; Frouz, 2024; Labouyrie et al., 2023; Liu et al., 2023).

2.3. Importance of forest soil microbial communities

Due to pronounced climatic stressors, nutrient limitation, and anthropogenic pressure in Mediterranean forests, soil microbial communities are of great importance for ecosystem resilience and health (Curiel Yuste et al., 2014; Delcourt et al., 2023). Composition and activity of soil microbial communities in Mediterranean forests are strongly determined by changes in vegetation, and in general, soil microbial activity is negatively affected by the degradation of vegetation cover (Garcia et al., 2002). Soil microbial communities represent a link between vegetation and ecosystem processes, with a crucial importance in global carbon sequestration and nutrient cycling (Grosso et al., 2018).

Forest soils are among the most diverse habitats for microorganisms (Hardoim et al., 2015; Nacke et al., 2012), supporting a wide range of microenvironments which may be only micrometres to millimetres apart, yet harbour different microbial communities and vary in rates of microbial activity (Fierer, 2017). Forest soils are characterised by strong vertical stratification, with the content and quality of organic matter, as well as microbial biomass, respiration, and extracellular enzyme activity, decreasing with soil depth (Lladó et al., 2017). Fungi and bacteria are the dominant microorganisms in forest soil and play essential, specific roles in nutrient cycling, decomposition, and overall forest ecosystem health. It is therefore crucial to consider fungi and bacteria not separately, but as interacting components of a complex soil microbial network (Onet et al., 2025). Forest soils harbour abundant and diverse saprotrophic and mycorrhizal fungal communities. Saprotrophic fungal communities are important as the main decomposers due to their wide range of extracellular enzymes, which allow them to degrade recalcitrant plant biomass efficiently (Bomble et al., 2017; Rousk & Bååth, 2011). Mycorrhizal fungal communities are root-associated fungi that form symbiotic relationships with >90% of terrestrial plant species, enhancing water and nutrient uptake (Sharma et al., 2013) and increasing plant biomass (Lagueux et al., 2021). Moreover, mycorrhizal fungi play a pivotal role in the mobilisation and sequestration of nitrogen and phosphorus, and are also responsible for the transport of carbon in soil (Averill et al., 2014; van der Heijden et al., 2015). Bacteria, on the other hand, represent another important, although often less explored, part of microbial communities in forest soils (Lladó et al., 2017). Bacteria contribute to a wide range of essential soil processes, primarily in the nitrogen cycle (Nelson et al., 2016) and in the decomposition of organic matter (Štursová et al., 2012). Most forest soils harbour bacterial taxa from phyla Proteobacteria, Acidobacteria, Actinobacteria, Bacteroidetes, and Firmicutes (Lauber et al., 2009). Due to the sharp vertical gradient in forest soils, the organic layer is richer in Proteobacteria and Bacteroidetes (Baldrian et al., 2012), which are also known to preferentially utilise easily accessible carbon substrates. In contrast, the mineral layer is richer in Firmicutes and Chloroflexi, which can utilise recalcitrant carbon substrates and inorganic nutrients (Lladó et al., 2017). Forest soil bacterial communities comprise abundant mineral-weathering taxa, which are important for nutrient-poor soils (Uroz et al., 2011), and taxa associated with EcM mycelia (Izumi et al., 2008). Also, bacterial communities in forest soil exhibit high spatial heterogeneity (Baldrian & Větrovský, 2012) and patchiness in activity (Šnajdr et al., 2008b).

Soil microbial communities are directly and indirectly linked to the soil processes, with the ability to produce and consume atmospheric trace gases (e.g., hydrogen, carbon dioxide, nitric oxide, nitrous oxide, methane, and other volatile organic compounds). Fungi and bacteria facilitate the transformation and mobilisation of essential nutrients such as nitrogen, phosphorus, and carbon. In nitrogen cycling, microorganisms entirely mediate nitrogen fixation, nitrification, and denitrification, regulating soil N availability (Stein & Klotz, 2016). In soil, all microbial communities are in complex interactions, which potentially contribute to the activity and abundance of nitrogen cycling microorganisms and subsequently determine variation in the nitrogen cycle across tree stands (Kelly et al., 2021; Qin et al., 2019; Ribbons et al., 2018). Soil bacteria dominate the nitrogen cycle compared to fungi (Nelson et al., 2016), but fungi play an important role in the nitrogen cycle as they can mineralise organic N and assimilate inorganic N to build their biomass, although their specific role in soil denitrification remains poorly understood (Aldossari & Ishii, 2021; Shoun et al., 2012). In the nitrogen cycle, soil microbial communities are important for nitrification and denitrification, involving the oxidation of ammonium to nitrite followed by the oxidation of nitrite to nitrate, resulting in a net loss of nitrogen from the ecosystem through leaching and/or denitrification (Prosser & Nicol, 2008, 2012).

In addition to nitrogen, phosphorus is another essential nutrient, and its availability is critical for plant growth (Wu et al., 2022). However, most of the phosphorus in forest soils is present in an insoluble form, thus only a small proportion is available for direct absorption and utilisation by plants and soil microbial communities (Han et al., 2021). Soil microbial communities are an important biotic factor for the phosphorus availability and play a crucial role in the phosphorus cycle (Pastore et al., 2020). For example, mycorrhizal and saprotrophic fungi, as well as phosphate-solubilising bacteria, can transform insoluble phosphorus to a bioavailable form (Baldrian, 2017; Koukol et al., 2006). Soil microorganisms thus contribute to phosphorus dynamics by effectively mineralising organic forms, solubilising precipitated phosphorus, and also immobilising excess phosphorus in biomass, which is then retained in necromass after cell death (Chen et al., 2023).

Another important role of soil microbial communities in forests is mediating organic matter turnover and stabilising carbon pools (Mason et al., 2023; Schimel & Schaeffer, 2012). They facilitate fundamental carbon cycle processes, namely the sequestration of atmospheric carbon and subsequent degradation of organic substrates. Soil microbial decomposition is a crucial process for transforming organic material into forms that can be utilised by other organisms, whereby microbes partition some carbon between biomass incorporation and CO_2

respiration (Hu et al., 2022; Liang & Balser, 2011; Trumbore, 2006). Soil microorganisms involved in the carbon cycle are extremely diverse, but fungi and bacteria dominate the decomposition processes, exhibiting distinct enzymatic abilities and metabolic requirements. Accordingly, some general patterns exist in distinguishing soil microbial communities into fungal and bacterial decomposers, with fungal communities often characterised as K-strategists and bacteria as r-strategists. In particular, soil fungal communities typically have lower nutrient requirements and can decompose complex substrates due to their broad enzymatic capabilities, whereas bacteria are characterised by higher nutrient requirements and favour labile substrates (Blagodatskaya & Kuzyakov, 2008; Rousk & Frey, 2015). Due to their nutrient demands, variety of extracellular enzymes and filamentous nature, fungi are considered as the primary decomposer of complex recalcitrant organic compounds originating from plant litter and deadwood, such as cellulose, hemicellulose, and lignin, whereas bacterial decomposers typically target simple organic compounds (Bomble et al., 2017; Rousk & Bååth, 2011; Rousk & Frey, 2015). In addition, ectomycorrhizal fungi play a major role in the forest carbon cycle by mediating the transfer of carbon from host photosynthesis into soil through their mycelium (Algora Gallardo et al., 2021; Žifčáková et al., 2017). However, increasing evidence shows that bacteria are also involved in the decomposition of complex carbon compounds in plant biomass (López-Mondéjar et al., 2020; Štursová et al., 2012).

Even though microbial community composition shifts in response to a variety of disturbances (Bevivino et al., 2014; De Vries & Shade, 2013; Jiang et al., 2021; Sun et al., 2020), ecosystem function could remain relatively stable due to functional redundancy when there is a coexistence of taxa with the same functional traits (Chen et al., 2022a; Eisenhauer et al., 2023). In particular, ecosystem functions could be maintained under disturbances through the selection of taxa with similar effect traits but different response traits (Violle et al., 2012). On the other hand, a lack of functional redundancy implies that functions are easily lost under disturbances, through the loss of taxa solely responsible for specific functions (Philippot et al., 2021). Soil microbial communities respond differently to disturbances (Jurburg et al., 2024; Shade et al., 2012), which suggests that functional redundancy also varies, from microbial communities with low redundancy and more prone to shift under disturbances to high redundancy and potentially more stable and resilient under disturbances (Ramond et al., 2025). Soil microbial communities that perform broad soil processes as “generalists”, such as carbon decomposition, are commonly phylogenetically widespread (Crowther et al., 2019) and are associated with high functional redundancy (Beier et al., 2017; Rivett & Bell, 2018). On the other hand, narrow functions in soil processes, such as nitrification, performed by “specialists”, may be restricted to a few taxa and are hypothesised to exhibit lower redundancy than “generalists” (Rocca et al., 2015). Consequently, changes in soil processes and ecosystem functioning will not be disrupted by shifts in the community composition of “generalists” with broader functions, but the loss or exclusion of “specialists” with narrow functions can significantly reduce ecosystem functioning (Grządziel, 2017). Therefore, understanding how microbial community composition and ecosystem functioning interact remains a major goal, especially in the face of climate change (Cavicchioli et al., 2019).

2.3.1. Soil microbial biomass

The importance of soil microbial communities in ecosystem functioning has caused great interest in determining microbial properties (Azam et al., 2003), such as microbial biomass or enzyme activities. Soil microorganisms are the living component of soil organic matter (SOM), and soil microbial properties exhibit rapid responses to environmental shifts, serving as senstitive indicators of soil health. Soil microbial biomass, comprising 1-4% of SOM, represents an important source of bioavailable nitrogen, phosphorus, and sulphate, which are held in a form protected from loss due to leaching or fixation (Babur & Dindaroglu, 2020; Brookes, 2001).

Soil microbial biomass incorporates ecologically and functionally relevant information for the ecosystem. Using specific methods for the differentiation of fungal and bacterial biomass (phospholipid-derived fatty acids, gene quantification), it is possible to determine the balance between fungal and bacterial decomposers given their distinct functional capabilities (metabolism and life strategies) (Strickland & Rousk, 2010). Fungal biomass usually exhibits a higher C/N ratio than bacterial biomass, therefore fungi often thrive in soils with elevated C/N ratios due to their ability to degrade recalcitrant carbon compounds (Fierer et al., 2009). Even though bacterial biomass is rich in nitrogen, its rapid turnover promotes fast nitrogen cycling and potential losses, whereas slower fungal turnover increases carbon stabilisation and nitrogen retention in soils (Frey et al., 2004; Strickland & Rousk, 2010). Soil fungal biomass relative to soil bacterial biomass, expressed as fungal-to-bacterial (F/B ratio) (Malik et al., 2016; Strickland & Rousk, 2010) is widely used in soil ecology to assess ecosystem functioning, particularly in the context of land-use and carbon sequestration (Strickland & Rousk, 2010). Soils subject to intensive management or disturbance typically exhibit lower F/B biomass ratios compared to less disturbed soils. This is primarily due to tillage, heavy fertilisation, and lower C/N ratios that favour bacterial proliferation over fungal growth. Lower F/B biomass ratios in intensively managed soils correlate with reduced carbon sequestration capacity. In contrast, soils with relatively higher fungal biomass are generally associated with slower decomposition rates and a greater potential for organic carbon accumulation, although this relationship depends on environmental context (Bruni et al., 2025; Six et al., 2006).

2.3.2. Soil microbial enzymes

Soil microbial enzymes play a significant role in microbial processes and are fundamental drivers of soil ecosystem functioning. They catalyse the breakdown of complex organic polymers like cellulose, hemicellulose, and lignin into forms accessible for microbial and plant uptake, thereby driving key biogeochemical cycles such as carbon and nitrogen transformations (Baldrian, 2009; Burns et al., 2013). A common misconception is that soil enzyme assays are directly equivalent to microbial activity, however, this is not always the case as microbial enzymes can be extracellular or intracellular (Bhardwaj et al., 2024; Rahul et al., 2022). Extracellular microbial enzymes constitute the majority of enzymes present in soil and are often attached to the soil particles, while intracellular enzymes are present only in living and metabolically active cells, and are released after cell death (Ch et al., 2017; Saha et al., 2000). In many studies, soil enzyme assays predominantly reflect extracellular enzymes, referring to catalytic enzymes of microbial origin that are no longer under the control of viable cells, and therefore may not directly represent current microbial activity. As stated by Nannipieri et al. (2018), by enzyme activity assays, it is not possible to determine how much of the activity originates from enzymes produced by viable, active cells or from stabilised enzymes accumulated in soil over a long period. Rather, with enzyme assays, we analyse extracellular enzymes to obtain brief insights into the potential of soil microbial communities to catalyse specific reactions. Despite these limitations, soil enzyme activities are frequently used as proxies for microbial functional potential in the cycling of key nutrients.

Acid phosphatases are produced both by fungi and bacteria and have a key role in phosphorus cycling by transforming complex and unavailable forms of organic phosphorus into assimilable phosphate under acidic conditions (Caldwell, 2005). Alpha (α)-glucosidase is an integral part of the carbon cycle, mediating polysaccharide degradation (e.g., maltose or starch) to monosaccharide glucose, and it is common in both fungi and bacteria (Daunoras et al., 2024; Seidl, 2008). Beta (β)-galactosidase, another important enzyme in the carbon cycle produced by fungi and bacteria, is involved in hydrolysing the disaccharide lactose to glucose and galactose (Neti & Peshwe, 2025). Cellulases are a group of several enzymes that can catalyse the decomposition of cellulose in dead plant biomass, and among the most important enzymes in this group are cellobiohydrolase and β-glucosidase (Baldrian & Valášková, 2008). Cellobiohydrolase is important for the degradation of cellulose to cellobiose (Baldrian & Valášková, 2008; Horn et al., 2012), whereas β-glucosidase catalyses the hydrolysis of cellobiose to glucose. Cellobiose is a largely available substrate, and β-glucosidases are produced by both fungi and bacteria (Baldrian & Valášková, 2008; Lynd et al., 2002). Fungi may have a higher capacity to produce cellulases and/or more efficiently contribute to the decomposition of complex plant biomass than bacteria (Boer et al., 2005; Mondal et al., 2022). However, with advanced approaches such as stable isotope probing and metagenomics, there is clear evidence of the active contribution of bacteria in the degradation of cellulose in forest soils (López-Mondéjar et al., 2020; Martinovic et al., 2022; Štursová et al., 2012).

Each enzyme binds to its specific substrate via an active site, forming a temporary enzyme-substrate complex. When the enzymatic reaction is done, the released products contribute to the nutrient pool (Saha et al., 2000), nutrient cycling, and ecosystem functioning. Soil microbial enzymes, as one of the microbial properties, along with soil microbial biomass, provide reliable insights into biological and biochemical changes since their dynamics reflect subtle modifications in the soil environment (Erdel et al., 2023; Li et al., 2019a). Consequently, soil microbial enzyme assays serve as robust tools for assessing soil degradation/soil health caused by a wide range of disturbances.

2.4. Drivers of forest soil microbial communities

Forest soils are of great importance, acting as both sinks and sources of carbon, while exhibiting high sensitivity to climate change. Therefore, forest soils became the key target for microbial ecologists in recent years (Lladó et al., 2017). Fungi and bacteria are the key players in regulating soil processes for maintaining forest ecosystem functions (Cavicchioli et al., 2019), responding differently to environmental changes and disturbances. For example, fungal communities respond more strongly to vegetation than bacterial communities (Martiny et al., 2006; Zinger et al., 2011). A comprehensive understanding of how and which factors drive the composition of forest soil microbial communities is essential for modelling environmental changes and disturbances that impact forest ecosystem services (Baldrian et al., 2012; Romanowicz et al., 2016). However, a significant level of uncertainty regarding the potential drivers of soil microbial community composition is still present, as drivers and their relative contribution differ from local to continental scales or differ based on the climate properties or edaphic factors (Fierer et al., 2012; Plassart et al., 2019; Rousk et al., 2010). Therefore, it is also important to highlight the importance of the spatial structuring of soil microbial communities (Wilhelm et al., 2017).

Nevertheless, all findings provide valuable information about drivers of soil microbial community composition, particularly when interpreted within a specific environmental context. In Mediterranean forests, which are shaped by constraints such as droughts, heat waves, low nutrient availability, and recalcitrant organic compounds, the drivers of soil microbial communities remain insufficiently understood (Brunel et al., 2020).

2.4.1. Spatial structuring of soil microbial communities

Spatial structuring, as a driver of soil microbial community composition, often accounting for variation independent of measured environmental variables (Martiny et al., 2006). This spatial component reflects multiple underlying processes, including dispersal limitation, historical contingency, stochastic colonisation events, priority effects, or unmeasured fine-scale environmental heterogeneity (Hanson et al., 2012). Fungal communities, particularly EcM fungi (Odriozola et al., 2024), frequently exhibit strong spatial structuring due to dispersal limitation and obligate dependence on host plants and their distribution patterns (Bahram et al., 2012; Peay et al., 2012). Although many fungal species produce abundant spores capable of long-distance dispersal, effective colonisation and establishment are constrained by host availability, priority effects, and local environmental conditions, resulting in pronounced distance-decay relationships where compositional similarity often decreases with geographic distance (Hanson et al., 2012; Odriozola et al., 2024). Bacterial communities also display spatial structuring, though not as pronounced as fungal communities, and more often vary with spatial scales and environmental conditions (Peguero et al., 2022; Zinger et al., 2019). The similarity of bacterial communities, even at regional scales, is usually attributed to their enhanced dispersal capacity, arising from small propagules, large populations, and short generation times (Hanson et al., 2012; Martiny et al., 2006; Peguero et al., 2022). In Mediterranean forest ecosystems, spatial structuring could be particularly pronounced due to the fragmented island landscape (water barriers, habitat isolation), which restricts microbial dispersal and gene flow, especially for EcM fungi.

2.4.2. Environmental drivers of soil microbial communities

Soil microbial community composition is associated with ecological niches that provide specific environmental conditions for the growth, distribution, and survival of microbial taxa (He et al., 2020; Hibbing et al., 2010). Among environmental, site-specific properties, soil pH (Delgado-Baquerizo et al., 2017), nutrient limitation, moisture (Naylor & Coleman-Derr, 2018; Schimel et al., 2007), and vegetation (Barbi et al., 2016; Han et al., 2021) are important factors influencing soil microbial diversity and composition, especially when these drivers act as constraints within Mediterranean forest ecosystems.

Soil pH strongly correlates with the composition and often the diversity of soil bacterial communities (Jenkins et al., 2009; Lauber et al., 2009). This pattern applies to the overall bacterial community composition (Fierer & Jackson, 2006; Lauber et al., 2009), as well as specific bacterial groups (Davis et al., 2009; Jenkins et al., 2009; Jones et al., 2009). Furthermore, this pattern persists across various scales, from sub-metre (Philippot et al., 2009) to continental (Lauber et al., 2009) and across different land-use types (Jenkins et al., 2009). There is a pronounced influence of pH on soil bacterial communities from *in situ* measurements, where bacteria exhibited narrow pH tolerances and deviations of 1.5 pH units, which constantly reduced their activity by 50% (Fernández-Calviño & Bååth, 2010), also causing the shifts in the relative abundances of Acidobacteria, increasing toward lower pH (Dimitriu & Grayston, 2010; Rousk et al., 2010), while Actinobacteria and Bacteroidetes increased toward higher pH (Lauber et al., 2009). On the other hand, fungal community composition often responds weakly to soil pH, likely due to the wide pH optimum for fungi (Beales, 2004; Rousk et al., 2010). Furthermore, there are indications that fungal community composition is not directly influenced by soil pH, but rather indirectly through competition with bacteria, as bacteria respond dynamically to soil pH, leading to turnover and suppression of some bacterial taxa that could favour fungal growth (Rousk et al., 2008, 2010).

Nitrogen and phosphorus are frequent limiting nutrients in Mediterranean forests (Gauquelin et al., 2018) and their availability is considered to have a stronger effect on bacterial communities compared to fungal communities (Chen et al., 2022b; Li, et al., 2019b). Nitrogen inputs often elevate soil bioavailable ammonium and nitrate concentrations, leading to a shift in bacterial community composition toward copiotrophic/fast-growing taxa (e.g., Proteobacteria and Actinobacteria) and reducing oligotrophic/slow-growing bacteria (e.g., Acidobacteria, Verrucomicrobia, Planctomycetes) (Dai et al., 2018; Nie et al., 2018). Higher concentrations of bioavailable ammonium and nitrate cause soil acidification, so shifts towards copiotrophic bacterial taxa might conflict with the expectation that Acidobacteria would increase due to lower pH (Dimitriu & Grayston, 2010; Rousk et al., 2010). Several studies have shown that increased nitrogen availability can override the pH effect and suppress oligotrophic bacterial taxa (e.g., Acidobacteria) capable of degrading recalcitrant and nutrient-poor substrates that favour fast-growing copiotrophs (Fierer et al., 2012; Ling et al., 2017; Zhou et al., 2015). There are inconsistencies in these patterns, as Li et al. (2019b) showed that nitrogen availability had no significant relationship with soil fungal and bacterial diversity and community composition. Additionally, phosphorus availability has shown divergent effects on microbial communities, ranging from significant increases in bacterial richness and arbuscular fungi in phosphorus-limited systems (Li et al., 2015; Liu et al., 2013), to a complete lack of significant relationships (Li et al., 2019b). These differing patterns emphasise the importance of different soil and habitat types, management regimes, vegetation, and other abiotic and biotic factors on nutrient availability (Dai et al., 2018; Hartmann et al., 2015; Lopes et al., 2011; Lupwayi et al., 2012). Studies investigating the influence of nitrogen and phosphorus on soil microbial communities in the Mediterranean ecosystems remain scarce, with most evidence derived from other ecosystems. Consequently, the effects of these limiting nutrients on Mediterranean microbial communities may differ, given the distinct environmental constraints of these ecosystems.

Moisture can directly affect soil microbial communities due to reduced substrate diffusion at low levels of water availability through alteration of richness and activity of soil microbial communities (Naylor & Coleman-Derr, 2018; Preece et al., 2019; Schimel et al., 2007). In general, research suggests that fungi are more tolerant to water stress than bacteria (Bapiri et al., 2010; Barnard et al., 2015; Liu et al., 2022; De Vries et al., 2018) due to their ability to accumulate osmo-regulatory solutes, and their filamentous structure that facilitates substrate exploration (Manzoni et al., 2012). A similar pattern has been observed in Mediterranean soils (Flores-Rentería et al., 2015; Preece et al., 2019; Yuste et al., 2011). However, regarding bacteria, Gram-positive bacteria generally resist low moisture levels better than Gram-negative bacteria, probably due to their thicker cell walls (Schimel et al., 2007). According to Yuste et al. (2011), under climate change scenarios and increasingly arid conditions, a potential shift from bacterial to fungal dominance may occur in Mediterranean regions.

Vegetation and forest tree species can directly drive microbial community diversity and composition through litter chemistry (Barbi et al., 2016) with a stronger effect on fungal communities compared to bacterial communities (Lauber et al., 2009; Pole et al., 2026; Rousk et al., 2010). Previous studies showed that stand age strongly influences fungal community composition, driving shifts in dominant taxa and functional groups in forest soils (Martín-Pinto et al., 2022; Odriozola et al., 2020). In particular, older, late-successional stands tend to host higher ectomycorrhizal fungal richness than younger stands (Martín-Pinto et al., 2022). The ecological complexity of Mediterranean forests often makes it difficult to isolate the specific direct impact of dominant vegetation on soil microbial communities (De Vries & Shade, 2013; Labouyrie et al., 2023). Therefore, vegetation as a driver of soil microbial communities is taxon-specific and context-dependent, reflecting environmental heterogeneity and nutrient limitations of Mediterranean ecosystems (De Vries & Shade, 2013; Sardans & Peñuelas, 2013).

Due to increasingly recognised interactions between soil microbial communities and plants (i.e. plant-soil-microbe feedback) as key drivers of ecosystem functions (Lozano et al., 2014), there is a need to understand both direct and indirect effects on soil microbial communities to gain comprehensive insights into how and why microbial communities respond to environmental changes and disturbances (Bowd et al., 2021). For example, one of the indirect effect vegetation can have on soil microbial communities is the modification of soil properties: when vegetation alters soil pH or C/N ratio, these changes subsequently drive soil microbial communities (Prada-Salcedo et al., 2022). Furthermore, soil pH affects the solubility of various elements, including aluminium, which can be toxic to soil microorganisms and thereby indirectly affect them (Lammel et al., 2018).

2.4.3. Intrinsic habitat characteristics and tourism influence

The indented coast of the Mediterranean region, with numerous islands, provides an ideal system for analysing intrinsic habitat characteristics. Islands are characterised by their geographical isolation and varying sizes, and they have served as model systems for developing and testing fundamental evolutionary and ecological theories (Whittaker et al., 2017). Islands' discrete spatial structure provides an ideal framework for analysing microbial communities across spatial scales, reflecting microbial composition turnover within and between islands (Wang et al., 2020). Furthermore, islands feature distinct abiotic conditions, population size, and extent of dispersal limitation compared to the mainland, which hinders the direct application of assumptions about mainland microbial communities to those on islands (Stuart et al., 2012). In their theory of island biogeography, MacArthur & Wilson (1967), proposed that the equilibrium number of species on an island is determined by the balance between immigration and emigration and extinction rates. They further proposed that island characteristics, especially its size (area effect) and its distance (isolation effect) from the potential pool of other organisms (e.g., mainland), strongly influence these processes. Recently, this has been applied to microorganisms (Li et al., 2020; Davison et al., 2018), supporting patterns of microbial biogeography through commonly accepted patterns of 1) positive species-area relationship that implies increasing the number of observed taxa with increasing sampling area, emphasising richness accumulation (total taxa vs. area), and 2) negative species-isolation relationship with lower species richness on remote islands (MacArthur & Wilson, 1967; Patiño et al., 2017).

Positive species-area relationship would lead to a higher microbial species richness on large islands based on three possible assumptions: 1) larger islands support greater number of microbial species due to their ability to sustain more individuals (sample effect); 2) a higher species colonisation rate, coupled with lower extinction rates, contributes to greater diversity on larger islands (area per se effect) and 3) larger islands have more diverse habitats, which in turn support a wider variety of microbial species (habitat heterogeneity effect) (Li et al., 2020). Within the species-area relationship, larger sampled areas may capture more taxa by chance alone, which is not ecologically meaningful and does not reflect microbial biogeography (Martiny et al., 2006). Moreover, the edge effect operates through habitat quality degradation rather than spatial barriers, causing abiotic stress filtering (e.g., wind turbulence, desiccation, salinity), specifically pronounced on smaller islands. Habitat quality degradation then results in lower species diversity on smaller islands (Ewers & Didham, 2006; Phillips et al., 2018).

On the other hand, a negative species-isolation relationship leads to lower species richness with decreased island isolation (Whittaker et al., 2017). Unlike the straightforwardness of island size, isolation can be estimated by different indices: from the nearest mainland, from the nearest island, from the nearest largest island, and from the largest island in the archipelago (Itescu et al., 2020). Due to greater inter-island distances, a decrease in microbial diversity, as well as more simplified community composition, is expected (Gao et al., 2015), making more distant islands more susceptible to colonisation and the spread of invasive microorganisms (Moser et al., 2018; Pyšek et al., 2010). When trying to isolate the pure isolation effect on microbial communities, dispersal limitation can often act as a confounding effect (Martiny et al., 2006). Dispersal limitation refers to the inability of a species to reach and successfully establish in a new location (Li et al., 2020) and generally operates across all spatial scales. However, ubiquitous aerial spores and wind create a high baseline dispersal across near and far islands, resulting in weak differentiation based solely on isolation/distance (Hanson et al., 2012; Li et al., 2020; Martiny et al., 2006).

In addition to spatial processes such as dispersal and isolation, anthropogenic disturbances can further shape microbial community composition. The Mediterranean region is one of the world's leading tourism destinations (Bourlion & Ferrer, 2018), where tourism activities significantly affect the environment and ecology, as reflected in the term "tourism interference" (Li et al., 2022; Luo et al., 2019). Soil is the most vulnerable environment, susceptible to a range of tourism activities and disturbances (Mostafanezhad & Norum, 2019). As soil microbial communities play an important role in soil health (Curiel Yuste et al., 2014; Delcourt et al., 2023), it is important to understand how tourism disturbance affects soil microbial community composition, especially in areas with pronounced tourism. Although research on this topic specifically in the Mediterranean region is lacking, some studies have addressed the consequences of tourism for soil microbial communities. Increased vegetation trampling resulted in a significant alternation in soil microbial communities in wetland ecosystems (Li et al., 2018), destruction of microbial diversity of local soil (Hall & Page, 1999; Newsome et al., 2002), change of microbial community composition which cannot recover immediately afterwards (Li et al., 2022) and significant decrease of soil enzyme activities (Duan

& Zhu, 2019). Under different degrees of tourism disturbance, the relative abundance of nitrogen-fixing bacteria was significantly different under moderately disturbed conditions compared to the control, while the alpha diversity of soil bacterial communities in severe tourism disturbance was significantly decreased. Soil microbial communities changed significantly in response to tourism disturbance, identifying such pressure as one of the key drivers of soil microbial community composition (Li et al., 2022). These changes in microbial communities have important implications for soil functioning and, ultimately, soil health.

2.5. Soil health

Soil is a dynamic, living, top terrestrial layer, vital to the ecosystem functioning (Doran et al., 1996; European Environment Agency, 2022) and essential to agriculture and natural plant communities (Doran & Zeiss, 2000). To manage soil sustainability and protect it when needed, it is crucial to understand how soil responds and develops under current and future management and climate conditions. Healthy soils optimise the provision of ecosystem services (EEA, 2022).

Soil health is defined as the actual capacity of soil to perform its vital functions to provide ecosystem services (Bonfante et al., 2020; EEA, 2022). A more recent definition of soil health provided by the European Commission for the Soil Monitoring Law is: "physical, chemical and biological condition of the soil determining its capacity to function as a vital living system and to provide ecosystem services" (SML, 2025). It is a holistic approach coupling intrinsic and dynamic soil properties with soil sustainability. Intrinsic soil properties represent the soil's maximum functionality based on its inherent properties (Vogel et al., 2019), while dynamic soil properties represent the soil's present properties that shift with management practices or amendments. Therefore, soil health represents the continued capacity of soils to support ecosystem services and to function optimally as a living ecosystem to sustain plants, animals and human health, under both dynamic and intrinsic soil properties as its natural boundaries (Doran & Zeiss, 2000; EEA, 2022). The core of the soil health concept shifts the focus from intrinsic soil functions to their role within a specific ecosystem, to establish which functions this ecosystem can support, firstly for nature and then also for humans, continuously (Janzen et al., 2021). Soil health is often used synonymously with soil quality, but the two terms differ conceptually. Soil quality emphasises soil fitness for specific uses (agriculture, forestry, urbanisation) and relies more on inherent properties and mechanistic measurements, while soil health focuses on the living, dynamic component of soils and capacity to provide ecosystem services (Cahyana et al., 2026; Doran & Zeiss, 2000; Lehmann et al., 2020). Additional differences in soil quality and soil health are listed in **Table 1**. This distinction matters because soils unsuitable for crop production (e.g., wetlands, mangroves) can be ecologically healthy in their natural state, supporting biodiversity, carbon storage, and water regulation (Fierer et al., 2021). Soil health thus prioritises environmental sustainability and biological functioning over production potential.

Accordingly, three important elements of the soil health concept should be emphasised: functionality, vitality and sustainability or resilience (Janzen et al., 2021). Functionality in this concept embraces the soil's capacity to promote utility (Doran & Zeiss, 2000) and it is not limited just to services with a direct impact on human benefit (Comberti et al., 2015; Danley & Widmark, 2016), but also the processes that maintain the integrity and stability of the biosphere. Vitality refers to soil as a living system, with many interconnected processes mediated by numberless soil biota. Sustainability or resilience was introduced as an essential component of the soil health concept, reflecting the soil's consistency toward ideal and evolving conditions and its endurance to disturbances. It is difficult to fully achieve soil health, but it is in its nature to continually thrive, sustaining over time (Janzen et al., 2021).

Table 1. Differences between soil quality and soil health according to *Cahyana et al. (2026); Doran & Zeiss, (2000)*.

	SOIL QUALITY	**SOIL HEALTH**
Focus	-more technical and mechanical -specific function based on purpose	-more on dynamic -holistic, biological activity -ecosystem vitality, resilience and sustainability
Response to degradation	-decline in inherent soil properties from its natural state	- disruption of soil living component
Inherent soil properties	-assessed as standalone baseline -fixed reference point	-placed in the context -holistic integration
Dynamic soil properties	-distinguished from inherent for guiding specific purpose -production-oriented	-fully integrated with inherent properties -focus on resilience
Management target	-task-specific (e.g. crop yield via amendments)	-ecosystem-wide (e.g. biodiversity, reduced disturbance)

HEALTHY IF ECOSYSTEM SUSTAINS ITSELF

2.5.1. Assessment of soil health

Soil health threats are processes that damage soil and its functioning, consequently reducing the soil's capacity to provide ecosystem services. Soil threats are characterised by a negative trend in one or several soil properties or are indicated directly by observations in the field (e.g., erosion or sealing) (EEA, 2022). Soil degradation is the main threat, reducing soil functions such as production/biomass, water storage and quality, carbon storage, nutrient cycling, and biological activity. Degradation can be 1) physical - soil compaction, erosion, landslides, sealing; 2) chemical - accumulation of chemicals, salinisation, acidification; 3) biological - reduced biodiversity and activity, reduced metabolism of contaminants, decline in soil organic carbon (Adhikari & Hartemink, 2016; EEA, 2022). Soils are healthy when not subjected to degrading processes or declines in function; hence, it is essential to minimise or even eliminate significant soil degradation, as soil sustainability maintains ecosystem services and is more cost-effective than recovering soils after degradation has occurred (FAO & ITPS, 2015).

Soil degradation and impact on soil functions can be described by three possible scenarios/outcomes: 1) healthy – based on a threshold, there is no risk at present and current conditions and land-use, soil properties are optimal; 2) intermediate – warning level for soil degradation and 3) unhealthy – according to the threshold, current and future soil conditions are that the system is at risk because at least one soil property exceeds the established threshold for unacceptable degradation (**Figure 1**) (EEA, 2022).

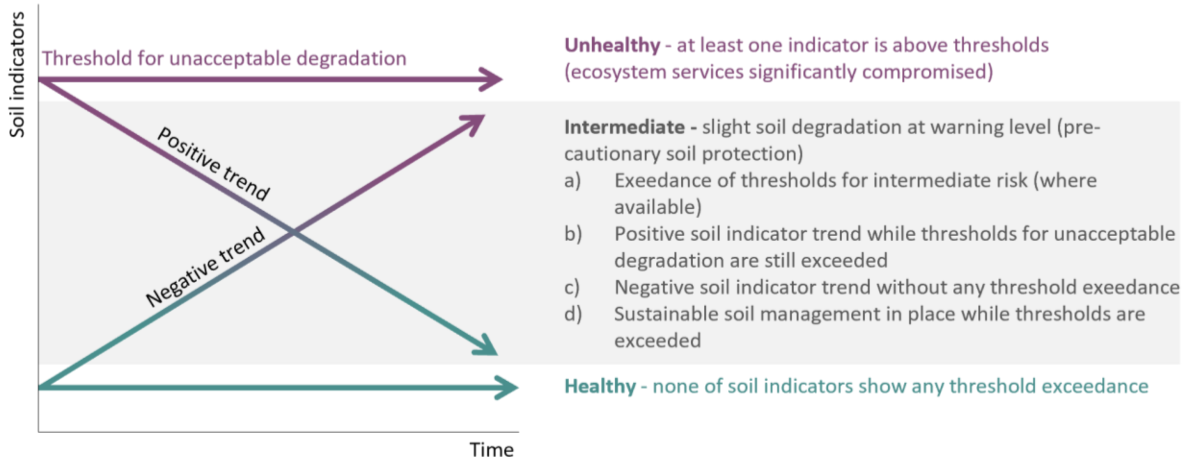

Figure 1. Dynamic assessment of soil health according to EEA (2022).

Soil health assessment indicates how well soil provides ecosystem services; however, it depends on complex physical, chemical, and biological interactions in space and time and varies within soil type, environmental conditions, climate and management practices (Doran & Zeiss, 2000; Ghimire et al., 2023; Lehman et al., 2015). The assessment of soil health should not focus on specific soil threats or functions, as such an approach cannot capture the overall condition of the soil. For example, Cornell's Comprehensive Assessment of Soil Health (Moebius-Clune et al., 2016), was entirely focused only on assessing healthy soil for crops and many assessments are oriented mainly on soil productivity during limited growing seasons and are not interpreted to reflect soil sustainability. To assess and interpret soil health, these steps are needed: 1) selecting and minimising the set of relevant soil properties; 2) quantifying selected soil properties and assigning an appropriate score; 3) defining the weight of each soil property or group of properties; 4) integration of weighted soil properties (Rinot et al., 2019).

This results in the formation of the soil health index (SHI), which should then be interpreted in a broader, holistic sense, rather than focusing on current and short-term uses of soil, such as crop productivity. It should be interpreted in the context of inherent soil properties, climate, biogeographical position, and management practices, with the ultimate goal of ensuring continuous ecosystem resilience and sustainability. Even though soil health is widely acknowledged as an important concept, the lack of standardised metrics makes it difficult to quantify and consistently define. At the same time, this could be both a weakness and a strength of soil health (Janzen et al., 2021), as its flexibility, context and interpretation always depend on stakeholders.

2.5.2. Soil health indicators and index

Considerable research effort is required to identify measurable indicators that facilitate robust soil health assessments (Mäkipää et al., 2024). Soil health indicators are a set of sensitive soil properties reflecting the capacity of soil to function (Bünemann et al., 2018), or soil properties that provide substantial information on soil functions (Vogel et al., 2019). According to Doran (2002), criteria for indicators should be that they: 1) define ecosystem processes; 2) enable synthesis of physical, chemical and biological properties into ecosystem processes; 3) are sensitive to management and climate variations; 4) are accessible and practical for land users, producers, conservationists and policymakers. Some important soil health indicators do not meet all 4 criteria; for example, total organic carbon does not change very quickly (it is not sensitive), on the other hand, soil texture and depth, as inherent soil properties, do not readily change and cannot be easily managed (Lehmann et al., 2020). Nevertheless, these inherent, unmanageable soil indicators provide context for soil health and can be interpreted as soil potential (Bouma et al., 2017), without which, dynamic, manageable soil indicators cannot be understood (Lehmann et al., 2020).

Chemical and physical soil properties are fundamental indicators of soil health that reflect nutrient availability, structural integrity, and the soil's capacity to support biological processes (Lehmann et al., 2020). Key chemical indicators include soil pH, which regulates nutrient availability and microbial activity; soil organic carbon and total nitrogen, reflecting nutrient availability and carbon storage; available phosphorus and potassium, which affect plant productivity; and the C/N ratio, indicating organic matter quality and decomposition dynamics (Bünemann et al., 2018; Grosso et al., 2016; Rinot et al., 2019). Important physical indicators include soil texture, which determines water-holding capacity, as well as bulk density. While these indicators provide essential baseline information, they are generally less sensitive to short-term changes than biological indicators (Cardoso et al., 2013).

The selection of soil health indicators should not rely solely on statistical significance, but also on their ecological relevance and roles in soil processes, considering specific characteristics of the respective ecosystem. The idea is to choose indicators with optimal thresholds or a range where considered soil functions are not violated, and critical thresholds beyond which soil functions are compromised (Vogel et al., 2019), as shown in **Figure 1**. Selecting the most relevant indicators to assess soil health and build SHI is a crucial step, primarily because of the precise thresholds or ranges for each indicator. (Rinot et al., 2019). European Environment Agency (2022) defines thresholds as critical limits at which soil functions decline, signal degradation or recovery, points out the need for preventive and restorative measures, and enables adjustments to management practices in response to varying conditions (**Figure 1**). Several considerations complicate the development of thresholds for soil health indicators. First, thresholds may not be comparable across countries, even under similar soil and land-use conditions. Second, thresholds require updating as risk assessment methods and scientific understanding advance. Third, when validated thresholds are unavailable, benchmarks or reference values can provide rough orientation for identifying degraded or unhealthy soils (EEA, 2022).

Acceptable thresholds for soil health indicators need to be soil- and land-use-specific, with consideration that they are spatially and temporally dependent (Lilburne et al., 2004). Creating the SHI is very challenging; it requires quantitative transformation and weighting of multiple indicators, including categorical indicators, to integrate them into a final score that encompasses ecosystem services. Indicator values need to be transformed using nonlinear relationships (Lehmann et al., 2020), because a higher value does not always indicate better soil health (Svoray et al., 2015). Therefore, establishment of scoring functions is needed: 1) more is better; 2) optimum range; 3) less is better; or 4) undesirable range (Bünemann et al., 2018). For example, high SOC is generally beneficial for soil functions, but it must be balanced with other soil properties (e.g., pH in the optimal range) to maintain ecosystem services (Lehmann et al., 2020).

2.6. Microbial communities in soil health

With advances in methodology, we can now characterise and quantify soil microbial communities, providing strong motivation to include microbes in soil health assessments. Moreover, there is a need for soil health indicators that apply beyond crop agriculture and provide broader information on ecosystem services and sustainability. Just like physical and chemical indicators, the interpretation of microbial indicators of what is “good/healthy” and “not good/unhealthy” is context-specific. So, the selection of valuable microbial indicators depends on the soil or site in question and the particular aspects of soil health of interest (Fierer et al., 2021).

Microorganisms drive fundamental nutrient cycling processes, soil structural dynamics, pollutant degradation, and other ecosystem services. They respond quickly to environmental stress and disturbances due to their short generation time and close relation with surroundings, attributed to their higher surface-to-volume ratio (Sharma et al., 2010). Microorganisms as indicators of soil health have been defined as “properties of the environment or impact that can be interpreted beyond the information that the measured or observed indicator represents itself” (Nielsen & Winding, 2002). The use of microbial metrics as bioindicators has been widely recognised, for example, the abundance of specific taxa or genes of interest, rates of microbial activities, and estimating microbial biomass or enzymes, which have proven useful, but often constrained by clear interpretability for management practices or policy purposes (Fierer et al., 2021). On the other hand, microbial community diversity and composition are often neglected or underused as indicators of soil health, but they can complement indicators such as microbial biomass or enzymes in the final assessment of soil health. Analyses of microbial communities, with advancements in high-throughput DNA sequencing, are very fast and affordable, offering valuable information about microbial communities in a short time (Fierer et al., 2021). Moreover, microbial communities exhibit spatial and temporal variability, but are not as variable as some soil characteristics that change very slowly or very quickly, and therefore are not always suitable for soil monitoring. On the other hand, microbial communities are dynamic enough to respond to environmental changes (seasons to years), whereas the DNA pool remains stable enough to avoid significant day-to-day fluctuations (Carini et al., 2020; Lauber et al., 2013). Furthermore, the high diversity of microbial communities enables the characterisation of numerous aspects of the soil environment (Fierer et al., 2021).

Microorganisms could be used to identify indicators of specific soil processes or conditions, and in such cases, they can help to prioritise when or where more detailed analyses of chemical and physical indicators are needed. For microbial properties as soil health indicators, it is important that they are consistently associated with particular aspects of soil health for the specific ecosystem in question (Fierer et al., 2021). Emphasising that soil health is context-dependent and that soil health assessment and goals are not the same for forests, agriculture, wetlands or tundra. However, microbial community diversity and composition metrics as indicators of soil health are not widely adopted yet, due to several limitations that need to be considered.

Methodological issues regarding molecular analyses should be considered, such as differences in DNA extraction protocols and efficiencies, PCR biases or bioinformatic data processing, which can hamper consistent comparison of results (Fierer et al., 2021). Also, not all DNA in soil is from active microorganisms, so the presence of relic DNA may obscure the relationship between microbes and soil conditions or processes (Carini et al., 2020). Soil health assessment lacks universally defined thresholds, and consequently, no "ideal" microbial communities are recognised as indicators of healthy soils. Moreover, higher microbial diversity does not necessarily indicate better ecosystem functioning (Jiang, 2007; Liu et al., 2025). Rather, microbial communities as indicators of soil health have the potential to track how soil conditions and ecosystem functioning change over time and space in response to disturbances (Fierer et al., 2021), considering them suitable for long-term soil health monitoring.

2.7. Knowledge gaps and scientific contribution

Despite advances in Mediterranean soil microbial ecology, numerous gaps persist that limit a comprehensive understanding of soil microbial communities and their significance to Mediterranean forest ecosystems.

Soil fungi and bacteria interact within complex networks to drive nutrient cycling, decomposition, and overall ecosystem health, yet most studies analyse them separately (Onet et al., 2025). It is well established that soil properties are highly important for bacterial community composition (Lauber et al., 2009; Rousk et al., 2010), whereas vegetation characteristics and traits affect fungal composition more strongly (Martiny et al., 2006; Zinger et al., 2011). In Mediterranean forests, the interplay of climatic stressors, land-use legacies, resource scarcity and environmental heterogeneity hampers the isolation of specific environmental drivers. Consequently, the relative contribution of environmental drivers on soil microbial community diversity and composition remains unclear (Curiel Yuste et al., 2014; Delcourt et al., 2023). Therefore, this dissertation provides an integrative analysis of environmental properties affecting both soil fungi and bacteria in the Mediterranean forest.

Different research debates whether classical biogeographic patterns are applicable to microorganisms due to their ubiquity, dispersal capacity or generation times (Green & Bohannan, 2006; Lindström & Langenheder, 2012; Martiny et al., 2006; Ramette & Tiedje, 2007). A first step toward better understanding microbial biogeography is identifying the drivers of microbial community composition not only in mainland ecosystems but also in insular systems, where isolation, area effects, and dispersal limitation may operate differently than for macroorganisms (Stuart et al., 2012). Furthermore, research on the effect of tourism disturbances on soil microbial community composition is scarce despite the widespread and intensifying tourism pressure across the Mediterranean region (Bourlion & Ferrer, 2018). The Croatian Adriatic coast, with its highly indented coastline comprising numerous islands of varying size, isolation, and tourism intensity, alongside mainland forests, offers an ideal environment to address these knowledge gaps.

Incorporating microbial communities into soil health assessments remains challenging due to their high taxonomic and functional diversity, substantial spatial and temporal variability, and the complexity of high-dimensional community data (Wilhelm et al., 2022). Consequently, microbial communities as indicators have been rarely integrated into soil health monitoring. Machine learning (ML) approaches offer powerful tools for overcoming the complexity of microbial community data, and their application based on microbiome data has already proven effective in predicting or assessing human health status (Topçuoğlu et al., 2020), crop yields (Chang et al., 2017) and physicochemical soil properties (Hermans et al., 2020). However, soil health assessment based on microbial communities in the Mediterranean forests, where natural environmental heterogeneity, varying management intensities, and tourism disturbances interact, remains understudied.

This dissertation provides a comprehensive understanding of soil properties, spatial structuring, vegetation, island traits, and tourism disturbance as drivers of soil microbial diversity and composition in Mediterranean forests, to develop a predictive soil health model based on microbial communities. Research emphasises the role of microbial communities in assessing the ecological importance of the soil health concept and its potential to reduce costs and the need for extensive soil monitoring. Furthermore, as this research was conducted within areas protected under the EU Habitats Directive (Brijuni National Park and Cape Kamenjak), the findings are essential for site-specific conservation. Assessing and preserving soil health in these ecosystems is of critical importance, given their vulnerability to anthropogenic activities and climate change.

3. Objectives and hypotheses

Considering that microbial communities play a leading role in reflecting soil stability and nutrient cycling, the main objective of this dissertation is to analyse microbial communities, their distribution, diversity, composition and their potential role as indicators for soil health assessment in a specific and heterogeneous, terrestrial and insular ecosystem of the Mediterranean region of Croatia.

- **Objective 1:** To analyse the diversity, composition, and role of microbial communities in heterogeneous and different types of Mediterranean habitats and to understand which physico-chemical soil properties and plant communities drive these microbial communities.

  Hypothesis 1 (H1): Soil microbial communities in the Mediterranean region are influenced by habitat types and are primarily driven by the physico-chemical soil properties and the composition of plant communities.

- **Objective 2:** To investigate the influence of habitat size and distance from the mainland (isolation) on the diversity and role of microbial communities in different types of habitats on Mediterranean islands.

  Hypothesis 2 (H2): Larger islands host more diverse soil microbial communities compared to smaller islands due to greater habitat heterogeneity and larger surface areas. Conversely, islands located further from the mainland harbour less diverse but more specialised soil communities, as the increased distance may limit the dispersal of microbial species.

- **Objective 3:** To develop a predictive model based on physico-chemical soil properties using machine learning algorithms to predict soil microbial communities and to evaluate whether microbial community compositions can serve as a reliable basis for predictive models to assess soil health.

  Hypothesis 3 (H3): The presence of specific microbial taxa or functional groups could serve as indicators or predictors of soil health, particularly in environments under different degrees of protection and anthropogenic influence.

4. Materials and methods

4.1. Sampling campaigns and sample processing

To address the hypotheses outlined in this dissertation, two independent sampling campaigns were conducted in protected areas of the Mediterranean region in Croatia (**Figure 2**). The first campaign targeted the first hypothesis, while the second addressed the second and third hypotheses. Both campaigns focused on soil sampling and analyses of soil microbial communities, but they differed in sampling design, subsequent soil property assessments, and statistical approaches. The sampling campaigns were designed to address distinct hypotheses and, accordingly, their results are not directly comparable, but together they contribute complementary evidence toward the broader research framework.

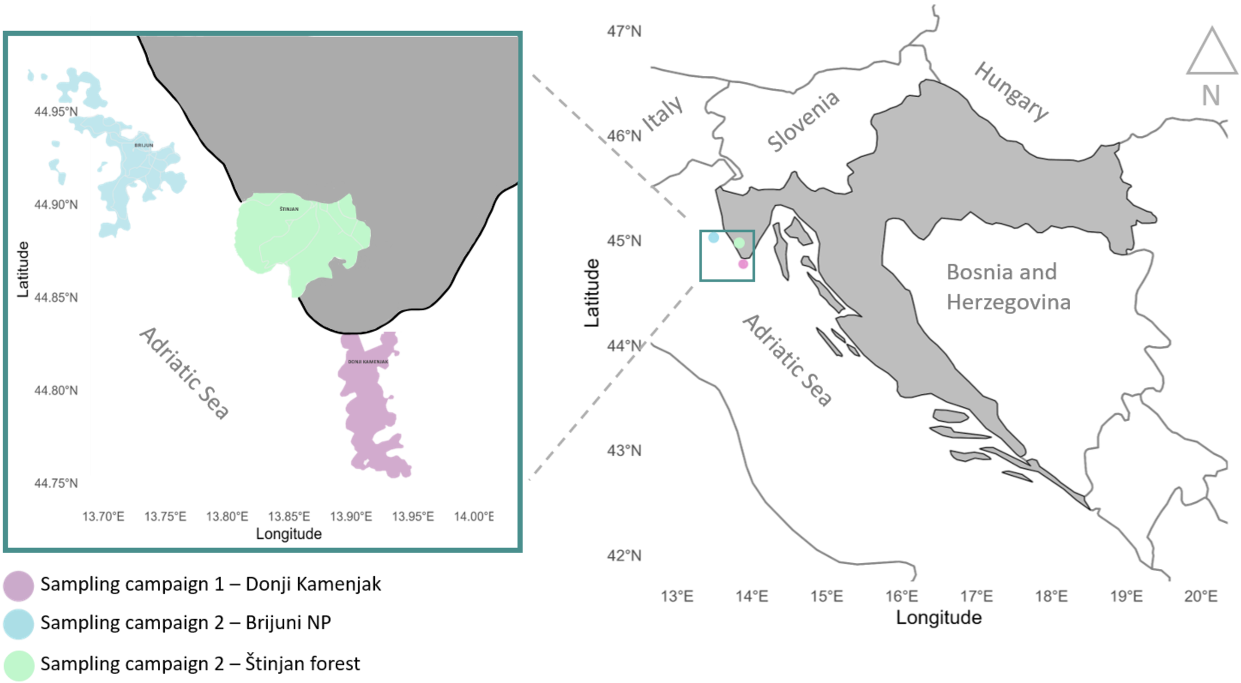

Figure 2. Study area and sampling campaigns at Donji Kamenjak, Brijuni NP and Štinjan forest.

4.1.1. Sampling campaign 1 – Donji Kamenjak

Donji Kamenjak is located at the southernmost part of the Kamenjak cape and the Istrian peninsula (**Figure 2**). It has been recognised as an important landscape and together with eight nearby islets, has been protected according to the Croatian Nature Conservation Act since 1996. Furthermore, its exceptional habitat biodiversity has been designated as part of the Natura 2000 ecological network (code HR2000616) since 2014. Prior to protection, land-use in this area was based on traditional agricultural practices, which, together with strong human activities over the past fifty years, reshaped this landscape. These anthropogenic activities degraded the original holm oak forests and transformed them into maquis, garrigue, and grasslands. Also, parts of this area were afforested with *P. halepensis* stands which caused the loss of pasture areas. Consequently, these actions have altered biodiversity patterns in this area (Ljubičić et al., 2020; Vuković et al., 2011). Donji Kamenjak is characterised by a typical Mediterranean climate and diverse habitat types in a relatively small area of approximately 370 ha. Main habitat types in this area are evergreen forests and maquis (197.09 ha), dry grasslands (67.63 ha), ruderal vegetation (57.18 ha), seaside (45.57 ha), and Mediterranean underbrush (16.55 ha) (Ljubičić et al., 2020).

To ensure systematic coverage for soil analysis, the study area was partitioned into 100 × 100 m plots, within which 20 × 20 m subplots were established for soil sampling (**Figure 3**). Most of the sampling plots were characterised by mixed and highly mosaic vegetation (**Table S1**). However, the focus in this sampling campaign was to capture different dominant ectomycorrhizal woody plants (*Cistus* spp. - *C. creticus* and *C. monspeliensis*, *Pinus halepensis* stands, and *Quercus ilex*) at each sampling subplot. A species was considered dominant if its canopy or ground cover accounted for at least 50% of the sampling subplots. In general, plots and subplots were chosen based on terrain accessibility and the presence of dominant vegetation species in forests, maquis and garrigue, while areas dominated by grasslands, ruderal vegetation, underbrush, or located near the seaside were not considered for soil sampling.

Soil samples were collected from each corner of the 20 x 20 m subplot (four samples per plot) using 4-cm metal cores to a depth of 20 cm (when possible). All litter was removed from the surface of sampling points before sampling. Immediately after sampling, material from all four soil cores was mixed to obtain a composite soil sample per subplot (n = 47), packed in zip-lock bags and stored at -20 °C. Frozen soil samples were then sieved and thoroughly homogenised using a vibratory sieve (2 mm) shaker Retsch AS 200 (Retsch GmbH, Germany), freeze-dried and stored again at -20 °C until further analyses. The geographical coordinates of the centre of each sampling plot were recorded using a GPSMAP 66s device (Garmin Ltd., USA) to account for any spatial structuring of samples. Sampling was conducted throughout 2021 (April: n = 8; October: n = 19; December: n = 20), with an uneven monthly spread due to terrain accessibility and weather conditions, while maintaining established protocols.

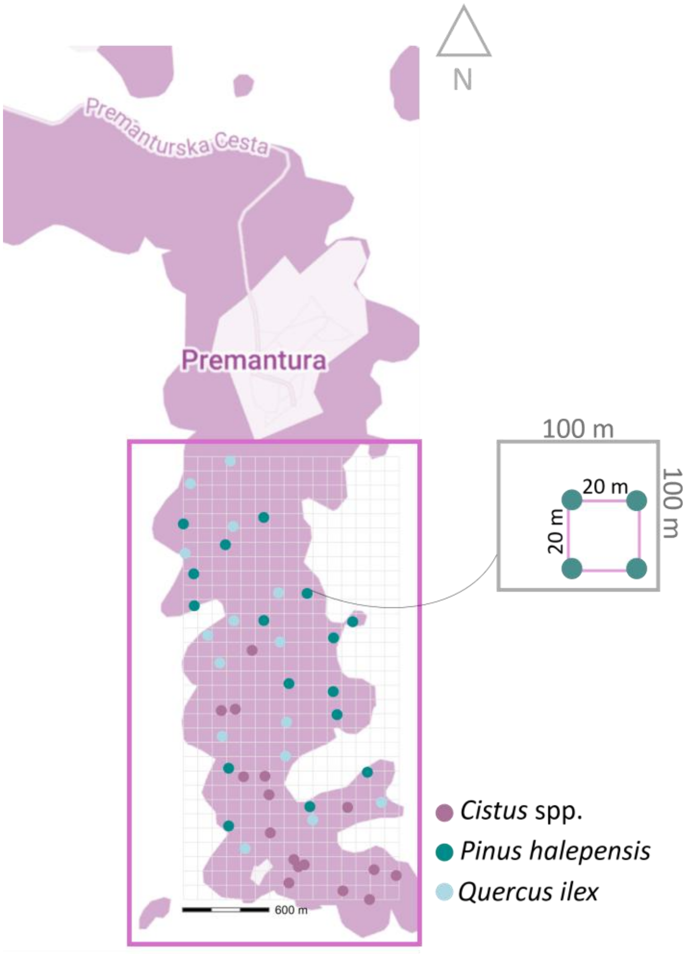

Figure 3. Sampling design at Donji Kamenjak, southernmost part of the Kamenjak cape. Gray square on the right is one sampling plot (100 x 100 m) with pink square within representing one sampling subplot (20 x 20 m). Colours are specific for the dominant vegetation species; pink = *Cistus* spp., green = *P. halepensis* and blue = *Q. ilex*.

4.1.2. Sampling campaign 2 – Brijuni NP and Štinjan forest

The Brijuni Islands were declared a national park (Brijuni NP) in 1983. Present boundaries of the Brijuni NP were set in 1999, including islands with surrounding sea and seabed, covering a total of 33.95 km^2. It is an extraordinary archipelago of natural, historical and cultural heritage, and therefore one of the most attractive tourist destinations in Croatia. Fourteen islands and islets constitute Brijuni NP: Veli Brijun (561.00 ha), Mali Brijun (108.85 ha), Vanga (19.84 ha), Sv. Jerolim (12.62 ha), Kozada (7.84 ha), Vrsar (6.79 ha), Gaz (6.28 ha), Pusti otok – Madona (5.06 ha), Galija (4.94 ha), Obljak (4.17 ha), Grunj (3.37 ha), Supin (1.28 ha), Supinić (0.37 ha), and Sv. Marko (0.89 ha) (JU Kamenjak, 2016; Petrić & Mikulić, 2012).

Soil sampling for this campaign targeted 7 of the 14 Brijuni islands and islets, selected based on forest cover, tourism intensity, size, and distance from the mainland. As in the first campaign, efforts focused exclusively on forested areas, whose extent was quantified using satellite imagery. To equitably represent biodiversity across islands, without disadvantaging smaller islands relative to larger ones, forest areas were logarithmically normalised. Specifically, each island's log-transformed forest surface was divided by the log of the smallest island's surface, yielding a proportional number of central plots. Forest areas on each island were then divided into 20 × 20 m central plots and randomly selected based on the number of plots needed (**Table 2**).

Table 2. Islands, their sizes, forest areas, and the number of samples collected in Brijuni NP.

Island	Size (ha)	Forest area (ha)	No. of central plots	No. of samples	Nearest island
Veli Brijun	561.00	**253.5**	8	32	Sv. Jerolim
Mali Brijun	108.85	**65.08**	6	24	Galija
Vanga	19.84	**9.58**	3	12	Galija
Kozada	7.84	**5.18**	2	8	Sv. Jerolim
Sv. Jerolim	12.62	**4.33**	2	8	Kozada
Galija	4.94	**2.99**	1	4	Vanga
Gaz	6.28	**2.11**	1	4	Galija

Prior to sampling, litter was removed from the surface of sampling points. Sampling was performed using plastic soil cores of 4 cm diameter to a depth of 20 cm (where possible). Within each selected central plot, soil was collected from four sampling subplots (10 x 10 m). At each subplot, five soil samples were collected; one positioned at the centre of the sampling subplot and four positioned 2 m away from the centre in cardinal directions. Centres of each sampling subplot were 10 m apart from one another (**Figure 4**).

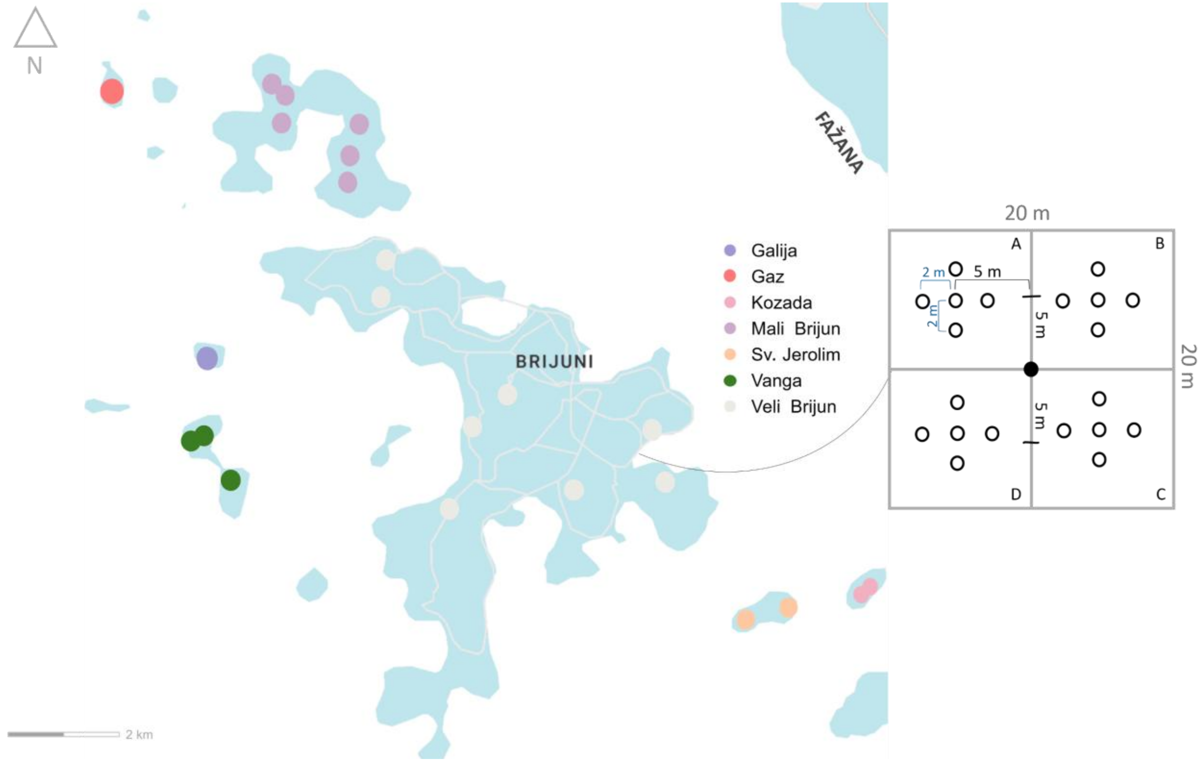

Figure 4. Sampling design in Brijuni NP. Different coloured points represent number of central plots at each respective island. Gray square on the right is one central plot with four sampling subplots (A, B, C and D). Empty points inside each sampling subplot represent five soil samples (central core represents sampling subplot coordinates), later combined in one composite sample, given four soil samples per one central plot.

The sampled islands in Brijuni NP exhibit differences in tourism pressure. Veli Brijun and Mali Brijun are the most visited islands, particularly Veli Brijun, as both are supported by organised boat services. Vanga, being under government control, is accessible only to high-level political officials. Kozada and Sv. Jerolim serve as a public day-trip destination, whereas the smallest islands, Galija and Gaz, remain fully closed to visitors.

The mainland site for this study was Štinjan forest, selected as the nearest unprotected forest to Brijuni NP (Figure 2), with pronounced human activities, which served as a control for the second hypothesis. The area of the Štinjan forest was gridded into 100 × 100 m sampling plots for soil sampling due to its much larger size. From each sampling plot, five replicates were sampled; one in the centre and four 2 m away from the centre in the cardinal directions (**Figure 5**). Sampling in Brijuni NP and Štinjan forest was conducted one month apart, in April and May 2024, respectively.

The soil samples obtained were stored at 4 °C and processed within 24 h after sampling. Remanent litter, roots and all other material indicating dead plant biomass were removed.

Material from respective soil cores was mixed to obtain a composite soil sample. Samples were then sieved and thoroughly homogenised, and the required amount of soil samples were packed in 50 mL Falcon tubes and stored at -20 °C. In the laboratory, samples were freeze-dried and stored again at -20 °C until further analyses. The total number of soil samples collected from Brijuni NP was 92 (Table 2), and from Štinjan forest was 20.

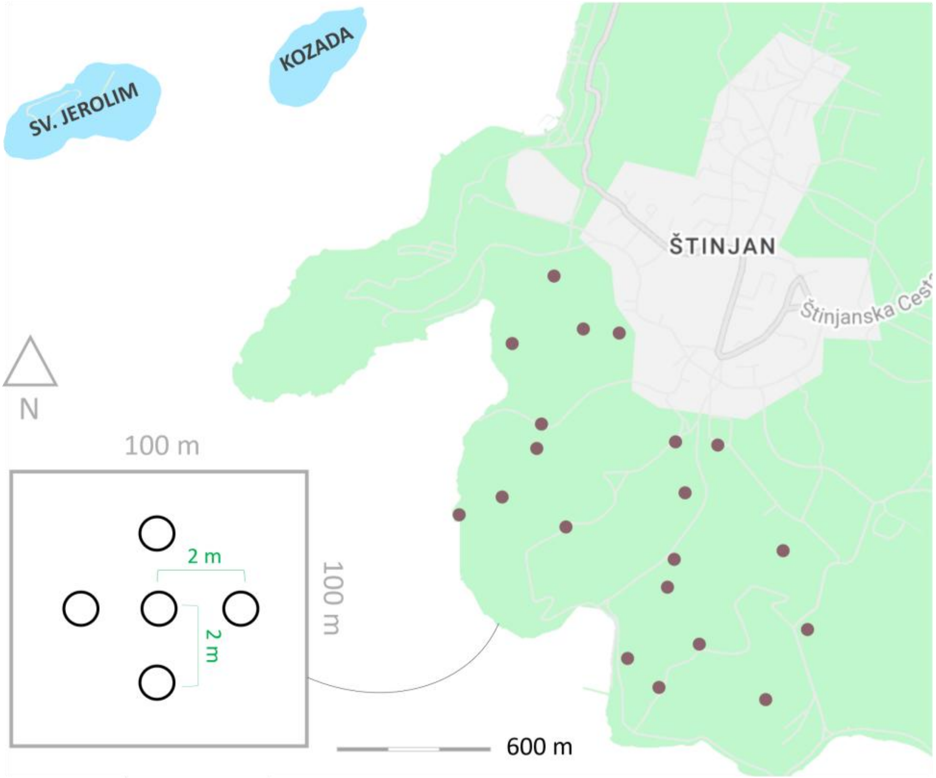

Figure 5. Sampling design in Štinjan forest. Coloured points are center of sampling plots (n = 20), and gray square on the left represents one sampling plot with five soil samples, later combined in one composite sample, given one soil sample per one sampling plot.

4.2. Soil physical and chemical properties

4.2.1. Sampling campaign 1 – Donji Kamenjak

Soil water content (moisture) was measured as a loss of mass during freeze-drying. Soil pH was measured in distilled water (1:10 w/w), and total nitrogen (N) and carbon (C) were quantified by combustion in a FLASH 2000 analyser (Thermo Fisher Scientific, USA) at the external laboratory of the Institute of Botany of the Czech Academy of Sciences (Průhonice, Czech Republic).

4.2.2. Sampling campaign 2 – Brijuni NP and Štinjan forest

Soil water content (moisture) was measured as a loss of mass during freeze-drying. Soil pH was measured in 5 mL of samples according to HRN ISO 10390:2022. Total nitrogen (N%) was measured by the Kjeldahl method from 1 g of soil, total carbon (TC) and total organic carbon (TOC%) were quantified from 1 g of soil using a total organic carbon analyser with a solid sample module (TOC-L, SSM-500A, Shimadzu, Japan). Humus (%) was quantified using the Tjurin method. Bioavailable phosphorus (P_2O_5 mg/100 g) and potassium (K_2O mg/100 g) were analysed from 5 g of soil using the AL-method (Ammonium Lactate method); P_2O_5 was determined spectrophotometrically (UV-VIS spectrophotometer), while K_2O was determined using a flame photometer. Total macro- and microelements were determined using ICP-OES (*Inductively Coupled Plasma Optical Emission Spectrometry*) (ICPE-9820, Shimadzu, Japan) after microwave digestion of 0.2 g of soil.

Particle size analyses included determination of five soil fractions: coarse sand (2.0 – 0.2 mm), fine sand (0.2 – 0.063 mm), coarse silt (0.063 – 0.02 mm), fine silt (0.02 – 0.002 mm) and clay (<0.002 mm) from 10 g of soil using the sodium pyrophosphate method.

Analyses for sampling campaign 2 were conducted in collaboration with the Soil, Plant and Water Laboratory of the Institute of Agriculture and Tourism (Poreč, Croatia).

4.3. Soil biological properties

4.3.1. Soil microbial biomass

Soil microbial biomass was quantified using two approaches: phospholipid-derived fatty acids (PLFAs) for sampling campaign 1 (Donji Kamenjak), and absolute abundances of fungal and bacterial genes using digital droplet PCR (ddPCR) for campaign 2 (Brijuni NP and Štinjan forest).

4.3.1.1. *Phospholipid-derived fatty acids (PLFAs)*

For sampling campaign 1 (Donji Kamenjak), phospholipid-derived fatty acids (PLFAs) were analysed by gas chromatography - mass spectrometry (450-GC with 240-MS ion trap detector, Varian, USA) to estimate microbial biomass, following established protocols (Frouz et al., 2016; Šnajdr et al., 2008a). The 18:2ω6,9 fatty acid served as a fungal biomass proxy (PLFA_Fungi), while the sum of i14:0, 16:1ω5, 10Me-16:0, 10Me-17:0, 18:1ω7, and cy19:0

indicated bacterial biomass (PLFA_Bacteria). Specific fatty acids were further used as a proxy for actinobacteria (10Me-16:0, 10Me-17:0, 10Me-18:0), Gram-positive bacteria (i14:0, i15:0, a15:0, i16:0, i17:0, a17:0), and Gram-negative bacteria (i16:1ω7, i16:1ω5, i18:1ω7, cy19:0, cy17:0). Total microbial biomass was the sum of all measured PLFAs (PLFA_Total). To get further insights, an important ecological indicator, the F/B ratio, was analysed, as PLFA Fungi/PLFA Bacteria. All different PLFA assignments followed (Joergensen, 2022; Štursová & Baldrian, 2011; Welc et al., 2012).

4.3.1.2. *Absolute abundance with ddPCR (digital droplet PCR)*

For sampling campaign 2 (Brijuni NP and Štinjan forest), microbial biomass was estimated by quantifying genes using ddPCR (*digital droplet Polymerase Chain Reaction*) on Bio-Rad QX200 system (Bio-Rad Laboratories, USA). Fungal and bacterial abundances were assessed by targeting the 18S rRNA and 16S rRNA genes, respectively. For fungi, the 18S rRNA gene was amplified using primers FF390/FR1 (amplicon size ~390 bp), and for bacteria, the 16S rRNA gene was amplified using primers 1108F/1132R (amplicon size ~59 bp).

Each 22 µL PCR reaction mix consisted of 11 µL 2× QX200 ddPCR EvaGreen Supermix (Bio-Rad Laboratories, USA), 0.3 µL of each forward and reverse primer (10 µM), 8.4 µL of DNA-free water and 2 µL of DNA template. The prepared mix was incubated at room temperature for 3 min. For droplet generation, 20 µL of PCR reaction mix and 70 µl of Droplet Generation Oil for EvaGreen were used, after which 40 µL of droplet emulsion was used for PCR amplification. For fungal 18S rRNA, 3-step PCR was used as follows: 5 min at 95 °C, (30 s at 95 °C, 30 s at 50 °C, 1 min at 72 °C) x 40 cycles, then 5 min at 4 °C, 5 min at 90 °C and incubation at least 1 h at 4 °C, with ramp 2 °C/s at each step. For bacterial 16S rRNA, a 2-step PCR was used as follows: 5 min at 95 °C, (30 s at 95 °C, 1 min at 57 °C) x 40 cycles, then 5 min at 4 °C, 5 min at 90 °C and incubation at least 1 h at 4 °C, ramp 2 °C/s at each step. Droplets were read on QX Droplet Reader (Bio-Rad Laboratories, USA). As proxies for fungal and bacterial biomass, abundances were expressed as 18S rRNA gene copies g^{-1} dry soil and 16S rRNA gene copies g^{-1} dry soil, respectively.

4.3.2. Potential enzymatic activity

For both sampling campaigns, potential enzymatic activity of eight enzymes, acid phosphatase, α-glucosidase, β-galactosidase, β-glucosidase, β-xylosidase, cellobiohydrolase, chitinase, and lipase, was assessed using 4-methylumbelliferone (MUF)-based fluorogenic substrates (Marx et al., 2001; Štursová & Baldrian, 2011; Vepsäläinen et al., 2001). Freeze-dried soil samples (250 mg) were homogenised in 25 mL acetate buffer (50 mM, pH 5) using Ultra Turrax homogeniser (IKA, Germany) for 20 s at 12,000 *rpm* to keep the pH constant, keeping samples on ice during homogenisation and before measurement to maintain pH stability. Enzymatic assays were performed in triplicate by mixing 200 µL of homogenised soil with 40 µL of each MUF substrate. A calibration curve was generated by mixing MUF standards with 200 µL of homogenised soil. MUF standards were prepared in different dilutions and added in microplates in different volumes: MUF 1X (10 µL, 20 µL), MUF 10X (20 µL, 50 µL) and MUF 100X (10 µL, 20 µL, 50 µL). Before each measurement, microplates were incubated at 40 °C to ensure sufficient enzyme activity without depleting the substrate. Fluorescence (excitation 355 nm, emission 460 nm) was measured after 5, 35, 65 and 125 min at 40 °C using an automatic fluorometric plate reader (Tecan, Switzerland). Readings were normalised to the dry weight of the soil and expressed as nM of MUF released per g of soil per unit of time in minutes [nM/min/g].

4.4. Molecular analyses

Prior to DNA extraction, lysis buffers from the commercial kit and their combinations were tested to identify optimal lysis conditions, given the variable soil compositions (organic/inorganic matter, humic substances, polysaccharides, etc.). Consequently, slightly different DNA extraction protocols were applied to soils from sampling campaigns 1 and 2. However, PCR amplification and amplicon sequencing were performed equally for both sampling campaigns.

4.4.1. DNA extraction

Total genomic DNA was extracted from 159 soil samples (47 from sampling campaign 1, 112 from sampling campaign 2) using NucleoSpin Soil kit (Macherey-Nagel, Germany). Extractions were performed in duplicate using 250 mg of freeze-dried soil, following the manufacturer’s protocol with slight modifications to the lysis step. For soils from sampling campaign 1, 800 µL SL1 buffer + 20 µL Enhancer SX was used; for soils from campaign 2, Enhancer SX was omitted. Samples were homogenised in a mixer mill Retsch MM 400 (Retsch, Germany) at 25-30 Hz for 5-10 min, followed by 10 min incubation at room temperature. Elution was conducted in two steps using the same column: 1) 25 µL, incubated 10 min at room temperature, centrifuged at 11,000 × *g* for 30 s; 2) another 25 µL, incubated 5 min, centrifuged at 11,000 × *g* for 1 min. DNA quantity was assessed using a NanoDrop spectrophotometer (Shimadzu, Japan), and integrity was confirmed by 1% (w/v) agarose horizontal gel electrophoresis.

Duplicates of extracted DNA were further purified using a Geneclean Turbo kit (MP Biomedicals, USA) following the manufacturer's protocol. Each duplicate was eluted in 30 µL, incubated at room temperature for 5 minutes, and centrifuged at 14,000 x g for 1 minute. Purified extracts were pooled and stored at −20° C until further analysis.

4.4.2. PCR amplification and Illumina amplicon sequencing

PCR amplification was performed in triplicate to reduce PCR bias. The fungal ITS2 region was amplified using barcoded gITS7 and ITS4 primers (Ihrmark et al., 2012; White et al., 1990), while the bacterial 16S rRNA V4 region was amplified using barcoded 515F and 806R primers (Caporaso et al., 2011). PCR reactions were carried out in a total volume of 25 µL and contained: 5 µL of 5X Q5 Reaction buffer (New England Biolabs, USA), 0.5 µL of 10 mM PCR Nucleotide mix (Promega, USA), 1.5 µL of 10 mg ml^{-1} BSA (GeneON, Germany), 1 µL of each 10 µM forward and reverse primer (Sigma-Aldrich, Germany), 0.25 µL of the Q5 High-Fidelity DNA polymerase (New England Biolabs, USA), 9.75 µL of DNA-free water and 1 µL of the template DNA. Cycler conditions for amplification of fungal ITS2 region were as follows: 4 min at 94 °C, (30 s at 94 °C, 30 s at 56 °C, 30 s at 72 °C) x 30 cycles and final extension for 7 min at 72 °C. For bacterial 16S rRNA V4 region amplification conditions were: 4 min at 94 °C, (45 s at 94 °C, 1 min at 50 °C, 75 s at 72 °C) x 25 cycles and final extension for 10 min at 72 °C (Martinović et al., 2021). To confirm amplification efficiency, amplicon triplicates were analysed on 1% (w/v) agarose horizontal gel electrophoresis.

Amplicons were pooled, purified with the MinElute kit (Qiagen, Germany), and quantified using the Qubit dsDNA BR Assay kit (Thermo Fisher Scientific, USA). Sequencing libraries were prepared with the TruSeq PCR-free kit (Illumina, USA) and sequenced on the Illumina MiSeq (2 × 250 bp paired-end reads) in the Laboratory of Environmental Microbiology, Institute of Microbiology of the CAS in Prague.

4.5. Bioinformatic processing and statistical analyses

Bioinformatic processing of amplicon sequencing data was conducted using the SEED 2.1.2 pipeline (Větrovský et al., 2018). Initial steps involved joining of raw paired-end reads with FASTQ-JOIN 1.1.2 (Aronesty, 2013) and quality filtering with a mean quality score of 30 as a cut-off (Phred score). Sequences were then grouped by barcodes, followed by primer and barcode trimming. For the fungal marker, the ITS2 region was extracted using ITSx 1.1.3 (Bengtsson-Palme et al., 2013). Chimeras were detected and removed with the UCHIME algorithm (Edgar et al., 2011), alongside sequences <40 bp. Remaining sequences were clustered into Operational Taxonomic Units (OTUs, molecular species) at 97% similarity using USEARCH 8.1.1681 (Edgar, 2010). For each OTU, the most abundant sequence was used as the representative for taxonomic identification.

Taxonomic identification for fungal OTUs was determined using BLASTn against the UNITE database release 10.0 (Abarenkov et al., 2023), while its putative ecological assignments were determined based on FungalTraits (Põlme et al., 2020), implemented in UNITE database release 10.0 in SEED 2.1.2. Taxonomic identification of bacterial OTUs was performed using Ribosomal Database Project (RDP) 11.5 (Cole et al., 2014) and Silva 138.1 database (Quast et al., 2013). All databases were downloaded from the SEED2 webpage (Větrovský, 2018). To more accurately identify fungal OTUs at different taxonomic levels, 90%, 85%, 80% and 75% sequence identity thresholds were used as criteria for assigning OTUs to genus, family, order or class level, respectively (Tedersoo et al., 2014). Similarly, for bacterial OTUs, 94.5%, 86.5%, 82.0%, 78.5%, 75.0% sequence identity thresholds were used for genus, family, order, class and phylum, respectively, based on (Yarza et al., 2014).

All statistical analyses were performed in R 4.5.2 (R Core Team, 2025). Metadata used for statistical analyses of sampling campaign 1 and sampling campaign 2 are available in Tables **S1/S2** and Tables **S3/S4**, respectively. Analyses of soil physical and chemical properties, including enzymes, microbial biomass, and alpha diversity metrics, were performed using a one-way analysis of variance (ANOVA) and a post-hoc Tukey test. One-way ANOVA and post-hoc Tukey test were also used to determine significant differences in relative abundances of specific fungal and bacterial taxa. Microbial community analyses were performed using the *phyloseq* (McMurdie & Holmes, 2013) and *vegan* (Oksanen et al., 2012) packages. Principal Coordinates of Neighbour Matrices (PCNM) were calculated to describe spatial distance using the latitude and longitude of each sample (Borcard & Legendre, 2002). Beta diversity was analysed using Bray-Curtis dissimilarity matrices and visualised with non-metric multidimensional scaling ordination (NMDS). PERMANOVA analysis with 999 permutations was used to test drivers of soil microbial community composition with subsequent multilevel comparisons. For the soil health modelling approach, PCA (Principal Component Analysis) was performed, followed by linear mixed-effects models and the Random Forest algorithm. All plots were created with the ggplot2 package (Wickham, 2016), and all statistically significant differences were observed at $p < 0.05$.

4.6. Analyses of microbial communities – Sampling campaign 1 (Donji Kamenjak)

4.6.1. Microbial diversity and composition of soil microbial communities

Alpha diversity metrics (Shannon diversity, OTU richness, and Pielou’s evenness) were used to summarise microbial community diversity and to assess differences in community diversity across dominant vegetation species. Using the *rrarefy* function, OTU tables were randomly subsampled to a uniform sequencing depth to account for uneven sequencing effort and to ensure a fair calculation (Schloss, 2024). Alpha diversity metrics for fungi and bacteria were calculated on 4,701 and 8,023 sequences per sample, respectively, and Spearman correlation was used to analyse correlations between alpha diversity metrics and soil properties separately for each dominant vegetation species. For further verification of alpha diversity metrics, multiple randomisations of subsampling OTU tables to even sequencing depth were performed. One-way ANOVA followed by a post-hoc Tukey test identified significant differences in microbial diversity among the dominant vegetation species (*Cistus* spp., *P. halepensis*, and *Q. ilex*).

For analysing beta diversity, raw OTU tables were first standardised using the Hellinger transformation. To visualise dissimilarity of the fungal and bacterial community composition, NMDS was applied based on Bray-Curtis dissimilarity matrices. NMDS was performed using *the metaMDS()* function from the vegan package. Microbial community composition was visualised with relative abundance plots at phylum and genus level, where OTUs unidentified at the respective level (e.g., "Incertae sedis", "Unclassified", "Gp") were grouped as “Others” to ensure ecological clarity.

4.6.2. Environmental drivers of soil microbial community composition

For the sampling campaign 1 (Donji Kamenjak), PERMANOVA analysis using the *adonis2()* function from the *vegan* package was conducted to test dominant vegetation species and soil properties (pH, C/N ratio, and moisture) as drivers of fungal and bacterial community composition on Donji Kamenjak, hypothesising the dominant vegetation species as the main driver. Additionally, PERMANOVA was used to examine the effect of these drivers on the composition and abundance of dominant fungal putative ecological categories (saprotrophs, EcM fungi, and plant pathogens), with OTU tables subsampled according to FungalTraits assignments (as described previously). Each respective OTU table was standardised using Hellinger transformation, followed by NMDS ordination based on Bray-Curtis dissimilarity matrices. Spatial autocorrelation was captured with PCNM vectors using *the pcnm()* function, and significant vectors were selected with forward selection using *forward.sel()* function and included in PERMANOVA. With significant spatial vectors, PCA using the *prcomp()* function was performed to retain the first principal component (PC1) as a spatial vector for NMDS plots, which explained the highest proportion of spatial variance. Spatial vector and environmental variables were added to the NMDS ordination as arrow vectors using the *envfit()* function.

Individual PERMANOVA was performed to test each driver separately while accounting for spatial autocorrelation, and the recalculated adjusted R^2 values were used to include them in the final PERMANOVA model. With *the pairwise.adonis2()* function, multilevel pairwise comparisons were tested between dominant vegetation species. To account for variation in sampling effort, the sampling month was included as *strata* in PERMANOVA analyses.

4.6.3. Core microbiome and indicator species analyses

For the sampling campaign 1 (Donji Kamenjak), core microbiome and indicator species analyses were performed. For this study, the core microbiome was defined as the set of OTUs common to all three dominant vegetation species (*Cistus* spp., *P. halepensis*, *Q. ilex*) (Walters et al., 2018). Venn diagrams of unique/shared OTUs were generated using the *ps_venn()* function from the *MicEco* package (Russel, 2022). To focus on core taxa, OTU tables were filtered to retain taxa present in ≥10% of samples for each dominant vegetation species, excluding rare taxa. Furthermore, with the *ps_venn()* function, both OTU counts and mean relative abundances of OTUs were calculated, emphasising OTUs with both high ubiquity and ecological dominance rather than just presence/absence. Relative abundance plots were generated to visualise core OTUs at the genus level, together with putative ecological assignments for fungi.

Indicator species analysis identified microbial taxa significantly associated with specific dominant vegetation using *multipatt()* function from the *indicspecies* package (De Cáceres & Legendre, 2009). Statistical significance was assessed with species-site group association function (*r.g.*) with 9,999 permutations. Analyses considered site group combinations but for interpretation prioritised exclusive associations (*duleg = FALSE*). OTUs were assigned to dominant vegetation groups based on their relative abundance and frequency of occurrence within those groups and resulting indicator values and p-values ($p < 0.05$, $p < 0.01$, $p < 0.001$) were extracted. Genus-level taxonomy was assigned using fungal/bacterial reference databases as described in section 3.4. Unidentified OTUs ("Incertae sedis", "Unclassified", "Gp") were excluded due to lack of ecological relevance. Significant indicators were further filtered by minimum relative abundance (≥0.1%) within associated dominant vegetation species. The putative ecological category for the fungal OTUs of the indicator genera was annotated, and only OTUs with indicator values greater than 0.25 that met the relative abundance threshold were selected for interpretation and visualisation.

4.7. Analyses of microbial communities – Sampling campaign 2 (Brijuni NP and Štinjan forest)

4.7.1. Microbial diversity and composition of soil microbial communities

For the sampling campaign 2 (Brijuni NP and Štinjan forest), alpha diversity metrics (Shannon diversity, OTU richness, and Pielou's evenness) were used to summarise microbial community diversity and to assess differences across islands and the mainland. Alpha diversity metrics for fungi and bacteria were calculated on 3,348 and 8,037 sequences per sample, respectively. One-way ANOVA followed by a post-hoc Tukey test identified significant differences in microbial diversity among islands of varying size (Veli Brijun, Mali Brijun, Vanga, Kozada, Sv. Jerolim, Galija, and Gaz).

For analysing beta diversity, raw OTU tables were first standardised using the Hellinger transformation. To visualise dissimilarity of the fungal and bacterial community composition, NMDS was applied based on Bray-Curtis dissimilarity matrices. NMDS was performed using *the metaMDS()* function from the vegan package. Microbial community composition was visualised with relative abundance plots at phylum and genus level, where OTUs unidentified at the respective level (e.g., "Incertae sedis", "Unclassified", "Gp") were grouped as “Others” to ensure ecological clarity.

4.7.2. Intrinsic habitat characteristics and tourism pressure as drivers of soil microbial communities

For the sampling campaign 2 (Brijuni NP and Štinjan forest), intrinsic habitat characteristics and tourism pressure were analysed as drivers of fungal and bacterial communities. Analyses focused on the seven islands sampled during sampling campaign 2 (Veli Brijun, Mali Brijun, Vanga, Sv. Jerolim, Kozada, Galija, Gaz) together with Štinjan forest as the mainland reference site. Intrinsic habitat characteristics were defined as three biogeographic variables: 1) island size, representing only the forest surface area (**Table 2**); 2) distance to nearest island (DNI) and 3) distance to mainland (DTM) (Štinjan forest). Due to the wide range in island sizes, island forest surface areas were log-transformed prior to analysis for normalisation.

Geographic distance metrics (DNI and DTM) were calculated using centroids. For each island and the mainland site, centroid coordinates (latitude and longitude) were determined by averaging the GPS coordinates of all sampling plots within each island and the mainland. DNI was computed as the minimum distance from each island’s centroid to the nearest other island’s centroid, derived from the distance matrix using the *distm()* function. DTM measured the distance from each island centroid to the Štinjan forest centroid using the *distGeo()* function. Tourism pressure, representing anthropogenic disturbance, was quantified using an arbitrary ordinal scale (0-3) developed based on the information mentioned in section 3.2.1. Values were assigned as follows: 3 to Veli Brijuni as the most visited island, 2 to Mali Brijuni and Štinjan forest, 1 to Vanga and Sv. Jerolim, 0 to Kozada, Galija and Gaz. Geographic distance metrics and tourism pressure were incorporated in models to disentangle biogeographic drivers and anthropogenic disturbance from environmental drivers of microbial community diversity and composition.

To ensure that differences in microbial alpha diversity among the islands were not due to unequal sampling effort, despite hierarchical sampling across islands, correlations between the number of samples per island and mean alpha diversity metrics per island were tested using *the cor.test()* function and further confirmed with linear models. Furthermore, linear models using *lm() function* were employed to assess drivers of microbial alpha diversity across islands. Variables were scaled and included intrinsic habitat characteristics (logarithmic values of island size, DTI and DTM) together with soil properties (pH, soil moisture and C/N ratio) selected for their relevance as drivers of soil microbial communities (Delgado-Baquerizo et al., 2017; Grosso et al., 2016; Naylor & Coleman-Derr, 2018; Schimel et al., 2007). To further refine the results of linear models, the Akaike Information Criterion (AICc) was applied. Even though simple linear models provided a general view of microbial diversity drivers, they assumed linearity and included all drivers simultaneously. In constrast, AICc evaluated all possible variable combinations, accounting for potential non-linear relationships and model uncertainty, thereby extending beyond single linear models and providing a more robust estimate of driver effects. AICc was applied using the *dredge()* function on the previous linear model and ranked the models; lower AICc indicated a better model, and models with ΔAICc $\leq$ 2 were retained as best models (Burnham et al., 2011).

PERMANOVA, with the *adonis2()* function, was used to assess geographic distance metrics (DTI, DTM) as drivers of microbial community composition between islands, using Bray-Curtis dissimilarities computed from Hellinger-transformed fungal and bacterial OTU tables. PERMANOVA included the following variables: pH (Delgado-Baquerizo et al., 2017), soil moisture (Naylor & Coleman-Derr, 2018), C/N ratio (Grosso et al., 2016), TOC/clay ratio, dominant vegetation (Pole et al., 2026) and canopy cover (Idbella et al., 2022). The island characteristics included DTI, DTM and tourism pressure. Spatial structuring was captured with PCNM vectors using *the pcnm()* function, and significant vectors were selected with forward selection using *forward.sel()* function and included in PERMANOVA. Initially, individual PERMANOVA analyses were performed to test each driver separately while accounting for spatial structuring. The drivers with significant recalculated adjusted R^2 were included in the final model. Afterwards, multilevel pairwise comparisons between islands were tested using the *pairwise.adonis2()* function.

4.8. Development of predictive soil health model – Sampling campaign 2 (Brijuni NP and Štinjan forest)

Previous analyses were conducted to examine differences in microbial communities and their explanatory drivers. Microbial communities respond to environmental and intrinsic habitat characteristics and thus could serve as predictors for soil health assessment. The first step included calculating the soil health index (SHI), followed by testing the effects of microbial properties on the SHI with linear mixed-effects models to account for fixed effects. The final step was to determine which microbial properties are the best predictors of soil health using the Random Forest machine learning algorithm.

4.8.1. Soil health index (SHI) calculation

The soil health index for this research was defined based on soil chemical and physical properties (abiotic SHI), thus serving as a proxy for soil health assessment and as a response variable for predicting soil health based on microbial properties. It is a data-driven index calculated from soil properties important for soil functioning, measured exclusively for this study, and thus can be applied to the studied Mediterranean forest ecosystems.

For the calculation of SHI, soil properties were selected based on the literature (Bünemann et al., 2018; Grosso et al., 2016; Rinot et al., 2019) addressing important soil health indicators, and the number of selected properties was reduced to minimise noise and ensure robustness and non-collinearity. Selected soil health indicators (minimum data set) were transformed and oriented for scores that higher values indicate healthier soil conditions or that lower values indicate healthier soil conditions, where applicable. Soil health indicators exhibiting optimum conditions, as they are often ecosystem-specific, were scored based on their optimum and tolerance ranges. These included pH, C/N and N/P ratios; for soil pH, the optimum was set at 6.5, with a tolerance of ±1.5 for healthy conditions. Similar values were set for the C/N ratio, where the optimum was 12 with a tolerance of ±6, and for the N/P ratio, where the optimum was 9 with a tolerance of ±4. Target values for stoichiometric indicators were selected based on typical ranges observed in forest soils (Cleveland & Liptzin, 2007; Kirkby et al., 2014; S. Li et al., 2024). Post-scoring, all indicators were normalised to a 0-1 scale (0 = unhealthy and 1 = healthy) to ensure their direct comparability (Pulido et al., 2017). Soil health indicator scores were weighted with PCA to objectively derive the contribution of each indicator to overall variability, as not all indicators contribute equally. PCA was performed with the *prcomp()* function, and PCA loadings (*$rotation*) on PC1 axis were used as weights for each indicator. The soil health index was then calculated as the sum of the products of each indicator score and its assigned weight: $SHI = \Sigma(indicator * weight)$, where $indicator$ is normalised indicator score on 0-1 scale and $weight$ represents the PCA loadings assigned to each $indicator$.

To see the variability of SHI based on different islands and the mainland, one-way ANOVA and post-hoc Tukey test were performed.

4.8.2. Microbes in soil health prediction

Linear mixed-effects models were employed to assess the extent to which the SHI is explained by microbial properties. Fungal and bacterial community composition were summarised using Principal Coordinates Analysis (PCoA) using the *cmdscale()* function on pre-computed Bray-Curtis dissimilarity matrices derived from Hellinger-transformed OTU tables, extracting the first two axes (PCoA1 and PCoA2).

Additive linear mixed-effects models were used, starting from the base null model that controlled for intrinsic habitat characteristics (island size, DNI, and DTM) and tourism pressure, with location included as a random intercept (sampling location). To assess whether microbial properties improved the base model, five additional models were tested, each adding a group of microbial properties to the base model. The likelihood ratio test (ANOVA) was used to determine if additional models significantly improved the base model. Groups of microbial properties included: 1) microbial biomass (fungal biomass, bacterial biomass and F/B ratio); 2) alpha diversity (fungal and bacterial richness); 3) fungal community composition (fungal PCoA1 and PCoA2 axes); 4) bacterial community composition (bacterial PCoA1 and PCoA2 axes) and 5) enzymes (acid phosphatase, α-glucosidase, β-galactosidase, β-glucosidase, β-xylosidase, cellobiohydrolase, chitinase and lipase). Models were compared and selected based on the lowest ΔAIC and the highest R^2. Final linear mixed-effects model integrated the best predictors from each group of microbial properties to determine which microbial properties have significant linear effects on the SHI after accounting for island size, DNI, DTM, and tourism pressure.

To evaluate the ecological relevance of dominant microbial taxa to soil health assessment, the next step was to identify which microbial communities were the most important predictors of SHI. First, linear mixed-effects models were employed to assess which ecologically relevant fungal guilds and bacterial phyla exert the strongest effects on SHI, and whether these effects are positive or negative. Relative abundances of fungal ecological traits and bacterial phyla were scaled. Linear mixed-effects models were performed separately for fungal ecological traits and bacterial phyla, using the same null base model that controlled for island size, DNI, DTM, and tourism pressure, with location included as a random intercept (sampling location). Models were compared based on ΔAIC and R^2.

To obtain comprehensive insights and rank the microbial predictors of SHI, a machine learning algorithm, Random Forest (RF), was employed, addressing the third hypothesis in this dissertation. Random Forest regression was performed using *randomForest()* function with 1000 trees. Variable importance was quantified using percentage increase in mean squared error (*%IncMSE*, higher = more important predictor) and node purity (*IncNodePurity*, higher = better splits). These importance matrices were extracted and sorted to identify the most important predictors of the SHI between microbial properties, fungal guilds and bacterial phyla.

5. Results

5.1. Overall results of soil physical, chemical and biological properties

5.1.1. Sampling campaign 1 – Donji Kamenjak

Soil pH ranged from 6.05 to 7.5, C/N ratio from 0.13 to 7.65, and moisture content from 9.42% to 20.87%. Plots dominated by *Cistus* spp. exhibited significantly lower soil pH compared to those dominated by *Pinus halepensis* or *Quercus ilex*. Neither the C/N ratio nor the moisture content differed significantly among dominant vegetation species. Potential enzymatic activity was highest in *Cistus*-dominated habitats across all measured enzymes, though differences remained non-significant. The F/B ratio was significantly elevated in *Cistus spp*. habitats compared to *P. halepensis* plots, with no significant difference between *P. halepensis* and *Q. ilex* plots (**Figure S1, Table S2**). Details of other measured soil properties results are presented in **Table S2**.

5.1.2. Sampling campaign 2 – Brijuni NP and Štinjan forest

Soil pH, moisture, and C/N ratio differed significantly among sampled locations. Soils from Kozada had the highest pH (above 7), while those from Galija had the lowest (below 6). The C/N ratio was the highest in Štinjan forest and the lowest in Kozada, whereas soil moisture peaked in Veli Brijun and was lowest in Galija. Potential enzymatic activity also varied significantly among locations, except for beta glucosidase and beta xylosidase. Overall, enzymatic activity was highest in Štinjan forest and lowest in Kozada. The F/B ratio showed the highest values in Galija and the lowest in Mali Brijun (**Figure S2, Table S4**). Details of other measured soil properties are presented in **Table S4**.

5.2. Dominant vegetation as driver of microbial communities – Hypothesis 1 (Donji Kamenjak)

5.2.1. Microbial alpha diversity at Donji Kamenjak

Alpha diversity metrics (Shannon diversity, OTU richness, and Pielou’s evenness) did not differ significantly between dominant vegetation species for either fungal or bacterial communities (**Figure S3**). However, dominant vegetation shaped distinct correlations between microbial alpha diversity and soil properties. In *Cistus*-dominated habitats, fungal alpha diversity correlated positively with soil pH - Shannon diversity (r = 0.68, p = 0.003), OTU richness (r = 0.55, p = 0.026), and evenness (r = 0.61, p = 0.012). OTU richness also correlated negatively with the F/B ratio (r = -0.59, p = 0.016). In *P. halepensis*-dominated habitats, fungal

Shannon diversity correlated negatively with alpha glucosidase activity (r = -0.54, p = 0.030) and evenness with alpha-glucosidase (r = -0.55, p = 0.027) and acid phosphatase activity (r = -0.60, p = 0.015). By contrast, no significant correlations appeared between fungal alpha diversity and soil properties in *Q. ilex*-dominated habitats (**Figure 6A**). Bacterial alpha diversity correlated with soil properties only in *Q. ilex*-dominated habitats, where Shannon diversity (r = 0.58, p = 0.025), OTU richness (r = 0.61, p = 0.016) and evenness (r = 0.55, p = 0.034) all showed positive associations with lipase activity (**Figure 6B**).

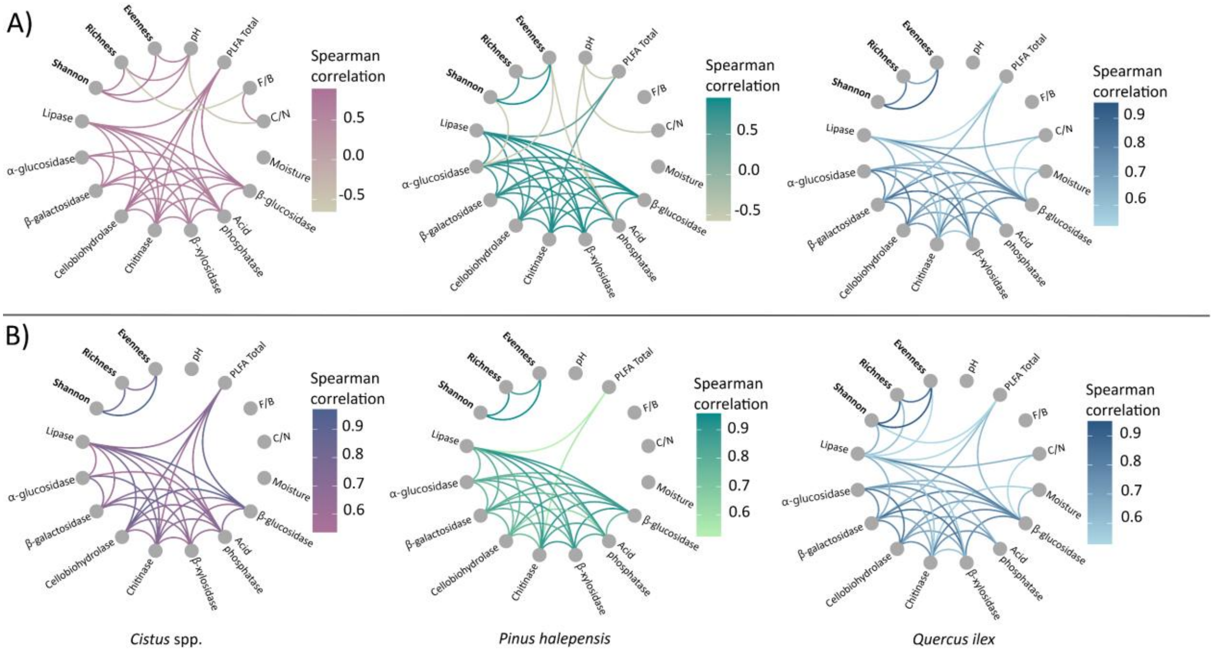

Figure 6. Correlation patterns of alpha microbial diversity metrics and soil properties at Donji Kamenjak. Only statistically significant correlations are presented ($p < 0.05$). **A)** Fungal communities; **B)** Bacterial communities. Each labelled grey node represents a variable, and the lines connecting the nodes represent significant Spearman correlations between the connected variables. Colours are specific to the dominant vegetation species; pink/purple = *Cistus* spp., green = *P. halepensis* and blue = *Q. ilex*. Colour shading corresponds to correlation: light shades are significant negative correlations, darker shades are significant positive correlations, depending on the r value range. **F/B** = PLFA Fungi/PLFA Bacteria, **C/N** = organic carbon/total nitrogen.

5.2.2. Microbial community composition at Donji Kamenjak

The fungal dataset comprised 7,299 OTUs, classified into 15 phyla and 1,164 genera. Across all vegetation species, Ascomycota and Basidiomycota were the dominant fungal phyla. In *Cistus*-dominated habitats, Ascomycota had a relative abundance of 54% (3 052 OTUs), followed by Basidiomycota at 41% (874 OTUs). *Pinus halepensis*-dominated habitats showed a similar pattern, with Ascomycota relative abundance of 51% (2,809 OTUs) and Basidiomycota 43% (1,025 OTUs). *Quercus ilex*-dominated habitats had a slightly higher relative abundance of Basidiomycota (52%; 991 OTUs) compared to Ascomycota (44%; 2 927 OTUs) (**Figure S4A**).

The fungal community composition varied by dominant vegetation species. In habitats dominated by *Cistus* spp., the most abundant genera were *Penicillium* (10%), *Hygrocybe* (10%), and *Inocybe* (1%). *Pinus halepensis* habitats were primarily dominated by *Penicillium* (12%) and *Inocybe* (8%), while *Q. ilex* habitats showed high relative abundances of *Penicillium* (12%), *Inocybe* (5%), and *Hygrocybe* (4%). The genus *Inocybe* was significantly more abundant in *P. halepensis* habitats than in those dominated by *Cistus* spp. In contrast, *Tomentella* was significantly more abundant in *Q. ilex* habitats (4%) than in those with *Cistus* spp. (1%) (**Figure S4B**). Analysis of ecological categories revealed a predominance of EcM fungi and saprotrophs at Donji Kamenjak (**Figure S4C, D**). Ectomycorrhizal fungi were significantly more abundant in *P. halepensis* (31%) and *Q. ilex* (32%) habitats compared to those dominated by *Cistus* spp. (12%). Additionally, *Cistus* spp.-dominated habitats hosted the lowest number of EcM genera, with *Cortinarius* (3%) being the most abundant (**Figure S4E**).

The bacterial dataset included 16 667 OTUs, classified into 45 phyla and 895 genera, with Actinobacteria and Proteobacteria consistently dominant in all habitats. In *Cistus* spp. habitats, Actinobacteria showed a relative abundance of 33% (1,648 OTUs) and Proteobacteria 26% (3,528 OTUs). *Pinus halepensis* and *Q. ilex* habitats had similar profiles, with Actinobacteria contributing ~30% and Proteobacteria ~26-27% of relative abundance (**Figure S5A**). Dominant bacterial genera included *Conexibacter*, *Solirubrobacter*, and *Gaiella*, all with a mean relative abundance of ~3-7% (**Figure S5B**).

The fungal core microbiome (shared across all three vegetation species) was dominated by saprotrophs, with a mean relative abundance of 57% (814 OTUs; **Figure 7A, left**). *Penicillium* (12%) and *Hygrocybe* (5%) were the most abundant genera in this core, while EcM fungi comprised 12%, dominated by *Tricholoma* (2%) (**Figure 7B, left**). Indicator species analysis identified 144 fungal OTUs strongly associated with *Cistus* spp.-dominated habitats, 122 with *P. halepensis*, and 62 with *Q. ilex*. *Hygrocybe*, *Cladophialophora*, and *Cortinarius* were strong indicators of *Cistus* spp. habitats; *Geopora*, *Inocybe*, *Penicillago*, and *Beauveria* were more associated with *P. halepensis*; and EcM genera *Sebacina* and *Tomentella*, along with *Penicillium*, were identified as indicators of *Q. ilex* habitats (**Figure S6A**).

The bacterial core microbiome comprised 5 313 OTUs shared across all vegetation species, accounting for 92% of the total bacterial relative abundance (**Figure 7A, right**). It was dominated by Actinobacteria (33%) and Proteobacteria (26%). Genus-level resolution was limited for many OTUs due to similarity thresholds, but *Rhodoplanes* and *Solirubrobacter* were the most abundant genera (both <3% mean relative abundance; **Figure 7B, right**). Indicator species analysis identified 144 bacterial OTUs associated with *Cistus* spp. habitats, 243 with *P. halepensis*, and 99 OTUs with *Q. ilex*. Main indicators included *Sphingomonas*, *Piscinibacter*, *Arenimicrobium*, and *Mycobacteriu*m for *Cistus*-dominated habitats; *Pseudonocardia* for *P. halepensis*; and *Acidibacter* and *Cupriavidus* for *Q. ilex* (**Figure S6B**).

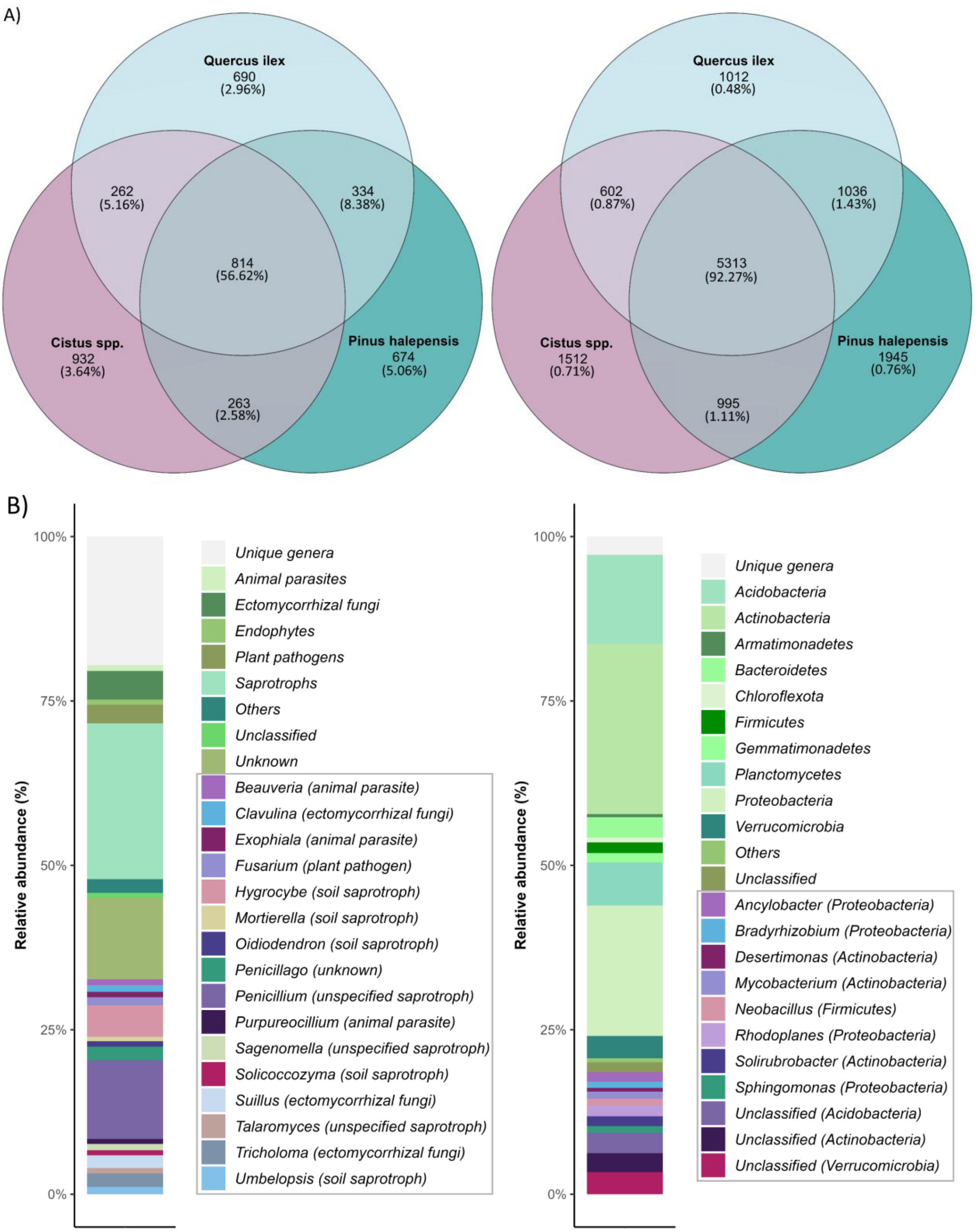

Figure 7. Fungal and bacterial core microbiome at Donji Kamenjak. **A)** Venn diagrams showing both OTUs common to all three dominant vegetation species and unique OTUs of fungal (left) and bacterial (right) communities between different dominant vegetation species with mean relative abundance in brackets. OTUs present in at least 10% of samples in each dominant vegetation species are shown. **B)** Relative abundance of core fungal putative ecological categories (left) and bacterial phyla (right). For the twenty most abundant fungal and bacterial genera, ecological category (fungi) or phylum (bacteria) is shown in brackets. For all other genera, only ecological category or phylum is shown. Unclassified are fungal and bacterial OTUs unidentified at genus and phylum level, respectively; Unknown are fungal OTUs with unassigned putative ecological category.

5.2.3. Drivers of community composition at Donji Kamenjak

NMDS ordination showed distinct clustering of fungal communities by dominant vegetation, with clear separation between *Cistus* spp. and *P. halepensis* habitats and an intermediate position for *Q. ilex* (**Figure 8A, left**). Bacterial communities, in contrast, showed considerable overlap between different dominant vegetation species (**Figure 8A, right**). NMDS ordinations of individual fungal ecological groups revealed similar separation patterns (**Figure 8B**), though high stress values (0.21) should be noted. Relative abundances of saprotrophs and plant pathogens were evenly distributed across dominant vegetation species, whereas EcM fungi were more abundant under *P. halepensis* and *Q. ilex* (**Figure 8B; Figure S4C, S4D**).

PERMANOVA analysis revealed that spatial structuring explained more variation in microbial community composition than dominant vegetation or soil properties. Fungal and bacterial communities were significantly structured by space (R^2 = 0.02341, R^2 = 0.02444), with EcM fungi showing stronger spatial structuring (R^2 = 0.05829) than total fungi. Dominant vegetation had a weaker but significant effect on EcM composition (R^2 = 0.07542), particularly distinguishing *Cistus* spp. shrubs from *P. halepensis* and *Q. ilex trees*. Overall, measured variables (space, dominant vegetation, soil properties) explained only a small fraction of total variation in microbial communities (**Table 3**).

Table 3. Summary of PERMANOVA analysis. The order of variables is based on adjusted R^2 values. Spatial PCNMs were always accounted for first. Significant p-values are in bold.

	Fungal community			Ectomycorrhizal fungi			Bacterial community		
Effect	Df	R^2	p-value	Df	R^2	p-value	Df	R^2	p-value
Spatial PCNMSs	5	0.0234	**0.001**	2	0.0583	**0.003**	2	0.0244	**0.008**
pH	1	0.0254	0.715	1	0.0204	0.399	1	0.0225	0.279
C/N	1	0.0279	0.646	1	0.0278	0.052	1	0.0226	0.294
Moisture	1	0.0262	0.799	1	0.0202	0.560	1	0.0322	0.064
Dominant vegetation	2	0.0847	0.864	2	0.0754	**0.001**	1	0.0554	0.057

Df = degrees of freedom (n = 47)

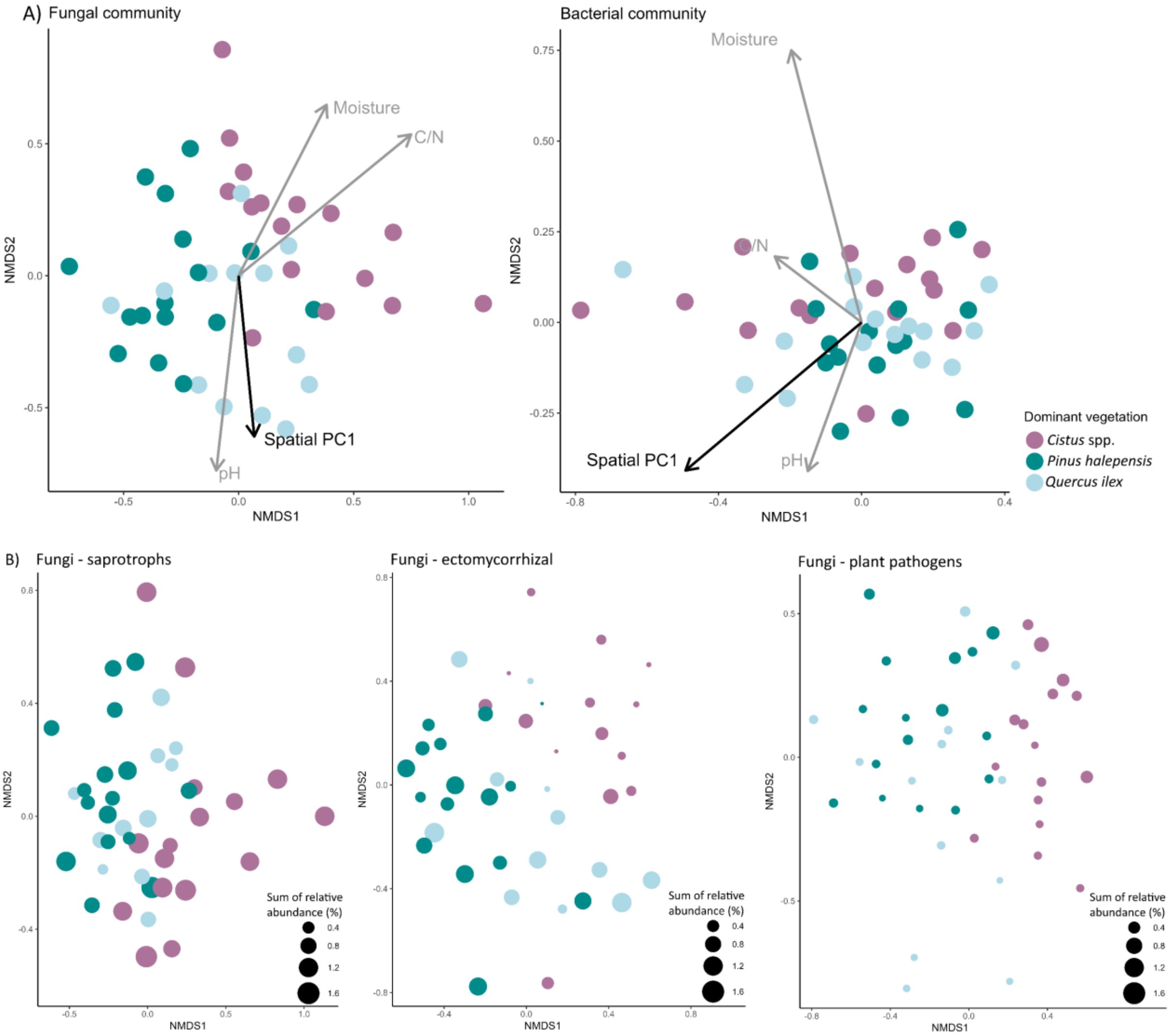

Figure 8. NMDS ordination plots of microbial community composition at Donji Kamenjak. **A)** Fungal community; **B)** Bacterial community; **C-E)** Fungal putative ecological categories. Circle size represents the sum of relative abundance (%). Vectors are fitted environmental variables: significant vectors ($p < 0.05$) are shown in black; non-significant vectors are grey. Stress value (fungal community, saprotrophs, ectomycorrhizal fungi and plant pathogens = 0.21; stress value (bacterial community) = 0.14.

While vegetation-specific correlations were confirmed at Donji Kamenjak, spatial structuring remained the primary driver of soil microbial communities, rather than dominant vegetation, with the exception of EcM fungi.

5.3. Intrinsic habitat characteristics and tourism gradient – Hypothesis 2 (Brijuni NP and Štinjan forest)

<u>5.3.1. Microbial alpha diversity at Brijuni NP and Štinjan forest</u>

Fungal alpha diversity metrics varied significantly between islands and the mainland. Post-hoc Tukey tests revealed a consistent pattern across metrics: Galija showed significantly lower fungal diversity than most locations; Shannon diversity and evenness were significantly lower at all locations, while richness was lower on Veli Brijun, Mali Brijun, and Kozada. The mainland Štinjan forest had significantly lower alpha diversity metrics compared to Veli Brijun and higher than Galija (**Figure 9A**).

Bacterial alpha diversity metrics exhibited weaker but significant differences between localities compared to fungi, with Shannon diversity as an exception. Bacterial richness was significantly higher in Veli Brijun compared to Mali Brijun, while evenness was higher in Sv. Jerolim compared to Vanga. No significant differences were observed between islands and the mainland (**Figure 9B**).

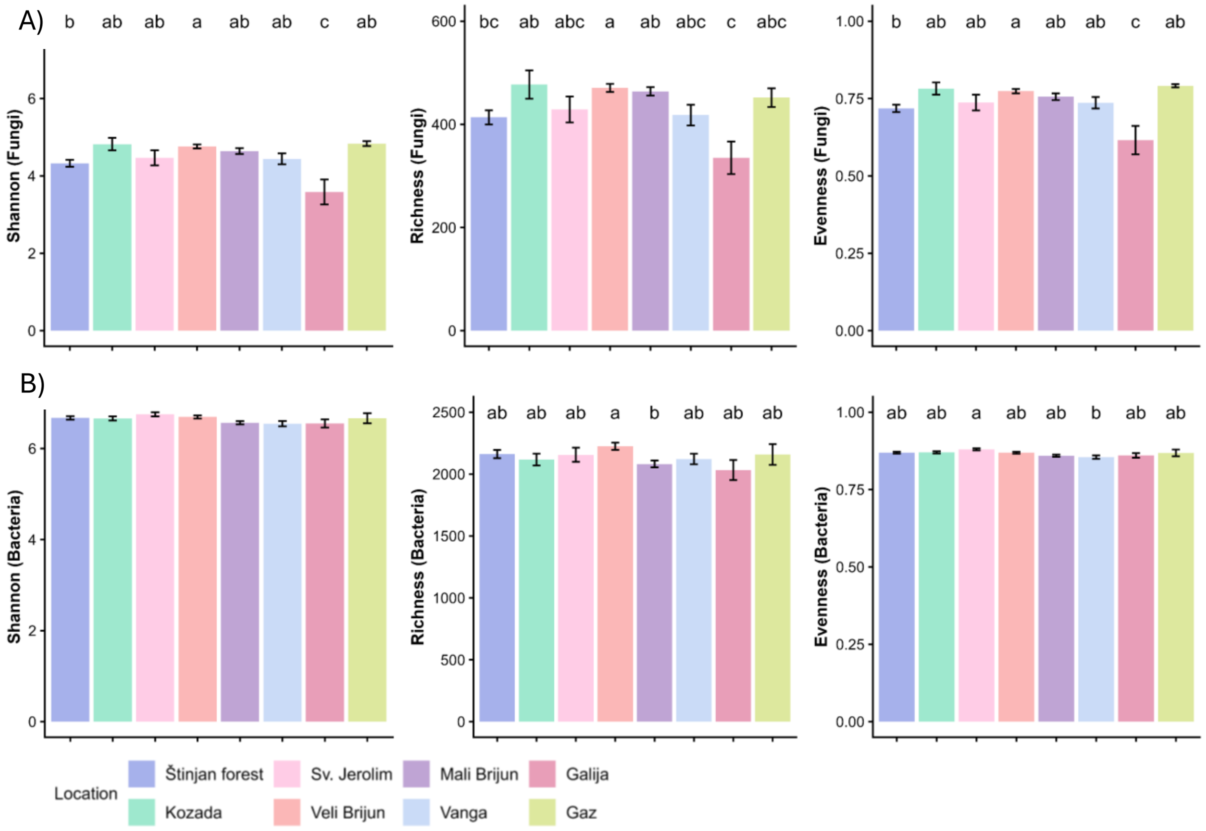

Figure 9. Microbial alpha diversity metrics (Shannon diversity, richness and Pielou's evenness) between islands of Brijuni NP and mainland Štinjan Forest. **A)** Fungal community; **B)** Bacterial community. Locations are ordered left-to-right by increasing distance from mainland (DTM). Error bars represent standard error of the mean; different letters indicate significant differences among locations.

To identify drivers of fungal and bacterial richness, linear models were employed using scaled variables: island size, DTM, DTI and soil properties (pH, moisture, C/N) ratio to account for known variability. For fungi and bacteria, models were significant and explained 24% and 19% of variation in richness, respectively. Geographic distance (DNI) and soil pH were the strongest, consistent positive drivers of fungal richness, while island size, DTM, moisture, and C/N showed no significant effects (**Table S5**). Soil moisture was a strong driver of bacterial richness (**Table S6**).

AICc model selection addressed limitations of full linear models by evaluating all possible combinations of six drivers (island size, DTM, DNI, soil pH, moisture and C/N ratio). Best models were ranked from lowest to highest AICc and selected based on ΔAICc ≤ 2. For fungal richness, the ten best models were selected; the leading model included DNI, island size, and pH (AICc = 1184.80, weight = 0.118), while the second included C/N, DNI, and pH (AICc = 1184.96, weight = 0.109). Parameters of all the best selected models for fungi are presented in **Table S7**. For bacterial richness, four best models were selected; the first included soil moisture (AICc = 1377.79, weight = 0.197), and the second included soil moisture and DTM (AICc = 1379.27, weight = 0.094). Parameters of the best-selected models for bacteria are presented in **Table S8**.

After selecting plausible models using AICc, model averaging was used to estimate model selection uncertainty across the plausible models, and importance was calculated as the sum of weights across models containing each variable. DNI and pH (sum of weights = 1.0) were confirmed as the main drivers of fungal richness. DNI and pH appeared in 100% of the best models, while island size appeared in 40% of the best models. For bacterial richness, soil moisture was confirmed as the main driver (sum of weights = 1.0) (**Figure 10**).

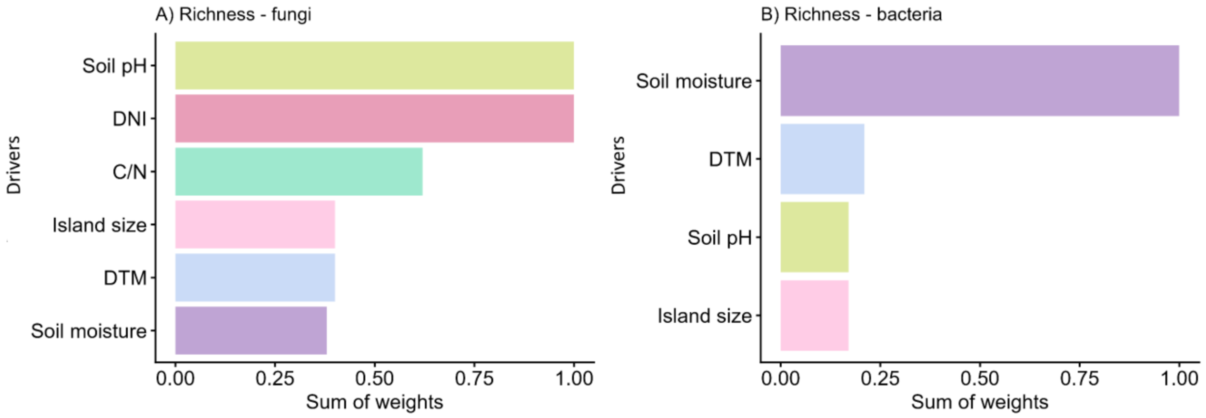

Figure 10. Drivers of fungal and bacterial richness at Brijuni NP and Štinjan forest. **A)** Fungal richness; **B)** bacterial richness. **C/N ratio** = total carbon/N; **DNI** = distance to the nearest island; **DTM** = distance to the mainland.

5.3.2. Microbial community composition

NMDS ordinations were used to visualise differences in microbial community composition between locations. Fungal communities showed clear separation, with Štinjan (mainland) and Gaz forming the most distinct clusters, as the two most distant locations. On the other hand, the two nearest islands, Galija and Vanga, appeared to show more similar fungal communities (**Figure 11A**). Bacterial communities exhibited tighter clustering compared to fungal communities **(Figure 11B)**.

Fungal communities were clustered into 9,269 OTUs and classified into 17 phyla and 1,365 genera. Among fungal communities, saprotrophs dominated, followed by plant pathogens and EcM fungi. There was a clear decrease in the abundance of EcM fungi with distance from the mainland. The relative abundance of EcM fungi at Štinjan was ~14%, while at Gaz it was significantly lower, ~0.01%. On the contrary, plant pathogens seemed to increase with distance from the mainland; at Štinjan, relative abundance was ~3%, whereas at the most distant island, Gaz, it was significantly higher at ~22% (**Figure 11C**). Among fungal genera, *Pencillium* was the most dominant at all locations (~8–22%), *Geastrum* (Basidiomycota) was significantly more abundant at Sv. Jerolim (~10%), *Hygrocybe* (Basidiomycota) significantly dominated at Galija (~24%) and *Incoybe* (Basidiomycota) at Veli Brijun (~2%). Genus *Oidiodendron* (Ascomycota) showed a tendency to decrease with distance, from ~6% at Štinjan to ~0.2% at Gaz (**Figure S7A**).

Bacterial communities were clustered into 18,277 OTUs and classified into 43 phyla and 1,013 genera. As observed in the NMDS ordination, bacterial community composition was similar across locations, with dominance of Actinobacteria (~22–36%) and Proteobacteria (~17–28%). The relative abundance of Proteobacteria was significantly higher at Štinjan (~28%) compared to Gaz (~19%) (**Figure 11D).**

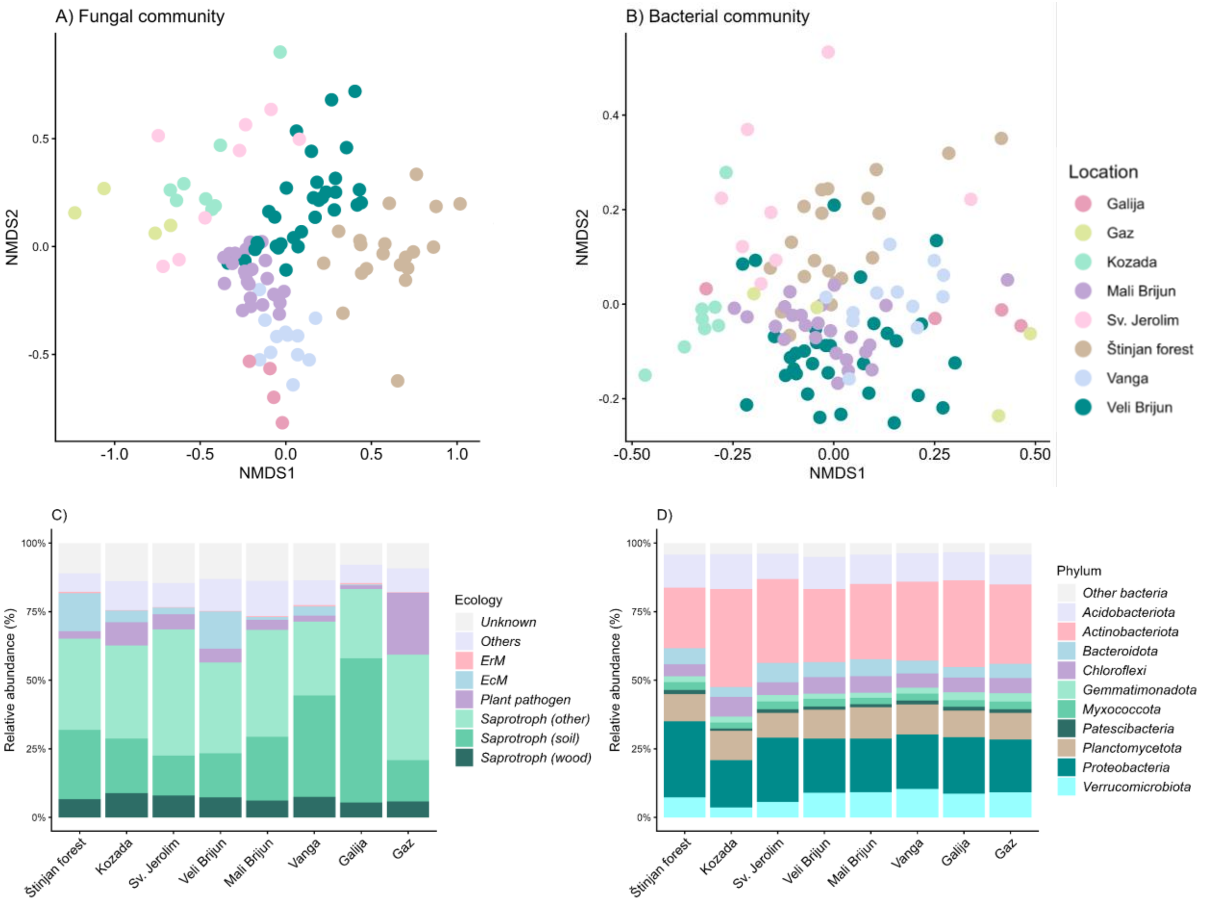

Figure 11. Microbial community composition at Brijuni NP and Štinjan forest. **A)** NMDS ordination of fungal community; **B)** NMDS of bacterial community; **C)** Relative abundance of ecology traits of fungi based on 30 most abundant genera; **D)** Relative abundance of 10 most abundant bacterial phyla. Localities are ordered left-to-right by increasing distance from mainland (DTM) on relative abundance plots (C and D).

5.3.3. Drivers of microbial community composition at Brijuni NP and Štinjan forest

For drivers of fungal community composition, PERMANOVA revealed strong spatial structuring explaining 16.0% of total variance. Beyond spatial structuring, tourism pressure contributed 2.7% of the variance, followed by dominant vegetation (2.5%) and soil pH (1%) (**Table 4**). The full model explained 63.4% of the total variance. Pairwise PERMANOVA was used to test differences in community composition, where each island was compared to the mainland and to each other. Mainland-island differences were consistently significant across all pairs confirming strong differences in fungal community composition between Štinjan forest and islands regardless of geographic distances (DTM or DNI). The most isolated islands (Vanga, Galija, and Gaz; farthest from the mainland) but mutually closer (Gaz to Galija and Galija to Vanga) showed non-significant differences, indicating similar microbial communities among distant from the mainland but mutually proximate islands (**Table S9**).

Table 4. Drivers of fungal community composition. Significant drivers ($p < 0.05$) are in bold. The order of variables is based on adjusted R^2 values. Spatial PCNMs were always accounted for first.

Drivers	Df	Sum of squares	R^2	F-statistics	Pr(>F)
Spatial PCNMs	15	2.159	0.1603	2.425	**0.001**
Tourism pressure	3	0.370	0.0274	2.0758	**0.003**
Dominant trees	3	0.337	0.025	1.8921	**0.001**
DNI	1	0.086	0.0064	1.4503	0.114
Soil pH	1	0.138	0.0102	2.3166	**0.012**
DTM	1	0.094	0.0069	1.5759	0.083
Canopy cover	1	0.075	0.0056	1.27	0.208
C/N	1	0.090	0.0067	1.5141	0.089
Soil moisture	1	0.112	0.0083	1.8904	**0.032**
TOC/clay	1	0.069	0.0051	1.1546	0.287
Residual	83	4.925	0.3657		
Total	111	13.466	1		

DTM = distance to the mainland; **DNI** = distance to the nearest island; **C/N ratio** = total carbon/N; **TOC/clay ratio** = total organic carbon/clay. **Df** = degrees of freedom (n = 112).

Similarly, for drivers of bacterial community composition, PERMANOVA revealed spatial structuring, explaining 15.96% of total variance. Soil pH was the strongest driver, explaining 5.9% of variance, followed by tourism pressure (4.8%) and soil moisture (1.1%) (**Table 5**). The full model explained 66.1% of the total variance. Pairwise PERMANOVA indicated weaker island differentiation for bacteria compared to fungi. Mainland Štinjan differed from most islands, but not from the farthest islands (Galija and Gaz). Island-island comparisons were frequently non-significant (**Table S9**), indicating lower island turnover among distant islands.

Table 5. Drivers of bacterial community composition. Significant drivers ($p < 0.05$) are in bold. The order of variables is based on adjusted R^2 values. Spatial PCNMs were always accounted for first.

Drivers	Df	Sum of squares	R2	F-statistics	Pr(>F)
Spatial PCNMs	14	0.226	0.1596	2.8238	**0.001**
Soil pH	1	0.084	0.059	14.6073	**0.001**
Tourism pressure	3	0.068	0.0478	3.9445	**0.001**
Dominant trees	3	0.023	0.0165	1.3656	0.114
C/N ratio	1	0.010	0.0069	1.7152	0.105
Soil moisture	1	0.015	0.0108	2.6808	**0.022**
DTM	1	0.010	0.0072	1.7955	0.096
DNI	1	0.011	0.0079	1.9465	0.07
TOC/clay ratio	1	0.007	0.005	1.2326	0.256
Canopy cover	1	0.008	0.0053	1.3131	0.227
Residual	84	0.480	0.3392		
Total	111	1.416	1		

DTM = distance to the mainland; **DNI** = distance to the nearest island; **C/N ratio** = total carbon/N; **TOC/clay ratio** = total organic carbon/clay. **Df =** degrees of freedom (n = 112).

Taken together, Brijuni NP and Štinjan forest, fungal richness was most strongly driven by geographic distance (DNI) and pH, while island size was important, but the effect was uncertain. On the other hand, soil moisture was the main driver of bacterial richness. Spatial structuring was the main driver of soil microbial community composition. Geographic distance metrics (DNI, DTM) were non-significant drivers of microbial community composition, however, the mutually closest islands but farthest from the mainland harboured similar microbial communities.

5.4. Soil health assessment – Hypothesis 3 (Brijuni NP and Štinjan forest)

5.4.1. Soil health index (SHI)

The soil health index was constructed by orienting and scoring 24 soil physical and chemical indicators and their weights (PCA loadings). Because PC1 captures the largest proportion of variance in the dataset, variables with larger loadings contributed more strongly to the SHI. Absolute values of the PC1 loadings were used to represent the magnitude of the contribution of each variable, irrespective of direction. Soil physical and chemical properties included as indicators for SHI were soil pH, soil moisture, TOC, N, C/N ratio, P, K, Ca, Mg, clay, TOC/clay ratio, P_2O_5, K_2O, humus, TC, Fe, Zn, Mn, Na, Al, Pb, N/P ratio, sand and silt. The scoring orientation and weight for each indicator are listed in **Table S11**.

Across all samples, the SHI ranged from 0.23 to 0.62 (median = 0.31, mean = 0.32). One-way ANOVA revealed significant differences in the SHI between islands (F = 4.281, p = 0.0003). Post-hoc Tukey test identified significant differences in the SHI between Gaz and Štinjan forest; Gaz had significantly higher SHI than Štinjan forest, with the two locations represented as unvisited and moderately visited, respectively. The SHI at Veli Brijun, as the most visited location, was not significantly different from other locations (**Figure 12**).

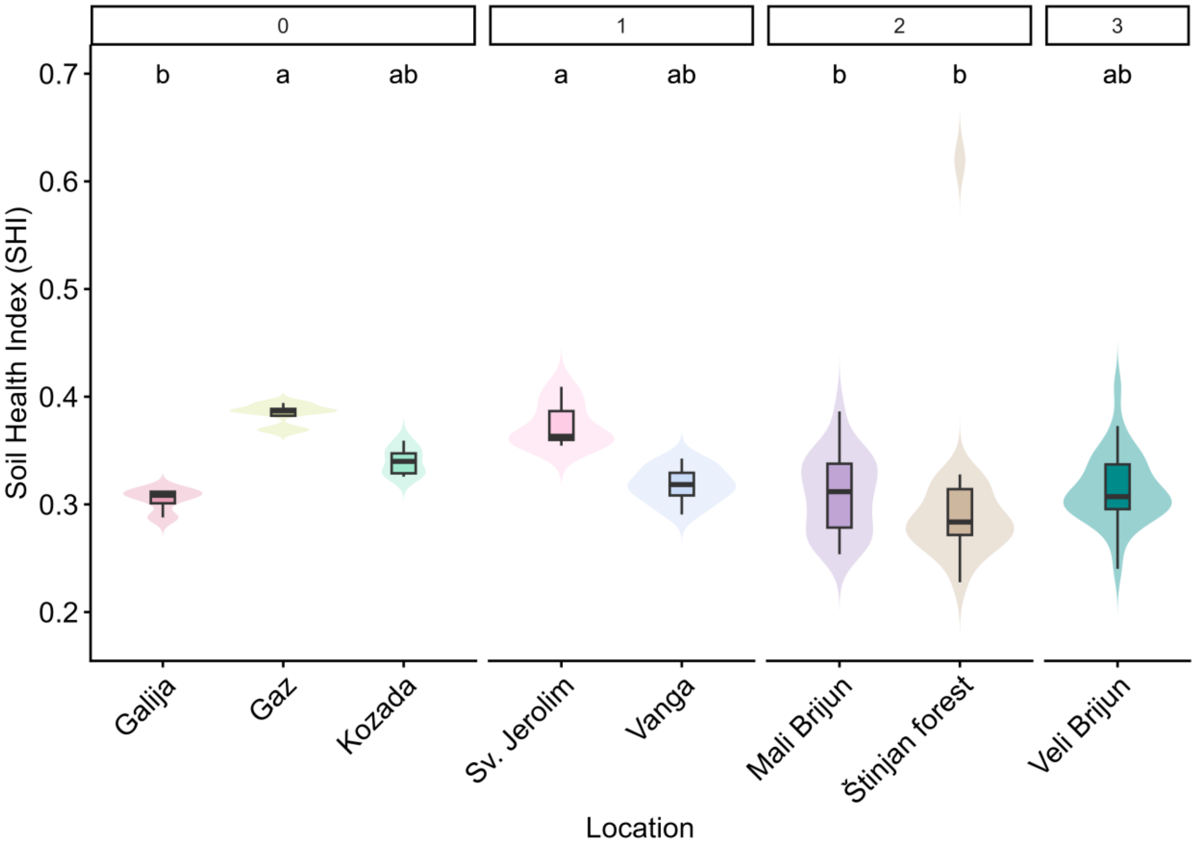

Figure 12. Soil Health Index (SHI) across Brijuni NP and Štinjan forest grouped by tourism pressure from 0-3. Tourism pressure 0 indicates locations fully closed to visitors or rarely visited; tourism pressure 1 indicates locations with restricted access; tourism pressure 2 indicates locations with moderate visiting; tourism pressure 3 indicates high visiting. Violins represent the full distribution of SHI values; embedded boxplots show the median, interquartile range (IQR), and whiskers extending to 1.5 × IQR. Different letters indicate significant differences between locations ($p < 0.05$). Higher SHI values indicate greater soil health.

5.4.2. Soil health prediction

To evaluate which properties of the soil microbial community best explain variation in the SHI, a series of linear mixed-effects models was constructed with location included as a random effect. A baseline model including island size, DNI, DTM, and tourism pressure explained ~22% of variance in SHI (marginal $R^2 = 0.216$, AIC = −377.2). Then, five candidate microbial predictor groups were separately added to the baseline model: microbial biomass, fungal community composition, bacterial community composition, alpha diversity and enzymes. The addition of microbial biomass did not significantly improve model fit over the baseline ($p = 0.2581$, marginal $R^2 = 0.243$, AIC = -375.23). Bacterial richness and fungal richness together significantly improved model fit ($p < 0.001$, marginal $R^2 = 0.328$, AIC = -390.33), driven primarily by bacterial richness, with the strongest individual effect among alpha diversity predictors ($t = 4.001$). The significant improvement in model fit also came from community composition: adding bacterial PCoA1 and PCoA2 to the baseline model produced a highly significant improvement ($p < 0.001$) and increased explained variance to ~50% (marginal $R^2 = 0.499$, AIC = -423.26), with bacterial PCoA2 being particularly influential ($t = -7.923$). Fungal community composition also showed a significant improvement over the baseline model ($p = 0.03$, marginal R2 = 0.263, AIC = -380.03), with the highest contribution from fungal PCoA2 ($t = 2.653$). The enzyme group most substantially improved model fit over the baseline model ($p < 0.001$, marginal $R^2 = 0.638$, AIC = -447.5), with β-xylosidase ($t = 2.068$), acid phosphatase ($t = 2.237$), and cellobiohydrolase ($t = -2.133$) emerging as the strongest individual enzyme predictors. Reduced model only with these three strongest enzyme predictors performed better (AIC = -450.08) compared to the full enzyme model (AIC = -447.5), indicating that the other five enzymes did not contribute additional explanatory power for SHI.

A final model was constructed by combining the strongest predictors from each group of microbial properties - bacterial richness, bacterial PCoA2, fungal PCoA2, β-xylosidase, acid phosphatase and cellobiohydrolase, together with the baseline predictors. This model explained ~72% of variance in the SHI (marginal $R^2 = 0.7190$, AIC = −479.64) and was significantly improved over the reduced enzyme model (marginal $R^2 = 0.6137$, AIC = -450.08). Enzymes explained a large proportion of the SHI variation, while community composition further improved model performance and captured additional variation in the SHI. Bacterial richness contributed little additional explanatory power once enzymes were accounted for (**Table S12**). Across all models, the random effect of location accounted for zero additional variance, indicating that island-level grouping did not introduce meaningful clustering beyond what was captured by the fixed predictors.

To complement the linear mixed-effects modelling framework and provide a non-parametric assessment of predictors' importance without assumptions of linearity, a random forest (RF) regression model was constructed using all microbial predictor variables as inputs and SHI as the response. Bacterial community composition was the most important predictor, with bacterial PCoA2 ranking first (%IncMSE = 15.4). Fungal community composition was also important for SHI, with fungal PCoA2 ranking second (%IncMSE = 14.91), followed by fungal PCoA1 (%IncMSE = 11.61). Among the enzyme group, cellobiohydrolase (%IncMSE = 6.8) and lipase (%IncMSE = 5.7) ranked highest. Fungal richness (%IncMSE = 0.3) was the least important predictor for SHI (**Figure 13**, **Table S13**).

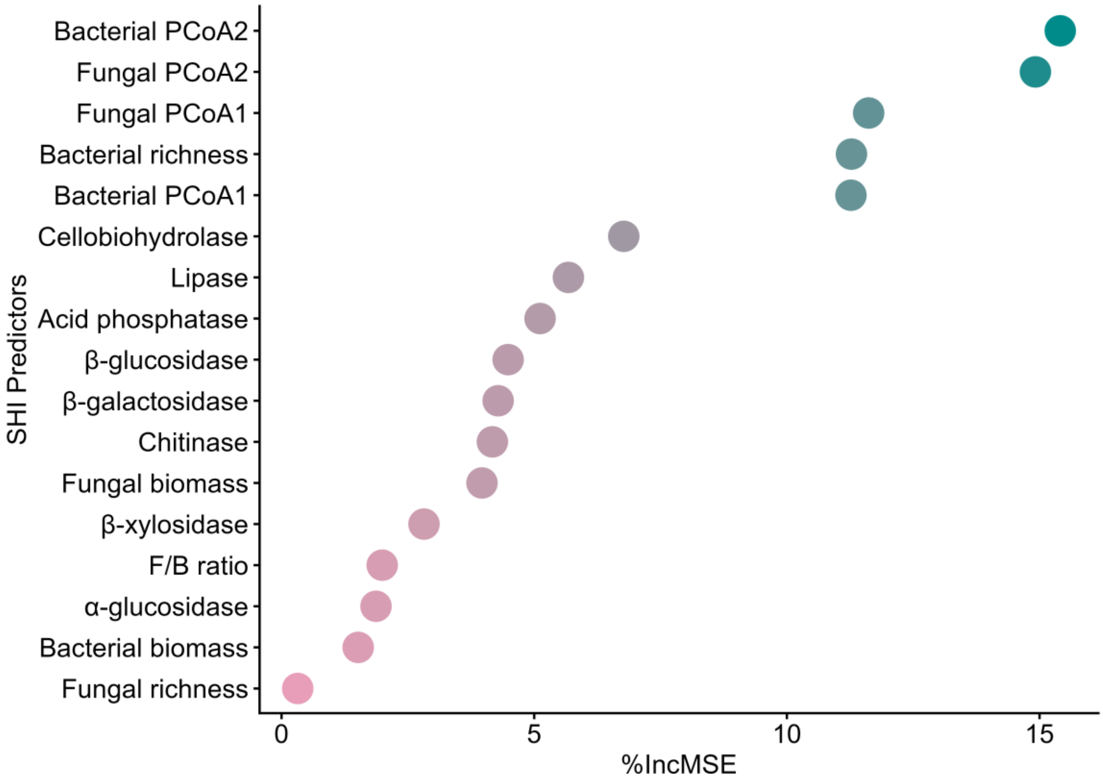

Figure 13. Variable importance of microbial predictors for soil health on Brijuni NP based on Random Forest algorithm. Point colour reflects variable importance along a gradient from pink (low importance) to dark green (high importance), visually reinforcing the ranked ordering.

To identify which specific taxonomic groups and functional guilds explained variation in SHI, a linear mixed-effects models were conducted using bacterial phylum relative abundances and fungal guild relative abundances as predictors. Adding the ten most abundant bacterial phyla to the baseline model produced a highly significant improvement in model fit ($p < 0.001$), with explained variance increasing from 22% (AIC = −377.2) to 52.6% (AIC = −413.1). Verrucomicrobiota showed the strongest individual effect (t = −2.282), followed by Gemmatimonadota (t = −2.179) and Patescibacteria (t = 3.888). In contrast, fungal guild composition did not significantly improve model fit over the baseline model (p = 0.838, marginal R² = 0.235, AIC = −367.97), indicating that the relative abundances of mycorrhizal fungi, plant pathogens, and saprotrophic fungi did not significantly explain variation in SHI once abiotic factors were accounted for.

The RF models complemented these findings. Phylum Verrucomicrobiota was the most important predictor of SHI (%IncMSE = 19.9), followed by Patescibacteria (%IncMSE = 10.4), Bacteroidota (%IncMSE = 8.8) and Actinobacteriota (%IncMSE = 3.7). Phyla Acidobacteriota, Planctomycetota and Myxococcota were the least important bacterial phyla predictors of SHI (**Table 6**). For fungal guilds, soil saprotrophic fungi emerged as the most important predictor (%IncMSE = 7.8), followed by plant pathogens (%IncMSE = 7.6) and EcM fungi (%IncMSE = 5.5) (**Table 7**).

Table 6. Importance of bacterial phyla as soil health predictors.

Bacterial phyla	%IncMSE
Verrucomicrobiota	19.9530
Patescibacteria	10.4213
Bacteroidota	8.76049
Actinobacteriota	3.71365
Chloroflexi	3.13756
Proteobacteria	1.73793
Gemmatimonadota	1.56507
Acidobacteriota	-0.1141
Planctomycetota	-0.4655
Myxococcota	-1.7926

Table 7. Importance of fungal guilds as soil health predictors.

Fungal guilds	%IncMSE
Saprotroph (soil)	7.8301
Plant pathogen	7.6349
EcM	5.4944
Saprotroph (other)	2.0473
Saprotroph (wood)	-0.6923
ErM	-0.8636

Taken together, the linear mixed-effects model and RF provided complementary findings: community composition together with enzymes were the strongest and most important microbial predictors of the SHI, while richness and microbial biomass offered little predictive value. The abundance of specific bacterial phyla, Verrucomicrobiota, Patescibacteria, and Bacteroidota, carried meaningful predictive importance for SHI, while fungal functional guilds offered little predictive importance for soil health.

6. Discussion

The aim of this dissertation was to identify drivers that shape soil microbial communities in Mediterranean forest ecosystems, and to evaluate whether these communities can serve as reliable predictors of soil health. Discussion is organised around three research questions: 1) contribution of dominant vegetation in shaping microbial diversity and composition, 2) how intrinsic island characteristics and tourism pressure drive microbial communities, and 3) the capacity of specific microbial properties to predict soil health.

6.1. Dominant vegetation, soil properties and spatial structuring as drivers of soil microbial communities

The relationship between vegetation and soil microbial communities has been extensively studied, yet the mechanisms by which vegetation shapes microbial communities remain taxon- and context-dependent (De Vries & Shade, 2013; Sardans & Peñuelas, 2013). In the presented research, dominant vegetation did not emerge as a strong driver of soil microbial communities, except for EcM fungi. Instead, spatial structuring was identified as the primary driver of community composition, while a stable core microbiome appeared to buffer vegetation-driven differences across dominant vegetation species.

Fungal and bacterial alpha diversity showed no statistically significant differences between habitats dominated by *Cistus* spp., *Pinus halepensis*, or *Quercus ilex* (**Figure S3**). This pattern may reflect similar soil properties across these dominant vegetation species, given that neither the C/N ratio nor the soil moisture differed significantly, with pH as the only exception. Microbial diversity is shaped by a combination of biotic and abiotic drivers, and pronounced seasonal fluctuations may further buffer any vegetation effects (Bissett et al., 2013; Docherty et al., 2012). It should also be noted that sampling campaign 1 was conducted over different seasons, causing temporal heterogeneity that may have further obscured vegetation effects on microbial diversity. Vegetation-specific correlation revealed differences in the coupling of fungal and bacterial alpha diversity with specific enzymes (**Figure 6**), which may reflect the effects of litter chemistry and substrate availability on potential microbial activity (Šnajdr et al., 2011; Voříšková & Baldrian, 2013). However, potential microbial activity had proven to be highly dynamic and sensitive to changes in environmental conditions (Kuzyakov & Blagodatskaya, 2015), which may have masked more pronounced vegetation-driven effects within temporally constrained sampling used here.

At the compositional level, fungal communities showed clearer separation among dominant vegetation species, while bacterial communities remained largely overlapped. This contrast was consistent with the pattern that fungi are more tightly coupled to vegetation than bacteria, due to their symbiotic and saprotrophic lifestyles, whereas bacterial community composition is more strongly governed by soil properties (Urbanová et al., 2015). Moreover, the significantly higher abundance of EcM fungi in habitats dominated by *P. halepensis* and *Q. ilex* compared to *Cistus* spp. could result from the obligate EcM symbiosis formed by these tree species (Nuñez et al., 2009; Smith & Read, 2008). Beyond these vegetation-driven compositional patterns, core microbiome analyses revealed high consistency of microbial taxa across all three dominant vegetation species. The fungal core microbiome accounted for half of total fungal relative abundance and was dominated by saprotrophs and EcM fungi, highlighting their central role in nutrient cycling and plant nutrient uptake (Baldrian et al., 2011; Smith & Read, 2008) (**Figure 7 left**). The bacterial core microbiome was larger, accounting for 92% of bacterial relative abundance, and was dominated by Actinobacteria and Proteobacteria (**Figure 7 right**). Although each dominant vegetation species harboured unique OTUs, they contributed minimally to total relative abundance, suggesting that soil microbial consistency is largely governed by shared core taxa that likely play an important role in ecosystem services (Luo et al., 2022; Zhang et al., 2022). This is further supported by Jiao et al., (2022), whose findings highlight the importance of core microbial communities in sustaining functional consistency in reforested ecosystems.

PERMANOVA revealed that spatial structuring was the main driver of soil microbial communities, with a more pronounced effect for fungi (**Table 3**), a pattern widely documented in Mediterranean soils (Adamo et al., 2021; Castaño et al., 2018; Pérez-Izquierdo et al., 2017; Richard et al., 2004; Saitta et al., 2018). Spatial structuring refers to the non-random geographic organisation of microbial communities and can arise from several underlying mechanisms such as dispersal limitation (Barbour et al., 2023), environmental gradients (Clark et al., 2021) or biotic interactions (Rożek et al., 2023). In the presented research, the spatial structuring of EcM communities likely reflects the non-random distribution of dominant vegetation species across the study area, with *Cistus* spp. dominated habitats are concentrated in the southern part of Donji Kamenjak, while *P. halepensis* and *Q. ilex* occurring further north. Furthermore, *Cistus*-dominated garrigues harboured distinctly different EcM communities compared to tree species of *P. halepensis* and *Q. ilex*, underscoring the importance of host identity in structuring EcM communities, consistent with studies from Mediterranean forests reporting host specificity or host preference in EcM associations (Buscardo et al., 2011; Pérez-Izquierdo et al., 2017; Rasmussen et al., 2017). In contrast, no significant differences in EcM community composition were detected between the two tree species, which may reflect the influence of relative abundance and co-occurrence of host trees in mixed and highly mosaic plots on the detectability of host-specific EcM patterns (Saitta et al., 2018) (**Table S1**).

Two factors not directly addressed in this study, which may help further resolve vegetation effects on EcM communities, are stand age and stand density (canopy cover). *Cistus* spp. are characteristic of early successional vegetation, and support EcM communities that shift across successional stages, with stand age emerging as a key driver of EcM community composition, particularly in *Cistus*-dominated areas (Martín-Pinto et al., 2022; Smith et al., 2002). In contrast, saprotrophic fungal communities, which dominated the study area, are more strongly influenced by stand density, as greater canopy cover reduces direct sunlight and maintains higher soil moisture, thereby favouring saprotroph development (Mediavilla et al., 2021).

Bacterial communities showed weaker spatial structuring and less pronounced vegetation-driven patterns, potentially pointing to a greater role of stochastic processes in shaping their composition (Caruso et al., 2011). Dini-Andreote et al. (2015) showed that microbial communities are initially shaped by stochastic processes, but as succession progresses, communities become increasingly shaped by deterministic processes. However, there is an ongoing debate about the relative contributions of stochastic and deterministic processes in shaping microbial communities (Zhou & Ning, 2017).

Taken together, these findings suggest that dominant vegetation is not a strong driver of soil microbial community composition at Donji Kamenjak, in contrast to studies that emphasise vegetation effects at local scales (Bastida et al., 2017, 2019; Iovieno et al., 2010). Rather, a stable shared core microbiome and highly mosaic habitats appeared to buffer vegetation-driven differences, promoting microbial community consistency across dominant vegetation species (Jiao et al., 2022). Additionally, the studied area of Donji Kamenjak is currently under passive management, the legacy effects of past land-use changes, including afforestation with *P. halepensis* and conversion of pastures, may continue to shape soil microbial communities in ways that persist beyond current vegetation patterns. These findings highlight the importance of considering both current vegetation and land-use history when evaluating microbial communities in ecosystem functioning.

While the research conducted at Donji Kamenjak aimed to analyse how local vegetation and space structure affect forest soil microbial communities on a local scale, the island system allows evaluation of drivers on the landscape scale, including habitat size, geographic distance, and anthropogenic pressures. Therefore, examining microbial communities across islands extends the analysis from local ecological drivers to the landscape scale.

6.2. Influence of intrinsic habitat characteristics and tourism gradient on soil microbial communities

Island biogeography predicts that larger islands support greater species diversity due to higher habitat heterogeneity and larger surface area, while more isolated islands harbour lower species diversity due to reduced immigration rates (MacArthur & Wilson, 1967). However, for this dissertation, the general postulates of island biogeography were used only as a conceptual framework to analyse whether island size and geographic distance can help explain observed patterns in microbial community composition between islands, not to explicitly test island biogeography theory. Results partially supported the second hypothesis and indicated that larger islands do not consistently support more diverse microbial communities and that distance does not always reduce richness. On the other hand, microbial community composition differed across islands and the mainland, but geographic distance metrics were not significant drivers of community composition.

Geographic distance, specifically DNI, had a positive rather than negative effect on fungal richness (**Figure 10A, Table S5**), indicating that more distant islands harboured greater fungal richness, a finding that contradicts the classical negative species-richness relationship (Davison et al., 2018). Given that beta diversity analyses showed no significant effects of geographic distance metrics (DTM, DNI) on fungal community composition, isolation per se may not be the primary mechanism driving richness patterns in this island system. Instead, the observed increase in fungal richness on more isolated islands likely reflects a combination of several factors. First, more distant islands in this study experienced lower direct anthropogenic pressure from tourism. Although tourism pressure was not tested as a driver of microbial species richness because the focus was on intrinsic habitat characteristics, it is worth noting that more isolated islands also experience lower anthropogenic pressure. This may have indirectly promoted fungal richness by maintaining more stable and less disturbed soil conditions (Duan & Zhu, 2019; Li et al., 2022). Second, differences in vegetation composition among islands (**Table S3)** could contribute to variation in fungal richness through host specificity and substrate availability (Saitta et al., 2018; Tedersoo et al., 2024). Third, more remote islands may exhibit greater microhabitat heterogeneity, providing a wider range of ecological niches for fungal taxa (Baldrian & Větrovský, 2012; Martiny et al., 2006). Another possibility could be in the dispersal biology of fungi, which is primarily passive, through different vectors such as wind, water, animals etc. (Golan & Pringle, 2017). Most fungal propagules settle close to their source (Schmidt et al., 2014), suggesting that immigration to more distant islands could depend on rare long-distance dispersal events (Golan & Pringle, 2017). Consequently, more distant islands may receive reduced propagule pressure (Golan & Pringle, 2017; Martiny et al., 2006), especially from highly abundant generalist fungi from the closest islands and might theoretically allow locally adapted specialists to persist (Mouquet & Loreau, 2003), reducing local richness. However, for a proper understanding of this mechanism as an explanation of increased fungal richness on more distant islands, further analyses are needed.

At the compositional level, spatial structuring was a dominant driver of both fungal and bacterial community composition (**Table 4, Table 5**), consistent with findings from sampling campaign 1 at Donji Kamenjak. Spatial structuring in PERMANOVA was accounted for using PCNM vectors to capture broad- and fine-scale spatial autocorrelation across all samples, thereby reflecting patterns of community similarity among nearby sites, regardless of the underlying causes. However, geographic distance metrics (DNI and DTM) were not significant drivers once spatial structuring and environmental variables were accounted for. Therefore, this suggests that space structures soil microbial communities, but compositional differences across island-mainland gradients are not due to isolation itself. Rather, the differences emerge from a combination of environmental filtering, vegetation and anthropogenic disturbance (Hanson et al., 2012; Odriozola et al., 2024). Notably, fungal community composition similarity between the mutually closest islands but farthest from the mainland could suggest that geographic proximity between islands promotes compositional similarity independently of their distance to the mainland (**Table S9**). On the other hand, bacterial communities showed weaker and less consistent island-mainland or island-island differentiation (**Table S10**), suggesting that bacteria are less sensitive to spatial structuring (Peguero et al., 2022; Zinger et al., 2019) across islands and mainland gradients than fungal communities (**Table 4, Table 5**).

The distinct spatial structuring between fungal and bacterial communities likely reflects differences in their ecology. Fungal communities may be more strongly structured by island proximity due to dispersal dynamics, where closer islands exchange propagules more effectively than with distant mainland (Norros et al., 2012) or due to stronger environmental filtering by similar vegetation (Martiny et al., 2006; Pole et al., 2026; Zinger et al., 2011) on proximate islands compared to vegetation on the mainland. In contrast, weaker spatial structuring of bacterial communities could suggest that they disperse more effectively across all distances (Zhang et al., 2021) and are not dependent on specific host plants compared to fungi (Martiny et al., 2006; Pole et al., 2026).

Tourism pressure was a significant driver of both fungal and bacterial community composition (**Table 4, Table 5**). As reported previously in section 4.6.2.2, tourism pressure was used as a proxy for anthropogenic disturbance, based on available visitor information. Nevertheless, its ecological and conservation relevance is significant given the substantial frequency of tourism in Brijuni NP, whose intensity varies across islands. Several studies have confirmed the negative effects of tourism pressure on soil microbial communities through vegetation trampling (Li et al., 2018; Newsome et al., 2002) which caused a significant decrease in microbial enzyme activities (Duan & Zhu, 2019), thereby pointing to the non-negligible effect of tourism disturbance on soil microbial communities (Li et al., 2022). These significant effects of tourism pressure on soil microbial communities underscore the need for conservation efforts to both prevent further degradation in less-disturbed areas and mitigate impacts in areas already experiencing pronounced anthropogenic pressure. This is further emphasised by our limited understanding of whether these tourism-driven disturbances are reversible and, if so, over what timescales.

The most pronounced pattern in community composition was a significant decrease in the relative abundance of EcM fungi with increasing distance to the mainland (DTM), from ~14% at Štinjan forest to almost total absence in the most isolated island, Gaz (**Figure 11C**). The loss of EcM host trees was observed in the field for sampling campaign 2, where Štinjan forest was dominated by *Q. ilex*, while at Gaz, there was almost total dominance of non-EcM *Phyillirea* *latifolia* (**Table S3**). This gradient most likely reflects the loss of EcM host trees with increasing distance, as EcM are obligate symbionts depending on host trees (Smith & Read, 2008). Parallel increase in relative abundance of plant pathogens on more isolated islands (**Figure 11C**) may reflect reduced plant host diversity and greater susceptibility of poor-diverse island plant communities to pathogenic fungi (Mitchell et al., 2002). This could also be a possible explanation for increased fungal richness on more distant islands, where the loss of EcM host trees could promote more favourable conditions for highly diverse saprotrophic taxa (Baldrian, 2017) as their competitive interaction would decrease (Bödeker et al., 2016).

Overall, island size did not influence soil microbial diversity, geographic distance had an unexpected effect on diversity, while spatial structuring showed a significant effect on soil microbial community composition. Baseline knowledge of the drivers of microbial communities on islands under different levels of tourism pressure is necessary to assess whether and to what extent these microbial communities can be utilised to predict soil health without additional analyses of soil parameters.

6.3. Microbial communities as predictors of soil health

While soil physical and chemical indicators formed a core of soil quality assessments (Cahyana et al., 2026), in a more holistic approach to soil health assessments, there is a growing recognition that the biological, live component of soil could be among the most sensitive and ecologically meaningful due to quick responses to disturbances and environmental changes (Lauber et al., 2013). However, which microbial properties and to what extent are important for predicting soil health remains incomplete. To address this and to gain more insight into the importance of microbial communities in soil health assessment, the central contribution of this dissertation was to evaluate microbial communities as predictors of soil health. Results revealed a clear ranking of predictive importance among tested microbial properties, with microbial community composition emerging as the strongest predictor, followed by enzyme activity, while bacterial biomass and fungal richness showed non-significant results for soil health and with little predictive importance (**Figure 13**).

To avoid mixing signals and oversimplifying microbial interactions and processing into a single number, a purely abiotic soil health index (SHI) was calculated exclusively from soil physical and chemical properties. Extensive analysis of soil properties contributed to a good representation of abiotic soil components, enabling the evaluation of microbes as biological components in the SHI prediction. This index is data-driven and applicable to Mediterranean ecosystems studied in this dissertation, as it was derived from soil properties measured specifically for this study. However, similar approaches have been reported in the literature (Andrés-Abellán et al., 2019; Bastida et al., 2006; Sánchez-Navarro et al., 2015) and could be applied to a variety of other ecosystems, where similar data are available. Abiotic SHI was used to avoid information loss by including many different indicators in SHI calculation and to avoid methodological confounding by adding microbial properties and using them again as predictors. Moreover, such SHI provides an objective and functionally interpretable baseline against which microbial communities could be evaluated as predictors. Although using composite SHI and awareness of microbial communities as important bioindicators is well established (Fierer et al., 2021), the methodological separation of soil properties as indicators from microbial predictors was a deliberate and relatively uncommon approach. This approach enabled independent evaluation of microbial community potential as a predictor of soil health, with the possibility of reducing the complexity of soil monitoring.

The SHI varied significantly across locations, with Gaz as the smallest, most isolated, and least visited island, showing significantly better soil health than the larger Štinjan forest on the mainland (**Figure 12**). However, the linear mixed-effects model revealed that while intrinsic habitat characteristics and tourism pressure collectively explained approximately 17% of the variance in the SHI, no single driver was statistically significant. This suggests that soil health in these Mediterranean ecosystems could be shaped by multiple and complex drivers. Also, the effects of individual drivers may be indirect, mediated by their influence on biological soil components, rather than acting directly on soil physical and chemical properties (Castro et al., 2010; Gatica-Saavedra et al., 2022). Moreover, it provides the ecological rationale for including and evaluating microbial communities as predictors.

The strongest predictors of SHI were enzyme activities and bacterial community composition. The enzyme group explained ~64% of variance in SHI, with β-xylosidase, acid phosphatase, and cellobiohydrolase emerging as the three strongest individual enzyme predictors (**Table S12**). These enzymes are functionally informative in the context of soil health: cellobiohydrolase and β-xylosidase are involved in carbon cycle in the degradation of cellulose (Baldrian & Valášková, 2008; Horn et al., 2012) linking them directly to organic matter decomposition and carbon availability, while acid phosphatase is responsible for the mineralisation of organic phosphorus, a key process determining phosphorus availability in soils (Caldwell, 2005). Cellobiohydrolase showed a negative association with SHI in the final linear mixed-effects model, indicating that higher activity of this enzyme is associated with lower SHI. Cellobiohydrolase activity is considered the slowest step in cellulose degradation processes because it acts on the crystalline, highly resistant part of cellulose (Ashraf et al., 2021; Li et al., 2026) therefore, it controls the overall speed of the whole process. High cellobiohydrolase activity is consistent with the accumulation of fresh, poorly decomposed cellulose-rich litter that is accumulating faster than it is being degraded, reflecting lower substrate quality with a high C/N ratio (Hicks et al., 2021). The better performance of these three enzymes over the full eight-enzyme model further confirms that a narrow set of functionally meaningful enzymes captured the essential variation in soil health more effectively than a broader enzymatic group, which contributed no additional explanatory power. These findings are consistent with findings that microbial extracellular activities provide strong information about biological changes because their dynamics quickly reflect minor modifications in the soil environment (Erdel et al., 2023; Li et al., 2019a), thereby representing an important functional bridge between microbial communities and soil health.

Bacterial community composition was the second strongest predictor in explaining variation in SHI (~50%), while fungal community composition explained 26% of variance in the SHI (**Table S12**). This suggests that bacterial community composition might reflect more directly soil health than fungi, probably due stronger dependence to soil physical and chemical properties than fungi (Lauber et al., 2009; Rousk et al., 2010) and their central roles in specific nutrient cycles such as nitrogen (Nelson et al., 2016), one of frequently limited nutrients in Mediterranean forests (Sardans & Peñuelas, 2013). On the other hand, fungal communities are critical for decomposition (Bomble et al., 2017; Rousk & Bååth, 2011; Rousk & Frey, 2015) and symbioses (Zinger et al., 2011) and their community composition may not so directly reflect soil health captured by the abiotic SHI. Furthermore, since tourism pressure was a significant driver of microbial community composition in this ecosystem (**Table 4, Table 5**), and community composition was a significant and important predictor of soil health (**Table S12, Figure 13**), these results collectively could suggest an indirect way through which anthropogenic disturbance may influence soil health through restructuring of microbial communities.

At the taxonomic level, bacterial phyla explained a substantial proportion of the SHI variation, with Verrucomicrobiota and Patescibacteria as the most important predictors (**Table 6**). Verrucomicrobiota are commonly found soil bacteria (Bergmann et al., 2011) known to participate in carbon (Naziębło et al., 2026) and nitrogen cycling (Bünger et al., 2020). The abundance of Verrucomicrobiota mostly depends on soil pH, SOM and nutrient availability (Naziębło et al., 2026). Generally, most members of Verrucomicrobiota are considered oligotrophs that thrive in nutrient-scarce environments (da Rocha et al., 2010; Lladó et al., 2017). Based on this, their negative association with SHI in the linear mixed-effects model may reflect their enrichment in low-quality organic matter environments. Also, their strong predictive importance for soil health may also reflect their high sensitivity to nitrogen availability, due to their important ecological role in nitrogen turnover (Naziębło et al., 2026). Patescibacteria, in contrast, showed a positive association with SHI in linear mixed-effects models. This phylum comprises ultrasmall-sized bacteria, and it is considered an advantage in low-energy environments due to the high surface-to-volume ratio (Sowell et al., 2009; Srinivas et al., 2024). Their positive association with SHI may therefore reflect their greater relative abundance in undisturbed areas. Potentially, where decomposition is efficient and SOM is stable, nutrient availability is lower, but consistent, and where there are no large resource inputs which could drive fast-growing opportunistic taxa. Rather, undisturbed environments could promote specialised, low-energy-adapted taxa such as Patescibacteria. However, the research about this phylum in soils is very scarce.

In contrast, fungal functional guilds did not emerge as important predictors for soil health in this Mediterranean ecosystem (**Table 7**). This is a surprising finding given that fungal guilds, especially ectomycorrhizal fungi and saprotrophs, are considered the main contributors to the carbon cycle and SOM dynamics (Algora Gallardo et al., 2021; Rousk & Bååth, 2011; Rousk & Frey, 2015; Žifčáková et al., 2017). This could be due to dominant vegetation, a significant driver of fungal community composition, suggesting that fungal guilds in this ecosystem may be more strongly coupled to vegetation than to soil properties captured by the abiotic composite SHI. Therefore, it is possible that the fungal contribution to soil health in this ecosystem is more indirect and captured through enzyme activities than at the functional guild level. This could be further supported with cellobiohydrolase, as one of the strongest predictors of the SHI, which is predominantly of fungal origin (Datta, 2024).

Taken together, these results support hypothesis 3 that soil microbial properties, particularly enzyme activities and bacterial community composition, are meaningful predictors of soil health in the Mediterranean ecosystems studied. Furthermore, the results suggest that tourism pressure may influence soil health indirectly, by reshaping the microbial community, rather than directly by altering soil physical and chemical properties. This is ecologically important and has direct implications for conservation management in Brijuni NP, as it suggests that even moderate tourist disturbance with no immediately detectable effects on soil properties may nonetheless compromise soil functioning by affecting microbial community composition.

7. Conclusions

This dissertation analysed drivers of soil microbial community diversity and composition in protected Mediterranean forest ecosystems and evaluated the potential of microbial community properties for predicting soil health, addressing three hypotheses across two distinct sampling campaigns, Donji Kamenjak and Brijuni NP, together with Štinjan forest. Findings from this dissertation could inform conservation management and soil monitoring, emphasising the ecological perspective of soil health assessments.

Hypothesis 1 - dominant vegetation was not a strong driver of soil microbial diversity or community composition in the studied Mediterranean ecosystems at Donji Kamenjak, except for EcM fungi. Spatial structuring consistently emerged as the primary driver of microbial community composition for both fungi and bacteria, while a stable and abundant core microbiome buffered vegetation-driven differences across dominant vegetation species. These findings indicate that vegetation effects on Mediterranean soil microbial communities are guild- and context-specific, and that spatial structuring, rather than dominant vegetation, dominates as a driver of community composition at local scales. Hypothesis 1 was therefore partially supported.

Hypothesis 2 - island size did not consistently predict microbial diversity, and geographic distance showed an unexpected positive rather than negative effect on fungal richness. At the compositional level, spatial structuring again emerged as the dominant driver, while geographic distance metrics were not significant once environmental variables were accounted for. Compositional differences across the island-mainland gradient were instead driven by a combination of environmental filtering, vegetation, and anthropogenic disturbance. The most pronounced compositional pattern was almost complete loss of EcM fungi with increasing distance from the mainland, reflecting the loss of obligate host trees across the distance gradient, followed by a parallel increase in plant pathogen relative abundance on more isolated islands. Hypothesis 2 was partially supported as scale- and taxon-dependent in the microbial context.

Hypothesis 3 - microbial community properties, specifically enzyme activities and bacterial community composition, were confirmed as the strongest and independent predictors of soil health, while microbial biomass and species richness contributed with little predictive importance. Among individual enzyme predictors, β-xylosidase, acid phosphatase and cellobiohydrolase were the strongest and most important predictors of soil health. At the taxonomic level, Verrucomicrobiota and Patescibacteria emerged as the strongest bacterial phylum predictors of SHI, while fungal functional guilds did not show direct association with soil health. Hypothesis 3 was supported.

Across both sampling campaigns, through mainland (mosaic forest at Donji Kamenjak and Štinjan forest) and insular forests at Brijuni NP, this thesis revealed several consistent ecological patterns. By examining microbial communities at both local (Donji Kamejnak) and landscape (Brijuni NP, Štinjan forest) scales, this research provided a comprehensive understanding of the drivers of soil microbial communities in the studied Mediterranean forests. Spatial structuring emerged as a dominant driver shaping microbial communities in both systems and at both scales, while classical drivers such as dominant vegetation or island size showed context-dependent or limited effects. Notably, tourism pressure emerged as a significant, often undervalued, driver of microbial community composition at the landscape scale. Most importantly, microbial properties, community composition and enzyme activities emerged as strong predictors of soil health, highlighting the potential of microbial communities as sensitive and ecologically meaningful indicators of soil health in Mediterranean forest ecosystems. Future research should focus on the functional roles of key taxa, including low-abundance groups, and testing the applicability of microbial indicators in broader spatial and temporal scales. Integration of microbial data into soil monitoring frameworks will be essential to assess their value as early warning indicators under increasing disturbance pressures. In addition, these insights should be translated into standardised and operational tools for soil health assessment. Such developments may also be relevant to the new EU Soil Monitoring Law (December 2025), particularly regarding the incorporation of microbial indicators into soil monitoring approaches.

8. References

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Supplementary

Table S7. Vegetation survey and GPS coordinates recorded at sampling plots at Donji Kamenjak.

Samples	Latitude_DD	Longitude_DD	Sampling month/year	*Dominant vegetation species	Other vegetation species
DK_004	44.79166667	13.90765	4/2021	*Quercus ilex*	*Quercus ilex, Pinus halepensis, Juniperus oxycedrus, Phillyrea latifolia, Ficus carica, Laurus nobilis,* *Myrtus communis*
DK_005	44.7957833	13.90733	4/2021	*Quercus ilex*	*Pinus halepensis, Quercus ilex, Juniperus oxycedrus, Laurus nobilis*
DK_006	44.79433333	13.90385	4/2021	*Quercus ilex*	*Quercus ilex, Myrtus communis, Phillyrea latifolia*
DK_007	44.79178333	13.90328333	4/2021	*Pinus halepensis*	*Pinus halepensis, Juniperus oxycedrus, Myrtus communis*
DK_008	44.78993333	13.90346667	4/2021	*Quercus ilex*	*Quercus ilex, Juniperus oxycedrus, Myrtus communis, Erica arborea*
DK_010	44.7728	13.90761667	4/2021	*Pinus halepensis*	*Pinus halepensis, Juniperus oxycedrus, Quercus ilex, Erica arborea, Cistus incanus, Spartium junceum, Cistus monspeliensis, Pistacia lentiscus*
DK_011	44.77643333	13.90755	4/2021	*Pinus halepensis*	*Erica arborea, Pinus halepensis, Cistus monspeliensis, Phillyrea latifolia, Juniperus oxycedrus*
DK_012	44.77591667	13.90886667	4/2021	*Cistus* spp.	*Cistus incanus, Cistus monspeliensis, Quercus pubescens, Phillyrea latifolia*
DK_013	44.78753333	13.91171667	10/2021	*Quercus ilex*	*Pinus halepensis, Quercus ilex, Juniperus oxycedrus, Laurus nobilis*
DK_014	44.78443333	13.91191667	10/2021	*Quercus ilex*	*Quercus ilex, Juniperus oxycedrus, Spartium junceum, Pinus halepensis*
DK_015	44.78181667	13.91275	10/2021	*Pinus halepensis*	*Pinus halepensis, Robinia pseudoacacia, Myrtus communis, Phragmites* sp., *Malus* sp., *Corylus avellane, Euonymus japonicus, Vitis vinifera*
DK_016	44.77938333	13.91258333	10/2021	*Quercus ilex*	*Quercus ilex, Pinus halepensis, Juniperus oxycedrus,* *Myrtus communis, Smilax aspera*
DK_017	44.77725	13.91253333	10/2021	*Quercus ilex*	*Pinus halepensis, Quercus ilex, Phillyrea latifolia, Juniperus oxycedrus, Myrtus communis, Pistacia lentiscus*
DK_018	44.7799	13.91701667	10/2021	*Pinus halepensis*	*Pinus halepensis, Spartium junceum, Quercus ilex, Quercus pubescens*
DK_019	44.78135	13.91665	10/2021	*Pinus halepensis*	*Pinus halepensis, Quercus ilex*
DK_020	44.78473333	13.91661667	10/2021	*Pinus halepensis*	*Pinus halepensis*
DK_021	44.78576667	13.91828333	10/2021	*Pinus halepensis*	*Pinus halepensis, Quercus ilex*
DK_022	44.77406667	13.91805	10/2021	*Cistus* spp.	*Juniperus oxycedrus, Quercus ilex, Salvia officinalis, Pistacia lentiscus, Pinus halepensis, Cistus incanus, Pinus pinea, Spartium junceum, Cistus monspeliensis*
DK_023	44.7763	13.91975	10/2021	*Pinus halepensis*	*Quercus ilex, Pinus halepensis, Erica arborea, Pistacia lentiscus*
DK_024	44.77441667	13.92101667	10/2021	*Quercus ilex*	*Quercus ilex, Quercus pubescens, Cistus incanus, Erica arborea, Pistacia lentiscus, Salvia officinalis*
DK_025	44.77325	13.915	10/2021	*Quercus ilex*	*Quercus ilex, Erica arborea, Pinus halepensis*
DK_026	44.77408333	13.91473333	10/2021	*Pinus halepensis*	*Pinus halepensis, Quercus ilex, Erica arborea, Myrtus communis, Paliurus spina-christi, Juniperus oxycedrus*
DK_027	44.76828333	13.92008333	10/2021	*Cistus* spp.	*Erica arborea, Juniperus oxycedrus, Cistus incanus, Cistus monspeliensis, Phillyrea latifolia, Pistacia lentiscus, Salvia officinalis*
DK_029	44.76981667	13.9224	10/2021	*Cistus* spp.	*Erica arborea, Juniperus oxycedrus, Salvia officinalis, Spartium junceum, Pinus halepensis*
DK_030	44.77016667	13.92045	10/2021	*Cistus* spp.	*Erica arborea, Juniperus oxycedrus*
DK_031	44.7748	13.91113333	10/2021	*Cistus* spp.	*Juniperus oxycedrus, Cistus monspeliensis, Cistus incanus, Quercus ilex, Salvia officinalis, Pistacia lentiscus*
DK_032	44.77596667	13.91075	10/2021	*Cistus* spp.	*Juniperus oxycedrus, Quercus ilex, Myrtus communis, Cistus monspeliensis, Pinus halepensis, Phillyrea latifolia, Spartium junceum, Cytisus scoparius*
DK_033	44.76926	13.91298	12/2021	*Cistus* spp.	*Phillyrea latifolia, Juniperus oxycedrus, Cistus monspeliensis, Pistacia lentiscus, Erica arborea, Cistus incanus*
DK_034	44.77029	13.91379	12/2021	*Cistus* spp.	*Cistus monspeliensis, Phillyrea latifolia, Juniperus oxycedrus, Myrtus communis, Spartium junceum*
DK_035	44.77042	13.91431	12/2021	*Cistus* spp.	*Juniperus oxycedrus, Spartium junceum, Cistus monspeliensis, Cistus incanus, Salvia officinalis, Pistacia lentiscus*
DK_036	44.77074	13.91339	12/2021	*Cistus* spp.	*Cistus monspeliensis, Cistus incanus, Salvia officinalis, Juniperus oxycedrus, Phillyrea latifolia, Helichrysum italicum, Pistacia lentiscus*
DK_037	44.77139	13.90909	12/2021	*Quercus ilex*	*Quercus ilex, Erica arborea, Juniperus oxycedrus*
DK_038	44.76881	13.91774	12/2021	*Cistus* spp.	*Erica arborea, Juniperus oxycedrus, Pinus halepensis, Cistus monspeliensis, Pistacia lentiscus, Phillyrea latifolia*
DK_039	44.7724	13.91129	12/2021	*Cistus* spp.	*Pistacia lentiscus, Cistus monspeliensis, Cistus incanus, Juniperus oxycedrus, Quercus ilex, Pinus halepensis, Erica arborea, Spartium junceum, Fraxinus ornus*
DK_040	44.77844	13.90694	12/2021	*Quercus ilex*	*Juniperus oxycedrus, Phillyrea latifolia, Pinus halepensis, Pistacia lentiscus, Quercus ilex*
DK_041	44.78006	13.90684	12/2021	*Cistus* spp.	*Juniperus oxycedrus, Pinus halepensis, Quercus ilex, Cistus incanus, Cistus monspeliensis, Phillyrea latifolia, Pinus pinea*

DK_042	44.78016	13.90807	12/2021	*Cistus* spp.	*Cistus incanus, Quercus ilex, Juniperus oxycedrus, Myrtus communis, Cistus monspeliensis, Salvia officinalis, Phillyrea latifolia*
DK_043	44.78306	13.90666	12/2021	*Quercus ilex*	*Quercus ilex, Phillyrea latifolia, Juniperus oxycedrus, Myrtus communis*
DK_044	44.78478	13.90556	12/2021	*Quercus ilex*	*Quercus ilex, Juniperus oxycedrus, Myrtus communis, Erica arborea, Quercus pubescens, Pinus halepensis, Salvia officinalis, Lonicera* sp., *Phillyrea latifolia*
DK_045	44.78573	13.90783	12/2021	*Quercus ilex*	*Juniperus oxycedrus, Quercus pubescens, Quercus ilex, Spartium junceum, Erica arborea, Ailanthus altissima, Phillyrea latifolia*
DK_046	44.78577	13.91047	12/2021	*Quercus ilex*	*Quercus ilex, Pinus halepensis, Phillyrea latifolia, Laurus nobilis*
DK_047	44.78388	13.90947	12/2021	*Cistus* spp.	*Helichrysum italicum, Cistus incanus, Pinus halepensis, Quercus ilex, Phillyrea latifolia*
DK_048	44.78751	13.91424	12/2021	*Pinus halepensis*	*Pinus halepensis, Quercus ilex*
DK_049	44.79051	13.907	12/2021	*Pinus halepensis*	*Pinus halepensis, Phillyrea latifolia, Quercus ilex, Juniperus oxycedrus, Laurus nobilis*
DK_050	44.78865	13.90425	12/2021	*Pinus halepensis*	*Pinus halepensis, Erica arborea, Quercus ilex, Myrtus communis*
DK_051	44.78664	13.90434	12/2021	*Pinus halepensis*	*Pinus halepensis, Phillyrea latifolia*
DK_052	44.79226	13.91034	12/2021	*Pinus halepensis*	*Pinus halepensis, Juniperus oxycedrus, Ligustrum japonicum, Pistacia terebinthus*

GPS coordinates recorded at centre of each sampling plot are recalculated to decimal degrees (Latitude_DD, Longitude_DD).
*Dominant vegetation - species whose canopy or ground cover accounted for at least 50% of the sampling subplots (20 x 20 m).
Other vegetation species - all plant species recorded at each sampling plot (100 x 100 m).

Table S8. Soil physical, chemical and biological properties in different dominant vegetation species at Donji Kamenjak.

SOIL PROPERTIES	**DOMINANT VEGETATION SPECIES**		
	Cistus spp.	*Pinus halepensis*	*Quercus ilex*
	n = 16	*n = 16*	*n = 15*
Soil moisture (%)	5.59 ± 0.48	4.18 ± 0.5	4.69 ± 0.54
pH	6.62 ± 0.09 $_{b}$	7.13 ± 0.08 $_{a}$	7.07 ± 0.07 $_{a}$
C/N ratio	12.19 ± 0.44	12.57 ± 0.43	13.74 ± 0.77
PLFA Total (µg/g)	11.16 ± 1.68	8.53 ± 0.96	8.94 ± 1.49
PLFA Fungi (µg/g)	0.51 ± 0.1	0.23 ± 0.05	0.39 ± 0.1
PLFA Bacteria (µg/g)	6.60 ± 1.07	5.35 ± 0.62	5.25 ± 0.85
F/B ratio	0.09 ± 0.02 $_{a}$	0.04 ± 0.004 $_{b}$	0.06 ± 0.01 $_{ab}$
PLFA Actinobacteria (µg/g)	0.99 ± 0.15	0.94 ± 0.09	0.94 ± 0.13
PLFA G+ (µg/g)	2.41 ± 0.37	1.82 ± 0.17	1.74 ± 0.26
PLFA G- (µg/g)	3.01 ± 0.53	2.44 ± 0.36	2.43 ± 0.45
Acid phosphatase (nM/min/g)	24516.56 ± 3693.63	21189.93 ± 4565.48	14678.43 ± 2104.29
α-glucosidase(nM/min/g)	818.91 ± 138.27	509.16 ± 95.35	752.23 ± 346.67
β-galactosidase (nM/min/g)	1211.18 ± 320.85	1001.7 ± 241.43	586.72 ± 123.79
β-glucosidase (nM/min/g)	11527.57 ± 1532.04	10273.3 ± 1535.96	7237.8 ± 998.36
β-xylosidase (nM/min/g)	1163.61 ±141.7	1023.23 ± 172.56	736.22 ± 103.34
Cellobiohydrolase (nM/min/g)	2301.45 ± 294.96	2118.02 ± 338.67	1331.87 ± 267.44
Chitinase (nM/min/g)	2099.11 ± 237.43	1758.66 ± 275.26	1509.98 ± 189.71
Lipase (nM/min/g)	63370.38 ± 14681.54	53799.63 ± 7982.74	46965.79 ± 5404.11

Values are the means ± SE and different lowercase letters indicate significant differences among dominant vegetation ($p < 0.05$).
C/N ratio = organic carbon/total nitrogen, **F/B ratio** = PLFA Fungi/PLFA Bacteria.

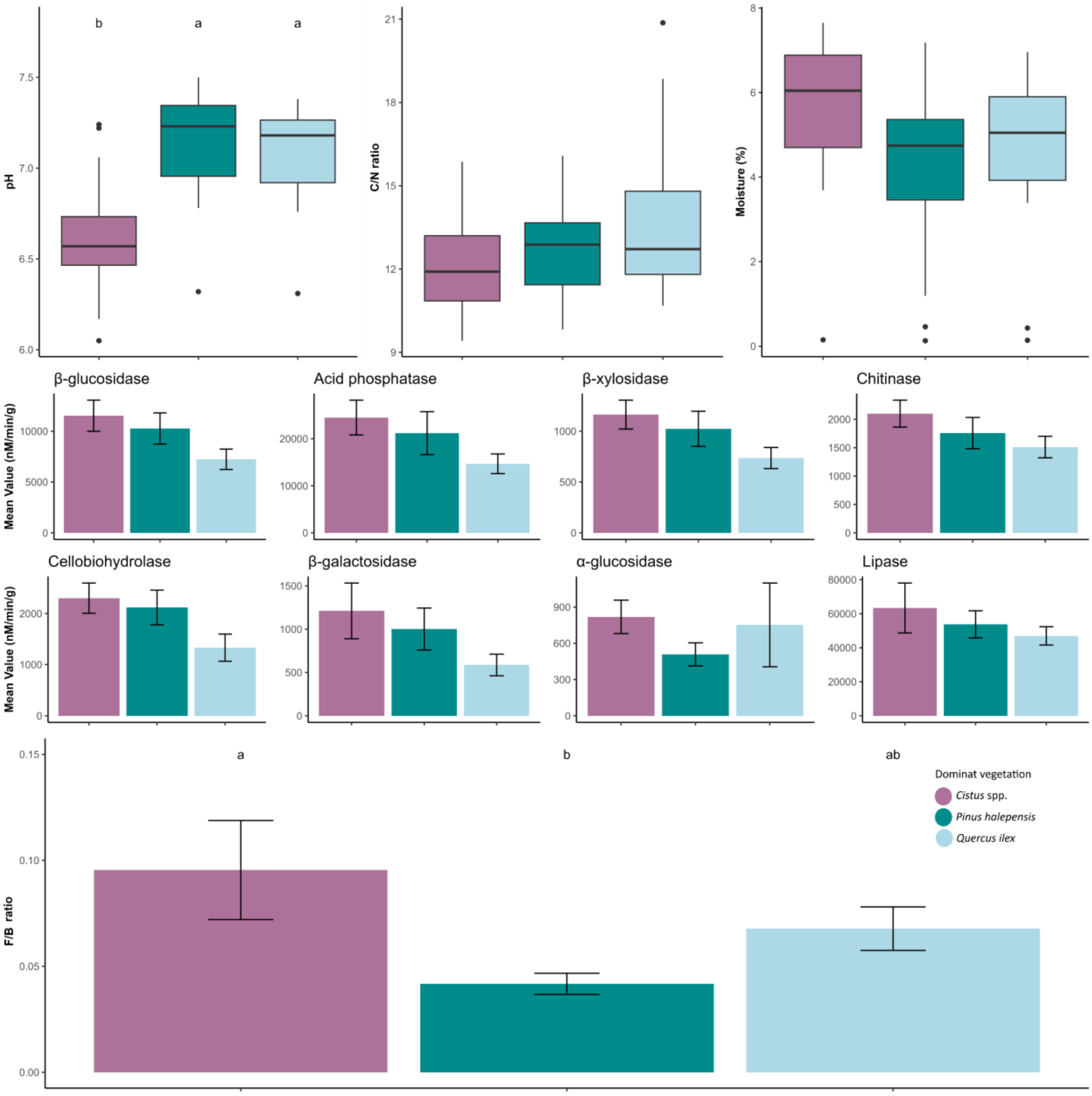


Figure S1. Analysis of soil biochemical properties at Donji Kamenjak. Values on the y-axis represent the mean, bars at bar plots indicate standard errors and different letters indicate significant differences among dominant vegetation species ($p < 0.05$). **C/N ratio** = organic carbon/total nitrogen, **F/B ratio** = PLFA Fungi/PLFA Bacteria

Table S9. Vegetation survey, GPS coordinates and geographic distance metrics recorded at sampling plots at Brijuni NP and Štinjan forest.

Sample ID	Sample location	Sampling date	Central LONG_DD	Central LAT_DD	Sampling LONG_DD	Sampling LAT_DD	Canopy cover_(%)	Dominant trees	DNI_m	DTM_m
VBS01A	Veli Brijun	12.4.2024.	44.90364	13.75675	44.90369	13.75671	30-40	*Quercus ilex, Phillyrea latifolia, Arbutus undeo*	3137.54	6288.486
VBS01B	Veli Brijun	12.4.2024.	44.90364	13.75675	44.90366	13.75683	30-40	*Quercus ilex, Phillyrea latifolia, Arbutus undeo*	3137.54	6288.486
VBS01C	Veli Brijun	12.4.2024.	44.90364	13.75675	44.90358	13.75681	30-40	*Quercus ilex, Phillyrea latifolia, Arbutus undeo*	3137.54	6288.486
VBS01D	Veli Brijun	12.4.2024.	44.90364	13.75675	44.90361	13.7567	30-40	*Quercus ilex, Phillyrea latifolia, Arbutus undeo*	3137.54	6288.486
VBS02A	Veli Brijun	12.4.2024.	44.90736	13.76979	44.90733	13.76967	30	*Quercus ilex*	3137.54	6288.486
VBS02B	Veli Brijun	12.4.2024.	44.90736	13.76979	44.90731	13.76985	30	*Quercus ilex*	3137.54	6288.486
VBS02C	Veli Brijun	12.4.2024.	44.90736	13.76979	44.90726	13.76978	30	*Quercus ilex*	3137.54	6288.486
VBS02D	Veli Brijun	12.4.2024.	44.90736	13.76979	44.90726	13.76964	30	*Quercus ilex*	3137.54	6288.486
VBS03A	Veli Brijun	12.4.2024.	44.90942	13.77931	44.90937	13.7794	10	*Quercus ilex*	3137.54	6288.486
VBS03B	Veli Brijun	12.4.2024.	44.90942	13.77931	44.90939	13.77926	10	*Quercus ilex*	3137.54	6288.486
VBS03C	Veli Brijun	12.4.2024.	44.90942	13.77931	44.90945	13.77929	10	*Quercus ilex*	3137.54	6288.486
VBS03D	Veli Brijun	12.4.2024.	44.90942	13.77931	44.90944	13.77941	10	*Quercus ilex*	3137.54	6288.486
VBS04A	Veli Brijun	12.4.2024.	44.91537	13.76152	44.91546	13.76143	30	*Quercus ilex, Phillyrea latifolia*	3137.54	6288.486
VBS04B	Veli Brijun	12.4.2024.	44.91537	13.76152	44.91543	13.76155	30	*Quercus ilex, Phillyrea latifolia*	3137.54	6288.486
VBS04C	Veli Brijun	12.4.2024.	44.91537	13.76152	44.9154	13.76154	30	*Quercus ilex, Phillyrea latifolia*	3137.54	6288.486
VBS04D	Veli Brijun	12.4.2024.	44.91537	13.76152	44.91534	13.76139	30	*Quercus ilex, Phillyrea latifolia*	3137.54	6288.486
VBS05A	Veli Brijun	12.4.2024.	44.91424	13.7773	44.91428	13.77722	40	*Quercus ilex*	3137.54	6288.486
VBS05B	Veli Brijun	12.4.2024.	44.91424	13.7773	44.91422	13.77737	40	*Quercus ilex*	3137.54	6288.486
VBS05C	Veli Brijun	12.4.2024.	44.91424	13.7773	44.91412	13.77735	40	*Quercus ilex*	3137.54	6288.486
VBS05D	Veli Brijun	12.4.2024.	44.91424	13.7773	44.91412	13.77723	40	*Quercus ilex*	3137.54	6288.486
VBS06A	Veli Brijun	12.4.2024.	44.91178	13.75818	44.91187	13.75811	40	*Quercus ilex, Phillyrea latifolia*	3137.54	6288.486
VBS06B	Veli Brijun	12.4.2024.	44.91178	13.75818	44.91183	13.7582	40	*Quercus ilex, Phillyrea latifolia*	3137.54	6288.486
VBS06C	Veli Brijun	12.4.2024.	44.91178	13.75818	44.9118	13.75822	40	*Quercus ilex, Phillyrea latifolia*	3137.54	6288.486
VBS06D	Veli Brijun	12.4.2024.	44.91178	13.75818	44.91178	13.7581	40	*Quercus ilex, Phillyrea latifolia*	3137.54	6288.486
VBS07A	Veli Brijun	12.4.2024.	44.92644	13.74685	44.92649	13.74681	60-80	*Quercus ilex, Phillyrea latifolia*	3137.54	6288.486

VBS07B	Veli Brijun	12.4.2024.	44.92644	13.74685	44.92651	13.74686	60-80	*Quercus ilex, Phillyrea latifolia*	3137.54	6288.486
VBS07C	Veli Brijun	12.4.2024.	44.92644	13.74685	44.92639	13.74685	60-80	*Quercus ilex, Phillyrea latifolia*	3137.54	6288.486
VBS07D	Veli Brijun	12.4.2024.	44.92644	13.74685	44.92639	13.74674	60-80	*Quercus ilex, Phillyrea latifolia*	3137.54	6288.486
VBS08A	Veli Brijun	12.4.2024.	*NA*	*NA*	44.92288	13.74674	50	*Quercus ilex, Phillyrea latifolia*	3137.54	6288.486
VBS08B	Veli Brijun	12.4.2024.	*NA*	*NA*	44.92286	13.74684	50	*Quercus ilex, Phillyrea latifolia*	3137.54	6288.486
VBS08C	Veli Brijun	12.4.2024.	*NA*	*NA*	44.92277	13.74684	50	*Quercus ilex, Phillyrea latifolia*	3137.54	6288.486
VBS08D	Veli Brijun	12.4.2024.	*NA*	*NA*	44.92274	13.74673	50	*Quercus ilex, Phillyrea latifolia*	3137.54	6288.486
MBS01A	Mali Brijun	15.4.2024.	44.93075	13.74542	44.93065	13.74544	30	*Quercus ilex, Phillyrea latifolia*	2333.743	9477.251
MBS01B	Mali Brijun	15.4.2024.	44.93075	13.74542	44.93072	13.74544	30	*Quercus ilex, Phillyrea latifolia*	2333.743	9477.251
MBS01C	Mali Brijun	15.4.2024.	44.93075	13.74542	44.93081	13.74535	30	*Quercus ilex, Phillyrea latifolia*	2333.743	9477.251
MBS01D	Mali Brijun	15.4.2024.	44.93075	13.74542	44.93073	13.74531	30	*Quercus ilex, Phillyrea latifolia*	2333.743	9477.251
MBS02A	Mali Brijun	15.4.2024.	44.93336	13.74527	44.9334	13.7452	50	*Phillyrea latifolia*	2333.743	9477.251
MBS02B	Mali Brijun	15.4.2024.	44.93336	13.74527	44.93339	13.74532	50	*Phillyrea latifolia*	2333.743	9477.251
MBS02C	Mali Brijun	15.4.2024.	44.93336	13.74527	44.93335	13.74534	50	*Phillyrea latifolia*	2333.743	9477.251
MBS02D	Mali Brijun	15.4.2024.	44.93336	13.74527	44.93327	13.74524	50	*Phillyrea latifolia*	2333.743	9477.251
MBS03A	Mali Brijun	15.4.2024.	44.93823	13.73763	44.93831	13.73758	50	*Quercus ilex, Phillyrea latifolia*	2333.743	9477.251
MBS03B	Mali Brijun	15.4.2024.	44.93823	13.73763	44.9383	13.73766	50	*Quercus ilex, Phillyrea latifolia*	2333.743	9477.251
MBS03C	Mali Brijun	15.4.2024.	44.93823	13.73763	44.93823	13.7377	50	*Quercus ilex, Phillyrea latifolia*	2333.743	9477.251
MBS03D	Mali Brijun	15.4.2024.	44.93823	13.73763	44.93823	13.7376	50	*Quercus ilex, Phillyrea latifolia*	2333.743	9477.251
MBS04A	Mali Brijun	15.4.2024.	44.93551	13.73762	44.93556	13.73758	30	*Phillyrea latifolia*	2333.743	9477.251
MBS04B	Mali Brijun	15.4.2024.	44.93551	13.73762	44.93558	13.73769	30	*Phillyrea latifolia*	2333.743	9477.251
MBS04C	Mali Brijun	15.4.2024.	44.93551	13.73762	44.93548	13.73766	30	*Phillyrea latifolia*	2333.743	9477.251
MBS04D	Mali Brijun	15.4.2024.	44.93551	13.73762	44.93547	13.73753	30	*Phillyrea latifolia*	2333.743	9477.251
MBS05A	Mali Brijun	15.4.2024.	44.93653	13.74584	44.93661	13.7458	60	*Quercus ilex, Phillyrea latifolia*	2333.743	9477.251
MBS05B	Mali Brijun	15.4.2024.	44.93653	13.74584	44.9366	13.74592	60	*Quercus ilex, Phillyrea latifolia*	2333.743	9477.251
MBS05C	Mali Brijun	15.4.2024.	44.93653	13.74584	44.93649	13.7459	60	*Quercus ilex, Phillyrea latifolia*	2333.743	9477.251
MBS05D	Mali Brijun	15.4.2024.	44.93653	13.74584	44.93653	13.74576	60	*Quercus ilex, Phillyrea latifolia*	2333.743	9477.251
MBS06A	Mali Brijun	15.4.2024.	44.93914	13.73607	44.93921	13.73605	30	*Phillyrea latifolia*	2333.743	9477.251
MBS06B	Mali Brijun	15.4.2024.	44.93914	13.73607	44.93921	13.73617	30	*Phillyrea latifolia*	2333.743	9477.251
MBS06C	Mali Brijun	15.4.2024.	44.93914	13.73607	44.93911	13.7361	30	*Phillyrea latifolia*	2333.743	9477.251

MBS06D	Mali Brijun	15.4.2024.	44.93914	13.73607	44.93913	13.73599	30	*Phillyrea latifolia*	2333.743	9477.251
VAS01A	Vanga	16.4.2024.	44.91266	13.7288	44.91272	13.72879	50	*Phillyrea latifolia*	760.6376	9569.609
VAS01B	Vanga	16.4.2024.	44.91266	13.7288	44.91272	13.72891	50	*Phillyrea latifolia*	760.6376	9569.609
VAS01C	Vanga	16.4.2024.	44.91266	13.7288	44.91264	13.7289	50	*Phillyrea latifolia*	760.6376	9569.609
VAS01D	Vanga	16.4.2024.	44.91266	13.7288	44.91268	13.7288	50	*Phillyrea latifolia*	760.6376	9569.609
VAS02A	Vanga	16.4.2024.	44.91255	13.72752	44.91259	13.72745	30-60	*Phillyrea latifolia*	760.6376	9569.609
VAS02B	Vanga	16.4.2024.	44.91255	13.72752	44.9126	13.72757	30-60	*Phillyrea latifolia*	760.6376	9569.609
VAS02C	Vanga	16.4.2024.	44.91255	13.72752	44.91252	13.72756	30-60	*Phillyrea latifolia*	760.6376	9569.609
VAS02D	Vanga	16.4.2024.	44.91255	13.72752	44.91251	13.72741	30-60	*Phillyrea latifolia*	760.6376	9569.609
VAS03A	Vanga	16.4.2024.	44.90773	13.73054	44.90778	13.73045	40-50	*Quercus ilex, Phillyrea latifolia*	760.6376	9569.609
VAS03B	Vanga	16.4.2024.	44.90773	13.73054	44.9078	13.73063	40-50	*Quercus ilex, Phillyrea latifolia*	760.6376	9569.609
VAS03C	Vanga	16.4.2024.	44.90773	13.73054	44.90772	13.73057	40-50	*Quercus ilex, Phillyrea latifolia*	760.6376	9569.609
VAS03D	Vanga	16.4.2024.	44.90773	13.73054	44.90769	13.73046	40-50	*Quercus ilex, Phillyrea latifolia*	760.6376	9569.609
KOS01A	Kozada	13.4.2024.	44.90032	13.79821	44.90034	13.79813	40	*Phillyrea latifolia*	1355.084	2171.066
KOS01B	Kozada	13.4.2024.	44.90032	13.79821	44.90035	13.79823	40	*Phillyrea latifolia*	1355.084	2171.066
KOS01C	Kozada	13.4.2024.	44.90032	13.79821	44.90034	13.7983	40	*Phillyrea latifolia*	1355.084	2171.066
KOS01D	Kozada	13.4.2024.	44.90032	13.79821	44.90025	13.79826	40	*Phillyrea latifolia*	1355.084	2171.066
KOS02A	Kozada	13.4.2024.	44.9014	13.7992	44.90146	13.79918	70	*Phillyrea latifolia*	1355.084	2171.066
KOS02B	Kozada	13.4.2024.	44.9014	13.7992	44.90144	13.79928	70	*Phillyrea latifolia*	1355.084	2171.066
KOS02C	Kozada	13.4.2024.	44.9014	13.7992	44.90134	13.79927	70	*Phillyrea latifolia*	1355.084	2171.066
KOS02D	Kozada	13.4.2024.	44.9014	13.7992	44.90136	13.79917	70	*Phillyrea latifolia*	1355.084	2171.066
JRS01A	Sv. Jerolim	13.4.2024.	44.89835	13.78427	44.8984	13.78424	30	*Phillyrea latifolia*	1355.084	3153.095
JRS01B	Sv. Jerolim	13.4.2024.	44.89835	13.78427	44.89843	13.78436	30	*Phillyrea latifolia*	1355.084	3153.095
JRS01C	Sv. Jerolim	13.4.2024.	44.89835	13.78427	44.89835	13.78431	30	*Phillyrea latifolia*	1355.084	3153.095
JRS01D	Sv. Jerolim	13.4.2024.	44.89835	13.78427	44.89832	13.78422	30	*Phillyrea latifolia*	1355.084	3153.095
JRS02A	Sv. Jerolim	13.4.2024.	44.90026	13.78886	44.9003	13.78881	20	*Phillyrea latifolia*	1355.084	3153.095
JRS02B	Sv. Jerolim	13.4.2024.	44.90026	13.78886	44.90029	13.78893	20	*Phillyrea latifolia*	1355.084	3153.095
JRS02C	Sv. Jerolim	13.4.2024.	44.90026	13.78886	44.90022	13.78889	20	*Phillyrea latifolia*	1355.084	3153.095
JRS02D	Sv. Jerolim	13.4.2024.	44.90026	13.78886	44.90021	13.78881	20	*Phillyrea latifolia*	1355.084	3153.095
GLS01A	Galija	16.4.2024.	44.91803	13.72906	44.91807	13.72899	60	*Phillyrea latifolia*	760.6376	9801.115

GLS01B	Galija	16.4.2024.	44.91803	13.72906	44.91807	13.72911	60	*Phillyrea latifolia*	760.6376	9801.115
GLS01C	Galija	16.4.2024.	44.91803	13.72906	44.91799	13.72911	60	*Phillyrea latifolia*	760.6376	9801.115
GLS01D	Galija	16.4.2024.	44.91803	13.72906	44.91799	13.72902	60	*Phillyrea latifolia*	760.6376	9801.115
GAS01A	Gaz	14.4.2024.	44.93849	13.71909	44.93855	13.71903	10	*Phillyrea latifolia*	2473.796	11736.81
GAS01B	Gaz	14.4.2024.	44.93849	13.71909	44.93853	13.71916	10	*Phillyrea latifolia*	2473.796	11736.81
GAS01C	Gaz	14.4.2024.	44.93849	13.71909	44.93846	13.71913	10	*Phillyrea latifolia*	2473.796	11736.81
GAS01D	Gaz	14.4.2024.	44.93849	13.71909	44.93846	13.719	10	*Phillyrea latifolia*	2473.796	11736.81
MNL01	Štinjan Forest	13.5.2024.	44.89382	13.80811	44.89382	13.80811	50	*Quercus ilex, Phillyrea latifolia*	2171.066	0
MNL02	Štinjan Forest	13.5.2024.	44.89207	13.80939	44.89207	13.80939	60	*Quercus ilex, Phillyrea latifolia*	2171.066	0
MNL03	Štinjan Forest	13.5.2024.	44.8921	13.81067	44.8921	13.81067	60	*Quercus ilex*	2171.066	0
MNL04	Štinjan Forest	13.5.2024.	44.89119	13.80696	44.89119	13.80696	70	*Quercus ilex*	2171.066	0
MNL05	Štinjan Forest	13.5.2024.	44.88845	13.80838	44.88845	13.80838	60	*Quercus ilex*	2171.066	0
MNL06	Štinjan Forest	13.5.2024.	44.88755	13.80833	44.88755	13.80833	30	*Quercus ilex*	2171.066	0
MNL07	Štinjan Forest	13.5.2024.	44.88847	13.81317	44.88847	13.81317	30	*Quercus ilex*	2171.066	0
MNL08	Štinjan Forest	13.5.2024.	44.88857	13.81466	44.88857	13.81466	60	*Quercus ilex, Phillyrea latifolia*	2171.066	0
MNL09	Štinjan Forest	13.5.2024.	44.88669	13.81375	44.88669	13.81375	15	*Quercus ilex*	2171.066	0
MNL10	Štinjan Forest	13.5.2024.	44.88564	13.80735	44.88564	13.80735	60	*Quercus ilex*	2171.066	0
MNL11	Štinjan Forest	13.5.2024.	44.88479	13.80593	44.88479	13.80593	35	*Quercus ilex, Phillyrea latifolia*	2171.066	0
MNL12	Štinjan Forest	13.5.2024.	44.88488	13.80973	44.88488	13.80973	20	*Quercus ilex*	2171.066	0
MNL13	Štinjan Forest	13.5.2024.	44.8851	13.81746	44.8851	13.81746	35	*Quercus ilex, Phillyrea latifolia*	2171.066	0
MNL14	Štinjan Forest	13.5.2024.	44.88425	13.81369	44.88425	13.81369	5-10	*Quercus ilex*	2171.066	0
MNL15	Štinjan Forest	13.5.2024.	44.88322	13.81358	44.88322	13.81358	40	*Quercus ilex, Phillyrea latifolia*	2171.066	0
MNL16	Štinjan Forest	13.5.2024.	44.88239	13.8187	44.88239	13.8187	45	*Quercus ilex, Phillyrea latifolia*	2171.066	0
MNL17	Štinjan Forest	13.5.2024.	44.88133	13.81497	44.88133	13.81497	50	*Quercus ilex*	2171.066	0
MNL18	Štinjan Forest	13.5.2024.	44.88047	13.81253	44.88047	13.81253	50	*Quercus ilex*	2171.066	0
MNL19	Štinjan Forest	13.5.2024.	44.87958	13.81377	44.87958	13.81377	10	*Quercus ilex, Phillyrea latifolia*	2171.066	0

MNL20	Štinjan Forest	13.5.2024.	44.87967	13.81757	44.87967	13.81757	50-55	*Quercus ilex, Phillyrea latifolia*	2171.066	0

GPS coordinates recorded at centre of each sampling plot are recalculated to decimal degrees (LAT_DD, LONG_DD).

Central LONG_DD and Central LAT_DD = longitude and latitude at central plots; **Sampling LONG_DD and Sampling LAT_DD** = longitude and latitude sampling plots within central plot; **Dominant trees** = trees whose canopy or ground cover accounted for at least 50% of the sampling plots; **DNI_m** = distance to the nearest island in meters; **DTM_m** = distance to the mainland (Štinjan forest) in meters.

Table S10. Soil physical, chemical and biological properties in Brijuni NP and Štinjan forest.

	Veli Brijun	**Mali Brijun**	**Vanga**	**Kozada**	**Sv. Jerolim**	**Galija**	**Gaz**	**Štinjan forest**
SOIL PROPERTIES								
pH	6.315±0.054 **cd**	6.608±0.06 **b**	6.491±0.079 **bcd**	7.237±0.069 **a**	6.834±0.167 **ab**	5.908±0.095 **d**	6.768±0.116 **abc**	6.695±0.094 **b**
Soil moisture (%)	50.238±1.562 **a**	37.777±1.47 **b**	30.792±1.395 **b**	34.663±1.448 **b**	43.705±2.722 **ab**	26.166±2.747 **b**	37.503±1.672 **ab**	41.708±4.315 **ab**
TOC (%)	8.31±0.503 **ab**	7.415±0.5 **b**	6.933±0.438 **b**	8.092±0.408 **ab**	12.126±0.847 **a**	5.229±0.382 **b**	9.588±0.26 **ab**	10.404±1.543 **ab**
N (%)	0.672±0.035 **abc**	0.617±0.041 **bc**	0.54±0.024 **c**	0.706±0.027 **abc**	0.874±0.043 **a**	0.436±0.029 **c**	0.869±0.032 **ab**	0.635±0.054 **bc**
C/N ratio	12.267±0.188 **b**	12.027±0.099 **b**	12.732±0.333 **b**	11.444±0.325 **b**	13.815±0.521 **ab**	11.973±0.233 **b**	11.043±0.15 **b**	15.425±0.726 **a**
P total (g/kg)	0.1±0.005 **c**	0.082±0.004 **d**	0.057±0.001 **e**	0.143±0.004 **a**	0.109±0.013 **bc**	0.049±0.002 **de**	0.145±0.008 **ab**	0.075±0.004 **de**
K total (g/kg)	0.813±0.026 **cd**	1.094±0.025 **b**	1.025±0.019 **b**	0.969±0.013 **bc**	1.546±0.132 **a**	0.989±0.028 **bc**	1.154±0.056 **b**	0.704±0.034 **d**
Ca total (g/kg)	1.143±0.058 **b**	1.088±0.093 **b**	0.878±0.03 **b**	1.435±0.058 **b**	2.543±0.617 **a**	0.752±0.019 **b**	1.17±0.05 **b**	1.364±0.131 **b**
Mg total (g/kg)	0.406±0.011 **d**	0.417±0.012 **d**	0.497±0.01 **bc**	0.473±0.009 **bcd**	0.6±0.027 **a**	0.44±0.01 **cd**	0.58±0.018 **ab**	0.48±0.017 **bc**
TOC/clay ratio	0.402±0.042	0.279±0.018	0.865±0.326	0.37±0.024	0.391±0.071	0.296±0.024	0.385±0.02	0.537±0.206
P_2O_5 (mg/100 g)	5.285±0.954 **bc**	3.123±0.419 **c**	2.946±0.26 **c**	3.296±0.474 **bc**	13.679±5.683 **a**	4.475±0.367 **abc**	12.928±5.384 **ab**	1.799±0.28 **c**
K_2O (mg/100 g)	44.688±2.874 **b**	56.629±4.012 **ab**	47.692±3.361 **b**	45.737±3.267 **bc**	73.875±9.978 **a**	42.375±3.275 **bc**	57.125±8.043 **ab**	28.185±1.592 **c**
Humus (%)	14.294±0.864 **ab**	12.754±0.859 **b**	11.925±0.753 **b**	13.918±0.701 **ab**	20.857±1.457 **a**	8.994±0.658 **b**	16.492±0.446 **ab**	17.896±2.654 **ab**
TC	10.16±0.534	8.995±0.489	7.942±0.311	9.448±0.45	13.024±1.13	6.84±0.306	10.51±0.334	12.3±1.777
Fe (g/kg)	2.504±0.072 **c**	2.887±0.065 **ab**	2.785±0.039 **abc**	2.988±0.047 **ab**	2.422±0.151 **bc**	2.896±0.072 **abc**	3.146±0.057 **a**	2.569±0.115 **abc**
Zn (mg/kg)	6.572±0.287	8.438±1.588	5.129±0.117	8.779±1.023	8.941±1.655	4.314±0.085	6.391±0.222	7.729±0.41
Mn (mg/kg)	62.124±1.859 **b**	64.349±1.929 **ab**	69.091±3.658 **ab**	67.304±1.542 **ab**	39.48±2.79 **c**	80.453±4.102 **a**	54.455±0.744 **bc**	64.39±3.053 **ab**
Na (mg/kg)	54.796±1.216 **c**	65.811±1.387 **ab**	74.588±2.465 **a**	59.775±3.519 **bc**	76.82±9.326 **a**	74.558±2.137 **ab**	65.089±3.192 **abc**	55.439±2.093 **c**
Al (g/kg)	4.705±0.133 **b**	5.312±0.111 **a**	4.625±0.051 **ab**	4.761±0.072 **ab**	5.539±0.281 **a**	4.522±0.1 **ab**	5.048±0.096 **ab**	4.671±0.212 **b**
Pb (g/kg)	10.719±0.322 **bc**	11.936±0.393 **b**	8.777±0.282 **c**	11.034±0.38 **bc**	11.512±0.647 **bc**	5.065±0.236 **d**	8.839±0.332 **bcd**	14.65±0.734 **a**
N/P ratio	6.737±0.202 **cd**	7.467±0.219 **bc**	9.479±0.422 **a**	4.968±0.225 **d**	8.41±0.625 **abc**	8.904±0.653 **abc**	6±0.138 **bcd**	8.492±0.546 **ab**
Sand (%)	12.3±0.987 **b**	11.075±0.751 **b**	11.543±0.732 **ab**	11.565±0.754 **ab**	15.956±3.078 **ab**	7.8±0.562 **b**	10.246±0.59 **ab**	17.311±1.583 **a**
Silt (%)	64.978±0.689 **b**	62.246±1.145 **b**	71.407±2.434 **a**	66.16±1.293 **ab**	48.344±1.402 **d**	74.424±0.973 **a**	64.704±0.724 **ab**	55.584±1.295 **c**
Clay (%)	22.722±0.903 **bc**	26.679±0.834 **b**	17.05±2.645 **c**	22.275±1.428 **bc**	35.7±3.749 **a**	17.775±0.925 **bc**	25.05±1.053 **abc**	27.105±1.422 **b**
Acid phosphatase (nM/min/g)	43323.368±2994.835 **b**	37478.736±2503.003 **b**	40826.78±3079.613 **b**	26862.276±1412.424 **b**	43461.111±6546.953 **ab**	37506.192±4255.55 **ab**	35053.534±4032.135 **ab**	70395.653±10179.528 **a**
β-xylosidase (nM/min/g)	3271.811±293.99	3462.5±405.572	2691.754±317.889	1765.193±129.118	3141.951±586.82	4842.263±1109.624	3003.517±599.46	4085.964±1090.312

β-glucosidase (nM/min/g)	24689.637±2322.874	28968.559±2012.419	24173.883±2247.896	19489.715±1457.738	32323.508±6266.219	22608.753±2408.158	28568.223±3731.356	33979.759±4341.273
Cellobiohydrolase (nM/min/g)	5752.564±657.329 **ab**	6283.245±682.707 **ab**	5360.561±793.695 **ab**	3816.608±378.722 **b**	7422.942±1989.789 **ab**	5399.306±915.522 **ab**	8964.64±2322.349 **ab**	9196.201±1302.225 **a**
β-galactosidase (nM/min/g)	1779.129±174.608 **b**	2002.237±283.777 **ab**	1503.29±121.353 **b**	2209.552±230.877 **ab**	2888.071±484.884 **ab**	1758.556±506.94 **ab**	1839.463±351.25 **ab**	3395.912±644.278 **a**
α-glucosidase (nM/min/g)	1179.918±105.014 **b**	1255.308±81.261 **ab**	1580.024±175.416 **ab**	965.273±62.834 **ab**	1514.263±303.619 **ab**	1191.548±242.005 **ab**	1220.034±327.039 **ab**	2567.92±691.265 **a**
Chitinase (nM/min/g)	3108.808±287.121 **b**	2844.139±247.245 **b**	3298.291±312.648 **b**	2132.514±200.326 **b**	5025.652±942.62 **ab**	2717.981±581.05 **ab**	4025.694±846.946 **ab**	5929.517±759.533 **a**
Lipase (nM/min/g)	93823.449±4869.714 **bc**	109633.749±10716.118 **bc**	90476.958±4674.338 **bc**	73825.601±10227.082 **c**	143515.425±10377.341 **ab**	93249.93±16150.022 **abc**	99018.41±11720.516 **abc**	155700.503±16442.753 **a**
Fungal biomass (copies/g dry soil)	19180574.201±978329.86 **b**	23928176.644±2093020.052 **b**	25477411.751±3126856.53 **ab**	27322440.585±5815687.772 **ab**	31295931.825±4329924.143 **ab**	46002472.01±7917214.114 **a**	31486630.34±4152563.194 **ab**	26785299.963±4423480.96 **ab**
Bacterial biomass (copeis/g dry soil)	1741232191.459±92814798.578 **b**	2607639151.583±141445440.444 **a**	1595094938.592±153508755.117 **b**	2009692503.125±121267896.726 **ab**	2128411480.25±251096464.242 **ab**	1757299891.75±382103739.466 **ab**	2124296599.75±290945685.291 **ab**	1985860603.9±115733097.17 **b**
F/B ratio	0.012±0.001 **c**	0.01±0.001 **c**	0.018±0.003 **ab**	0.014±0.003 **bc**	0.015±0.002 **abc**	0.027±0.002 **a**	0.015±0.002 **abc**	0.013±0.002 **bc**

Values are the means ± SE and different lowercase letters indicate significant differences among different islands and mainland (Štinjan forest) ($p < 0.05$). **TOC (%)** = total organic carbon; **C/N** = total carbon/N; **TC** = total carbon; **TOC/clay ratio** = total organic carbon/clay; **N/P ratio** = N/P total; **F/B ratio** = fungal biomass/fungal biomass.

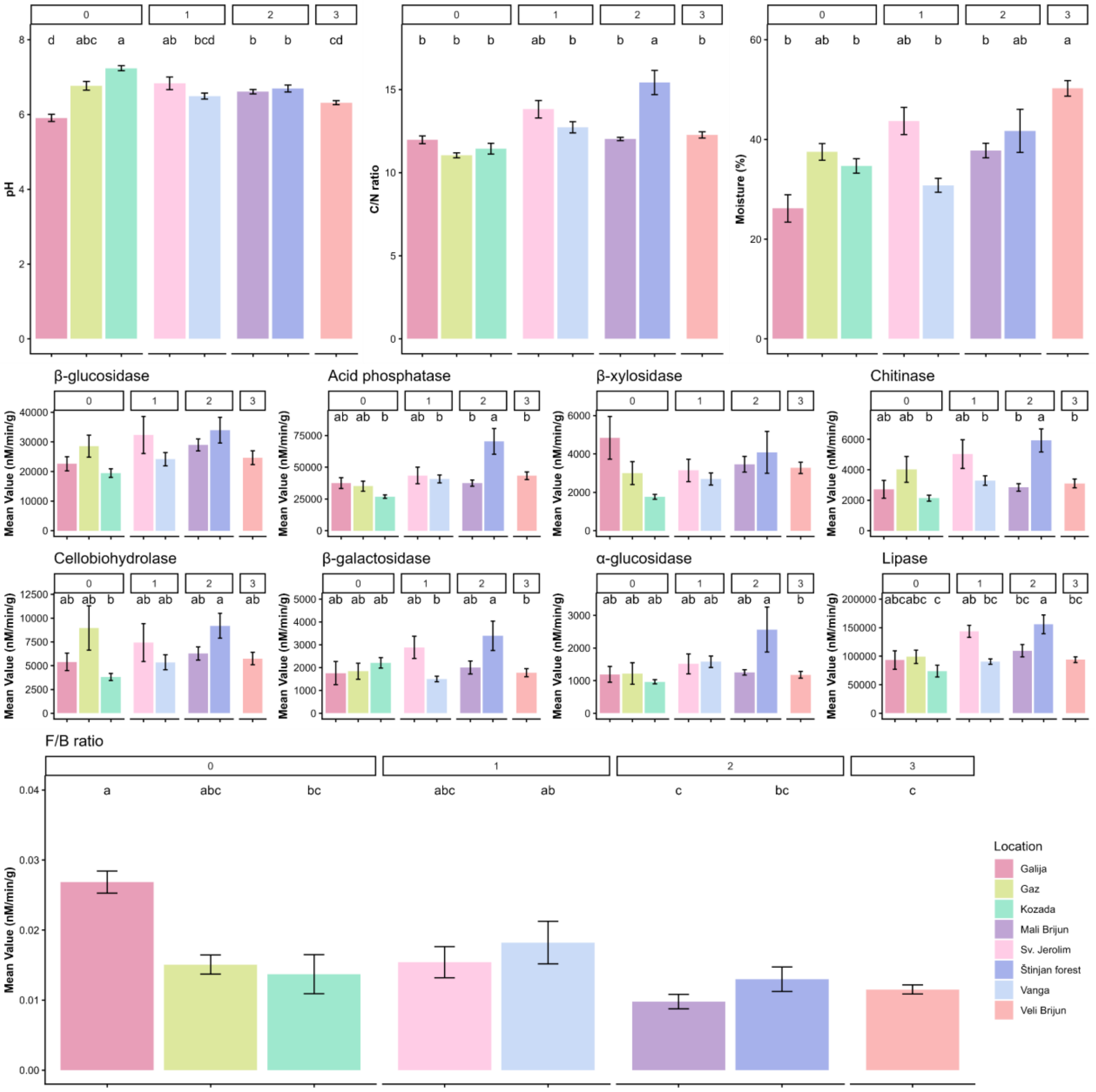


Figure S2. Analysis of soil biochemical properties at Brijuni NP and Štinjan forest grouped by tourism pressure. Panels show different levels of tourism pressure from 0 as unvisited locations to 3 as the most visited location. Error bars at bar plots indicate standard error of the mean and different letters indicate significant differences between locations ($p < 0.05$). **C/N ratio** = total carbon/N, **F/B ratio** = Fungal biomass/Bacterial biomass.

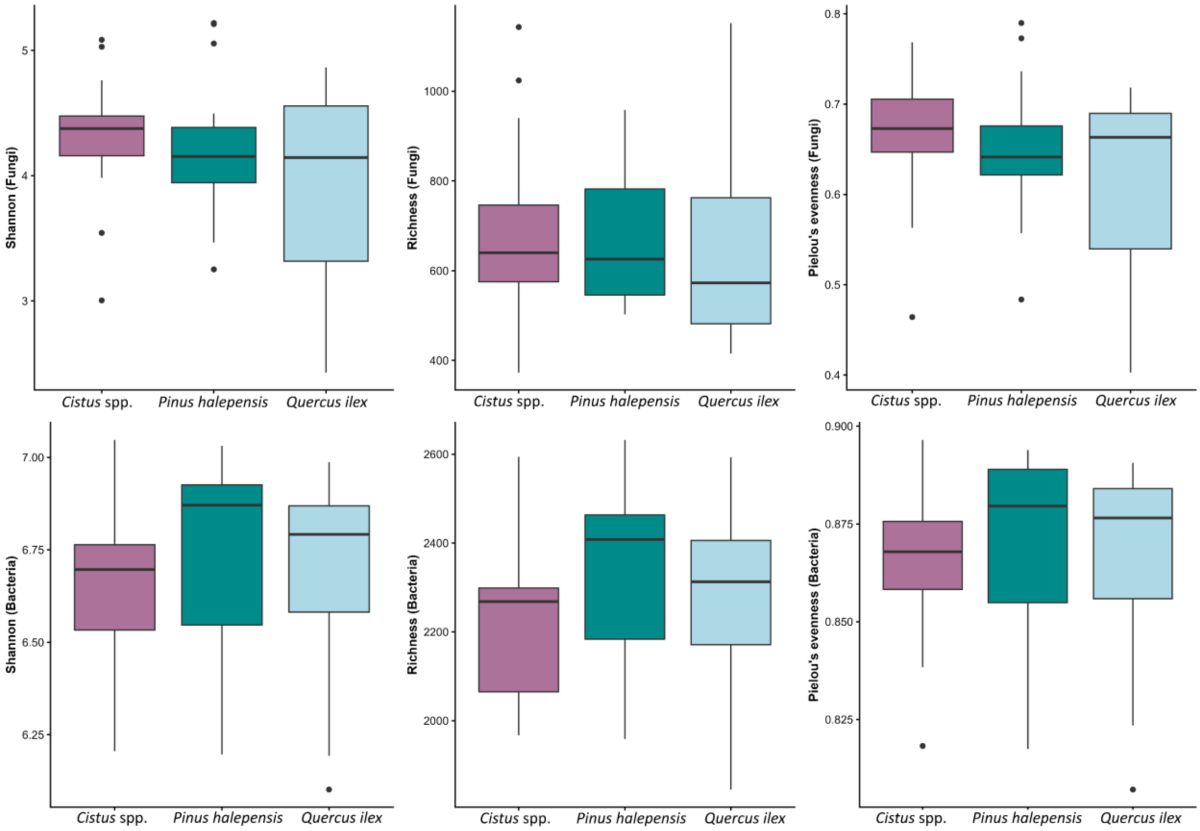
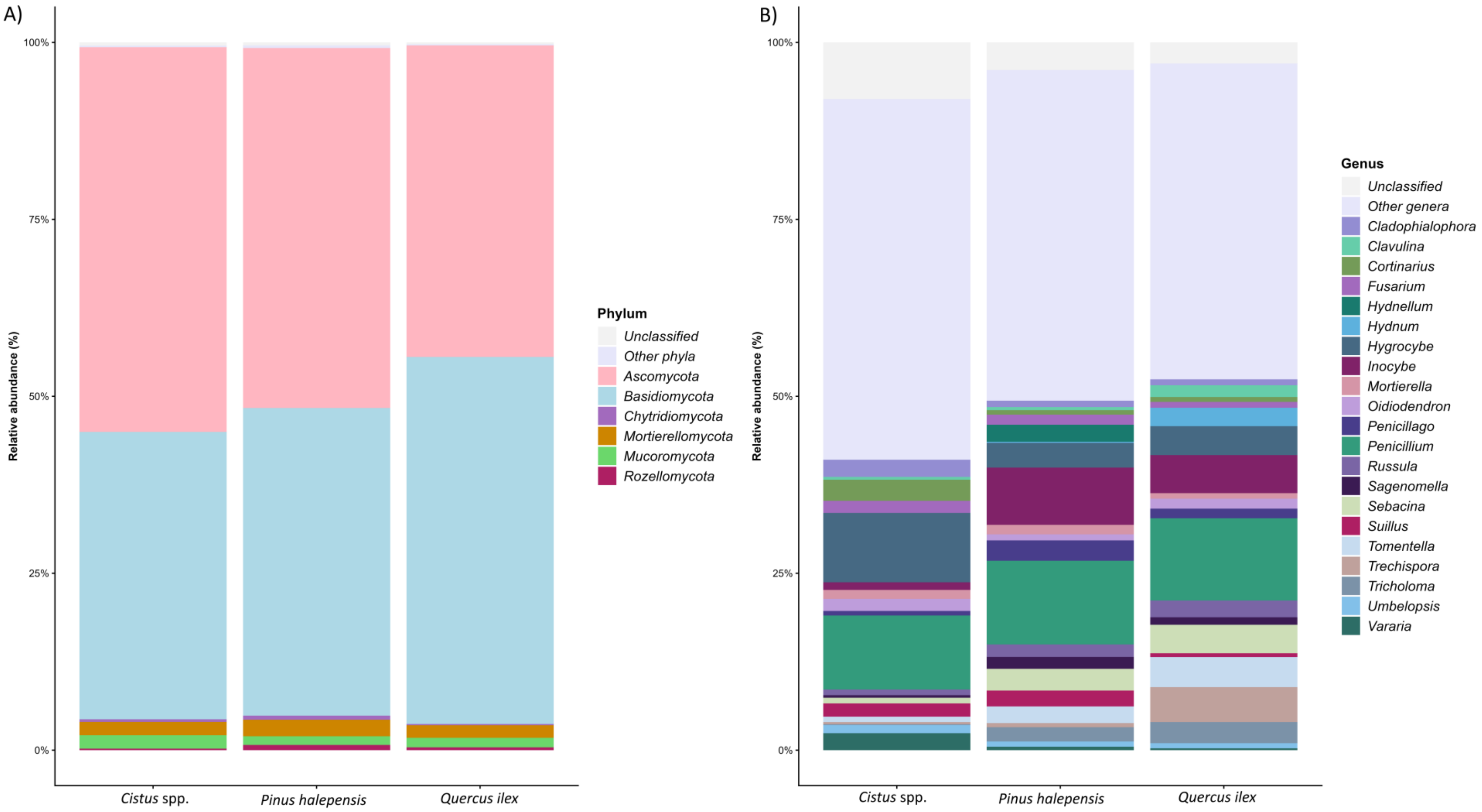


Figure S3. Alpha diversity metrics of fungal and bacterial communities between different dominant vegetation species at Donji Kamenjak.


A)
Relative abundance (%)
100%
75%
50%
25%
0%
Cistus spp.
Pinus halepensis
Quercus ilex
Phylum
Unclassified
Other phyla
Ascomycota
Basidiomycota
Chytridiomycota
Mortierellomycota
Mucoromycota
Rozellomycota
B)
Relative abundance (%)
100%
75%
50%
25%
0%
Cistus spp.
Pinus halepensis
Quercus ilex
Genus
Unclassified
Other genera
Cladophialophora
Clavulina
Cortinarius
Fusarium
Hydnellum
Hydnum
Hygrocybe
Inocybe
Mortierella
Oidiodendron
Penicillago
Penicillium
Russula
Sagenomella
Sebacina
Suillus
Tomentella
Trechispora
Tricholoma
Umbelopsis
Vararia

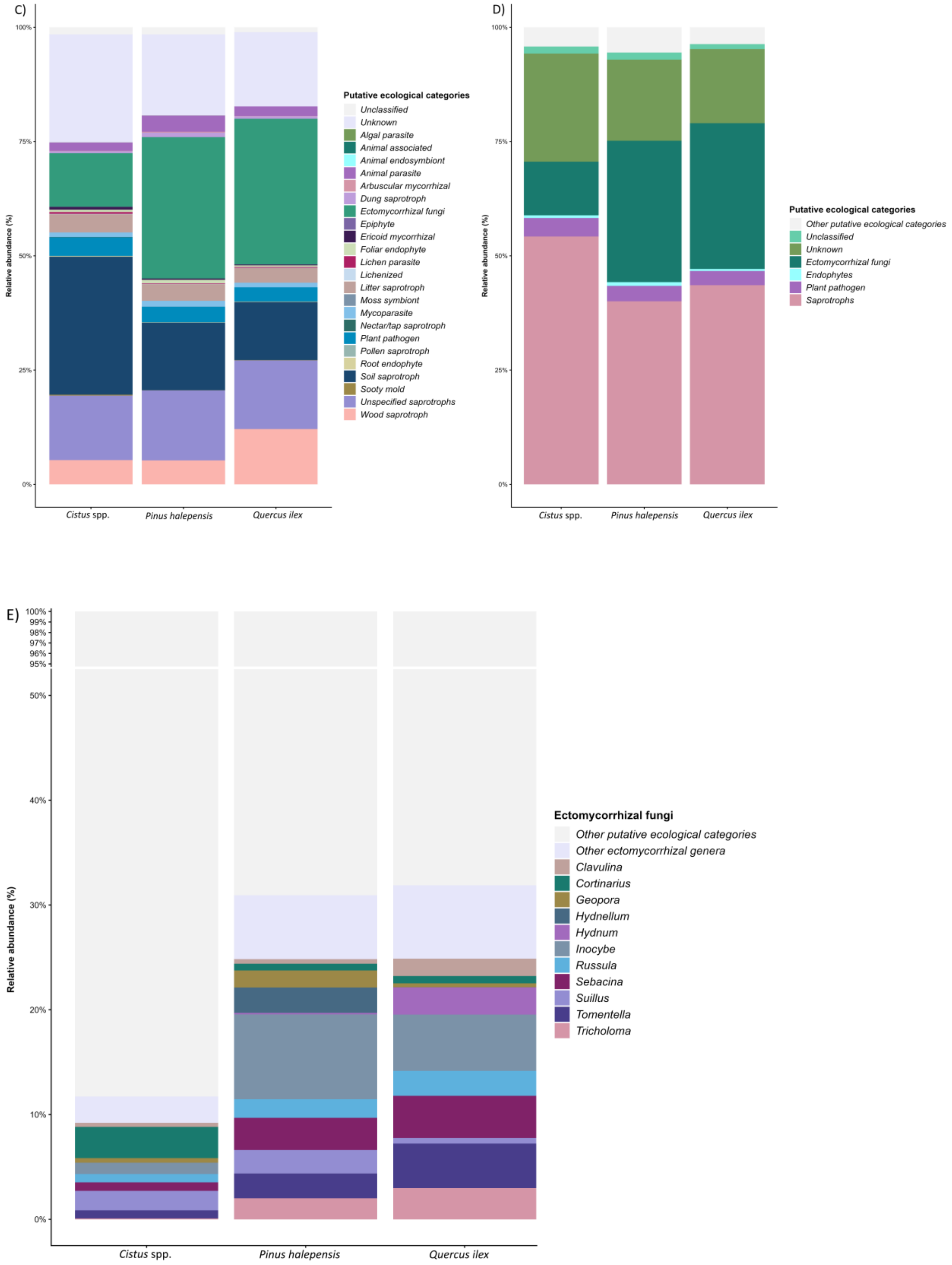


Figure S4. Relative abundance of fungal communities at Donji Kamenjak. **A)** Relative abundance of most abundant phyla; **B)** Relative abundance of 30 most abundant genera; **C)** Relative abundance of all putative ecological categories based on 30 most abundant genera; **D)** Relative abundance of the most abundant putative ecological categories based on 30 most abundant genera. Litter, wood, soil, unspecified, pollen, and dung saprotrophs are grouped. **E)** Relative abundance of most abundant ectomycorrhizal genera. **Unclassified A)**: fungal OTUs unidentified at phylum level; **Unclassified B), C), D)**: fungal OTUs unidentified at genus level; **Unknown D)**: unassigned putative ecological category.

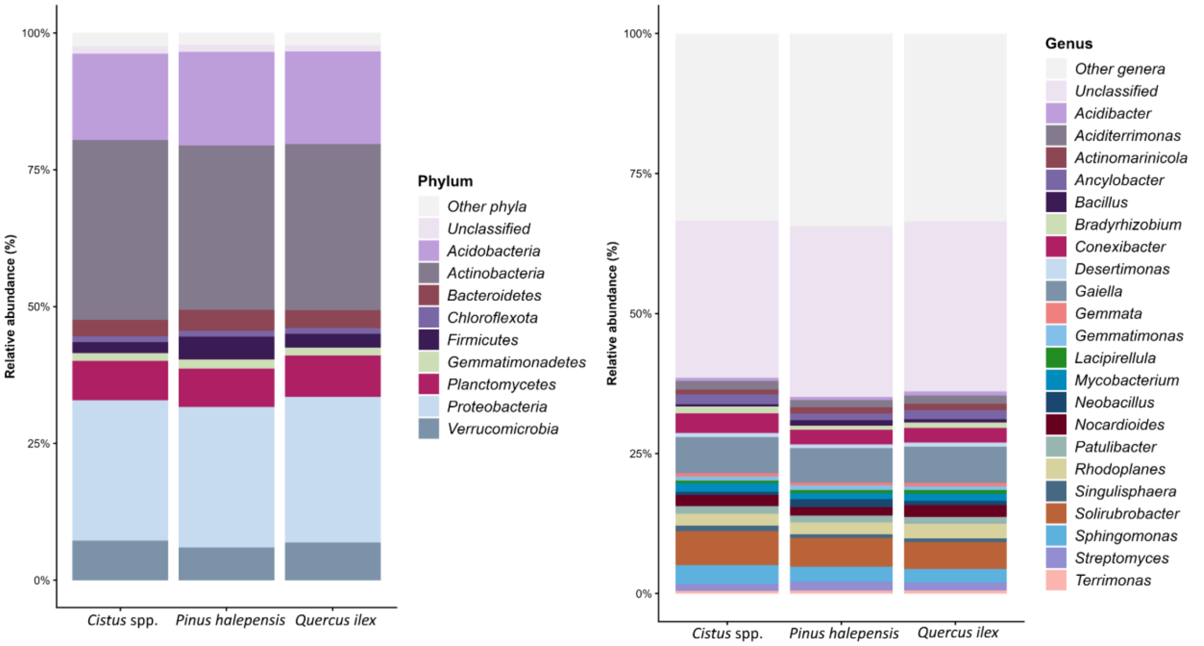


Figure S5. Relative abundance of bacterial communities at Donji Kamenjak. **A)** Relative abundance of most abundant phyla; **B)** Relative abundance of 30 most abundant genera. **Unclassified A)**: bacterial OTUs unidentified at phylum level; **Unclassified B)**: bacterial OTUs unidentified at genus level.

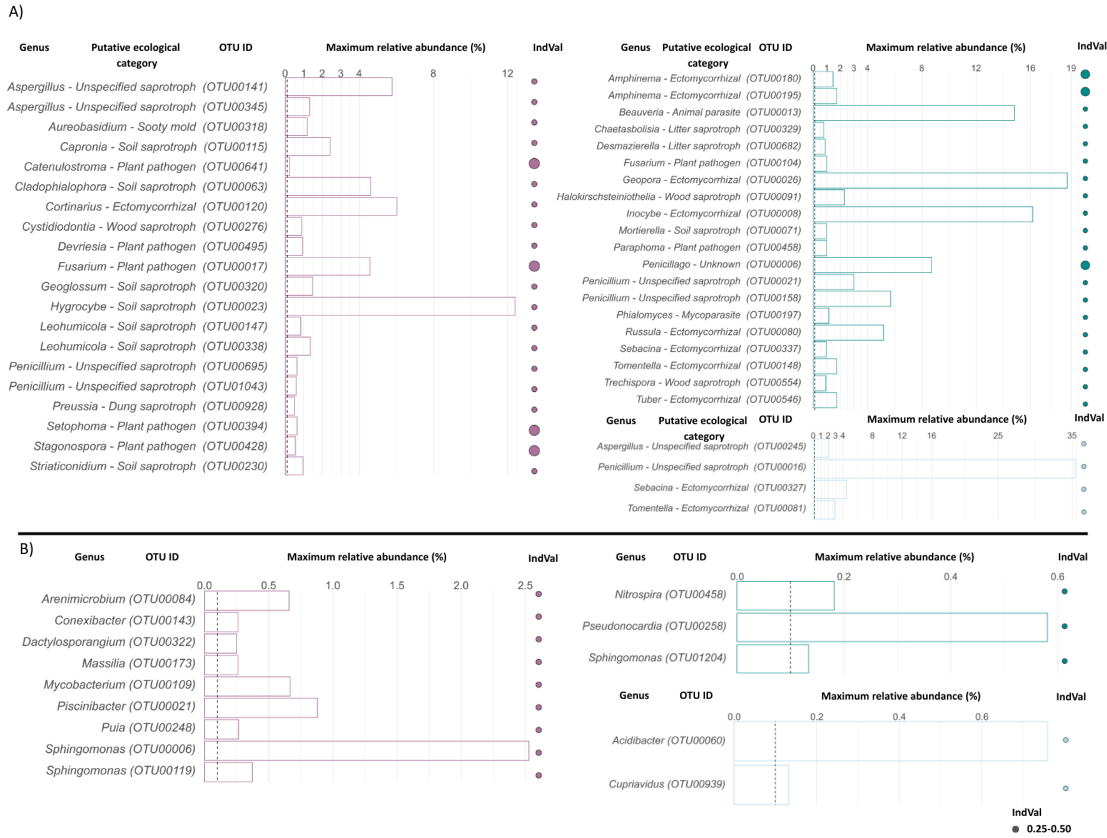


Figure S6. Indicator species analysis showing indicator taxa specific for each dominant vegetation species at Donji Kamenjak. **A)** Fungal communities; **B)** Bacterial communities. Bar plots represent maximum relative abundance. The dashed black line indicates the relative abundance threshold (0.1%). Circle size represents the indicator value, and colours correspond to dominant vegetation species: light purple = *Cistus* spp., dark green = *P. halepensis* and light blue = *Q. ilex*.

Table S5. Summary of linear models for fungal richness at Brijuni NP. Significant effects (p < 0.05) are in bold.

	Estimate	Std. error	Statistic	p. value
Intercept	446.57	5.55	80.48	**2.00E-93**
Island size	1.58	19.79	0.08	0.937
DTM	12.82	19.18	0.67	0.506
DNI	23.12	6.43	3.6	**5.00E-04**
Soil pH	23.72	7.08	3.35	**0.001**
Soil moisture	8.36	6.67	1.25	0.213
C/N ratio	-11.33	7.04	-1.61	0.111

DTM = distance to the mainland; **DNI** = distance to the nearest island; **C/N ratio** = total carbon/N.

Table S6. Summary of linear models for bacterial richness at Brijuni NP. Significant effects (p < 0.05) are in bold.

	Estimate	Std. error	Statistic	p. value
Intercept	2145.56	13.98	153.46	**1.70E-121**
Island size	-40.64	49.85	-0.82	0.417
DTM	-49.87	48.34	-1.03	0.305
DNI	4.06	16.19	0.25	8.03E-01
Soil pH	4.98	17.83	0.28	0.781
Soil moisture	67.59	16.8	4.02	**1.10E-04**
C/N	-0.13	17.74	-0.01	0.994

DTM = distance to the mainland; **DNI** = distance to the nearest island; **C/N ratio** = total carbon/N.

Table S7. Summary of AICc model selection for fungal richness at Brijuni NP.

Intercept	C/N	DNI	DTM	Soil moisture	Island size	Soil pH	Df	logLik	AICc	delta	weight
444.648		28.218			-15.773	28.415	5	-587.104	1184.797	0	0.118
444.648	-14.715	26.078				18.021	5	-587.185	1184.958	0.161	0.109
444.648	-10.062	27.289	9.762			22.735	6	-586.081	1184.993	0.197	0.107
444.648	-9.347	27.564			-10.305	23.726	6	-586.114	1185.059	0.263	0.104
444.648		27.764	14.874			27.155	5	-587.329	1185.247	0.45	0.095
444.648	-10.973	23.608	11.075	8.368		24.151	7	-585.223	1185.566	0.769	0.081
444.648	-10.456	24.295		7.253	-10.734	24.64	7	-585.458	1186.035	1.239	0.064
444.648	-15.953	22.985		6.732		18.649	6	-586.63	1186.091	1.294	0.062
444.648		25.661		5.808	-16.637	29.593	6	-586.683	1186.198	1.401	0.059
444.648		24.653	16.393	7.156		28.707	6	-586.71	1186.252	1.455	0.057

DTM = distance to the mainland; **DNI** = distance to the nearest island; **C/N ratio** = total carbon/N.

Table S8. Summary of AICc model selection for bacterial richness at Brijuni NP.

Intercept	C/N	DNI	DTM	Soil moisture	Island size	Soil pH	Df	logLik	AICc	delta	weight
2148.972				73.412			3	-685.780	1377.790	0.000	0.197
2148.972			-11.169	71.597			4	-685.440	1379.268	1.478	0.094
2148.972				72.721	7.198		4	-685.636	1379.661	1.871	0.077
2148.972				74.908		6.029	4	-685.684	1379.757	1.967	0.0737

DTM = distance to the mainland; **DNI** = distance to the nearest island; **C/N ratio** = total carbon/N.

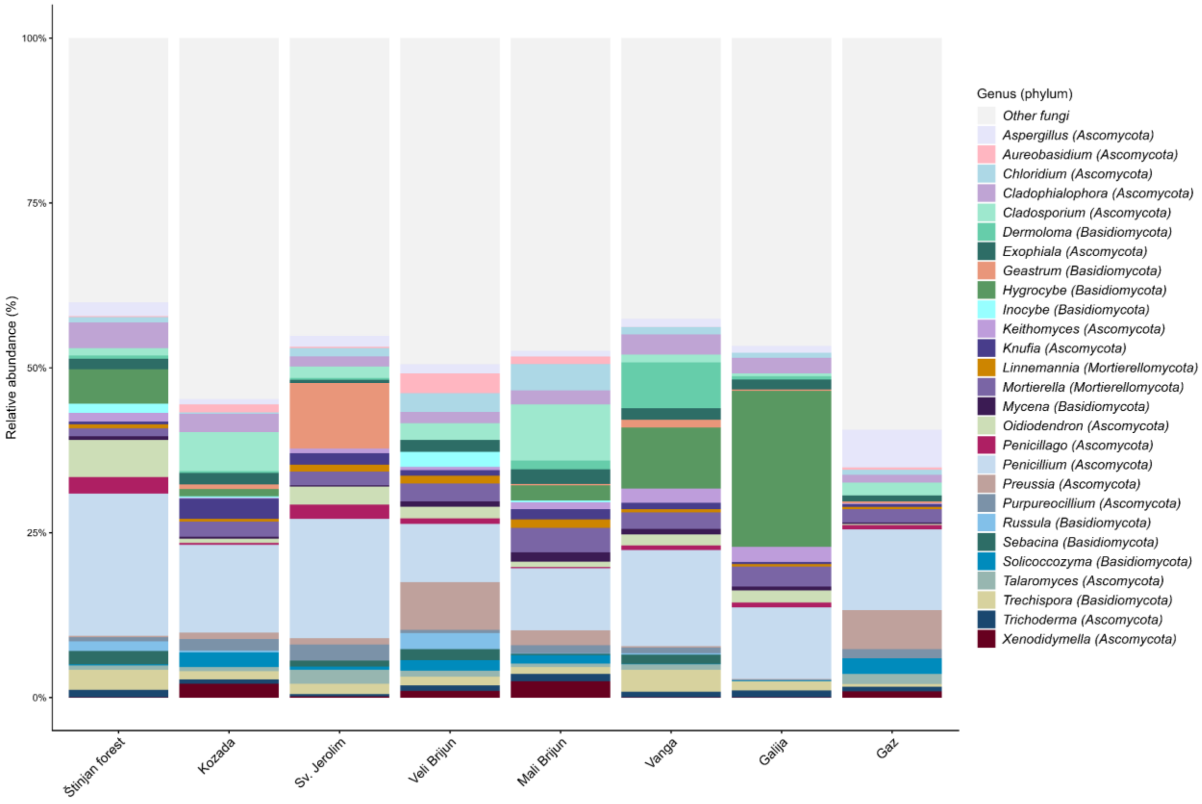


Figure S7. Relative abundance of 30 most abundant fungal genera at Brijuni NP and Štinjan forest, with phyla in the brackets. Localities are ordered left-to-right by increasing distance from mainland (DTM).

Table S9. Pairwise PERMANOVA comparisons between locations for fungal communities at Brijuni NP and Štinjan forest. Significant differences ($p < 0.05$) are in bold.

Location pairs	Sum of squares	F-statistics	R^2	p-value adjusted
Kozada vs Štinjan forest	1.4013796	6.80075	0.207335	**0.028**
Kozada vs Mali Brijun	1.0746698	6.352244	0.174741	**0.028**
Kozada vs Veli Brijun	1.3262287	6.645224	0.148845	**0.028**
Kozada vs Vanga	1.2797068	7.176576	0.28505	**0.028**
Kozada vs Sv. Jerolim	0.6168155	2.983656	0.175678	**0.028**
Kozada vs Galija	0.9746548	5.446192	0.352591	0.112
Kozada vs Gaz	0.6819478	3.746181	0.272525	**0.028**
Štinjan forest vs Mali Brijun	2.3185795	12.51438	0.229561	**0.028**
Štinjan forest vs Veli Brijun	1.8240569	8.863262	0.150574	**0.028**
Štinjan forest vs Vanga	1.4953986	7.583415	0.201776	**0.028**
Štinjan forest vs Sv. Jerolim	1.1434029	5.308343	0.16955	**0.028**
Štinjan forest vs Galija	0.9940441	4.864421	0.181073	**0.028**
Štinjan forest vs Gaz	1.2848302	6.244658	0.221092	**0.028**
Mali Brijun vs Veli Brijun	1.558357	8.408613	0.134735	**0.028**
Mali Brijun vs Vanga	0.9850068	5.944771	0.148825	**0.028**
Mali Brijun vs Sv. Jerolim	1.0164328	5.73382	0.160459	**0.028**
Mali Brijun vs Galija	0.7497302	4.626361	0.151058	**0.028**
Mali Brijun vs Gaz	1.0092857	6.182843	0.192116	**0.028**
Veli Brijun vs Vanga	1.6347985	8.432933	0.167211	**0.028**
Veli Brijun vs Sv. Jerolim	1.2089243	5.869613	0.133797	**0.028**
Veli Brijun vs Galija	0.9652571	4.882311	0.125566	**0.028**
Veli Brijun vs Gaz	1.0332882	5.202598	0.132711	**0.028**
Vanga vs Sv. Jerolim	0.959251	5.001287	0.217435	**0.028**
Vanga vs Galija	0.4205605	2.507834	0.151918	0.084
Vanga vs Gaz	1.0535285	6.200991	0.306965	**0.028**
Sv. Jerolim vs Galija	0.7640903	3.759707	0.27324	0.084
Sv. Jerolim vs Gaz	0.6914365	3.351466	0.251019	**0.028**
Galija vs Gaz	0.8199732	5.1331	0.461067	0.952

Table S10. Pairwise PERMANOVA comparisons between locations for bacterial communities at Brijuni NP and Štinjan forest.Significant differences ($p < 0.05$) are in bold.

Location pairs	Sum of squares	F-statistics	R^2	p-value adjusted
Štinjan forest vs Veli Brijun	0.509131	4.9086554	0.089397	**0.028**
Štinjan forest vs Mali Brijun	0.468744	4.923	0.104923	**0.028**
Štinjan forest vs Vanga	0.349424	3.620	0.107661	**0.028**
Štinjan forest vs Sv. Jerolim	0.292433	2.786	0.096776	**0.028**
Štinjan forest vs Kozada	0.581809	5.994	0.187357	**0.028**
Štinjan forest vs Galija	0.302727	2.9209295	0.117208	0.056
Štinjan forest vs Gaz	0.258778	2.4428801	0.099942	0.084
Veli Brijun vs Mali Brijun	0.256529	2.5816716	0.045627	**0.028**
Veli Brijun vs Vanga	0.36103	3.5568051	0.078074	**0.028**
Veli Brijun vs Sv. Jerolim	0.434213	4.0280482	0.095842	**0.028**
Veli Brijun vs Kozada	0.544774	5.320967	0.122827	**0.028**
Veli Brijun vs Galija	0.250828	2.3383497	0.064349	**0.028**
Veli Brijun vs Gaz	0.176603	1.6239474	0.045586	0.308
Mali Brijun vs Vanga	0.316772	3.5015392	0.093371	**0.028**
Mali Brijun vs Sv. Jerolim	0.374465	3.8617885	0.114046	**0.028**
Mali Brijun vs Kozada	0.494898	5.4922981	0.154746	**0.028**
Mali Brijun vs Galija	0.284527	3.0074715	0.103679	0.084
Mali Brijun vs Gaz	0.193486	2.0041008	0.071565	0.140
Vanga vs Sv. Jerolim	0.396427	3.9501618	0.17996	**0.028**
Vanga vs Kozada	0.678341	7.6281888	0.297648	**0.028**
Vanga vs Galija	0.171945	1.7736678	0.112445	0.756
Vanga vs Gaz	0.148108	1.4730732	0.095202	1.000
Sv. Jerolim vs Kozada	0.328517	3.2077232	0.186412	0.056
Sv. Jerolim vs Galija	0.274618	2.3070527	0.187458	0.420
Sv. Jerolim vs Gaz	0.237353	1.9129876	0.16058	0.560
Kozada vs Galija	0.394818	4.010038	0.286226	0.084
Kozada vs Gaz	0.330382	3.1921636	0.241974	0.168
Galija vs Gaz	0.106539	0.8076037	0.118633	1.000

Table S11. Scoring orientation and weight of each soil health indicator at Brijuni NP and Štinjan forest.

Soil health indicator	Orientation	Weight
TC	Higher -> healthier	0.07721
Humus	Higher -> healthier	0.076852
TOC	Higher -> healthier	0.076851
Sand	Lower -> healthier	0.072468
N	Higher -> healthier	0.070247
Fe	Higher -> healthier	0.068369
Soil moisture	Higher -> healthier	0.061303
Al	Lower -> healthier	0.060271
Ca	Higher -> healthier	0.059136
TOC/clay	Higher -> healthier	0.046565
Na	Higher -> healthier	0.044157
P	Higher -> healthier	0.037259
K	Higher -> healthier	0.035193
Mn	Higher -> healthier	0.033411
Silt	Higher -> healthier	0.031451
N/P ratio	Optimum 9 ± 4 -> healthier	0.031135
P_2O_5	Higher -> healthier	0.027694
Clay	Higher -> healthier	0.021445
Mg	Higher -> healthier	0.020045
Pb	Lower -> healthier	0.018411
C/N ratio	Optimum 12 ± 6 -> healthier	0.014983
Soil pH	Optimum 6.5 ± 1.5 -> healthier	0.006433
Zn	Higher -> healthier	0.005261
K_2O	Higher -> healthier	0.003853

TOC = total organic carbon; **C/N ratio** = total carbon/N; **TC** = total carbon; **TOC/clay ratio** = total organic carbon/clay; **N/P ratio** = N/P total.

Table S12. Final linear mixed-effects model for prediction of SHI at Brijuni NP and Štinjan forest.

	Estimate	Std. Error	t-value
Intercept	4.84E-01	5.17E-02	9.363
Island size	5.79E-02	2.40E-02	2.411
DNI	5.80E-02	1.25E-02	4.623
DTM	1.67E-02	3.88E-03	4.305
Tourism pressure 1	-1.48E-02	1.87E-02	-0.794
Tourism pressure 2	-2.35E-01	6.02E-02	-3.898
Tourism pressure 3	-3.32E-01	8.52E-02	-3.898
Bacterial richness	1.41E-03	3.12E-03	0.451
Bacterial PCoA2	-2.58E-02	4.88E-03	-5.286
Fungal PCoA2	-1.73E-02	6.42E-03	-2.689
β-xylosidase	7.47E-06	1.91E-06	3.908
Acid phosphatase	1.60E-02	5.15E-03	3.099
Cellobiohydrolase	-1.10E-02	4.15E-03	-2.636

Table S13. Variable importance of microbial predictors for soil health at Brijuni NP and Štinjan forest.

Microbial predictors	%IncMSE	Node purity
Bacterial PCoA2	15.4094949	0.027499555
Fungal PCoA2	14.9179609	0.014285543
Fungal PCoA1	11.6178296	0.013125253
Bacterial richness	11.2782375	0.014116464
Bacterial PCoA1	11.2676793	0.016848804
Cellobiohydrolase	6.7722254	0.018424855
Lipase	5.6765624	0.013984427
Acid phosphatase	5.1159301	0.015708147
β-glucosidase	4.4837447	0.022178027
β-galactosidase	4.2867289	0.016822251
Chitinase	4.1720463	0.008222464
Fungal biomass	3.9665511	0.007720812
β-xylosidase	2.8190920	0.017395266
F/B ratio	1.9920878	0.005839374
α-glucosidase	1.8694861	0.010556767
Bacterial biomass	1.5168697	0.005079533
Fungal richness	0.3200815	0.004599004

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