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Mycobiome and microbiome resilience of alpine Norway spruce forests in response to disturbances: Can soil and endophytic microorganisms help drive an efficient forest management?

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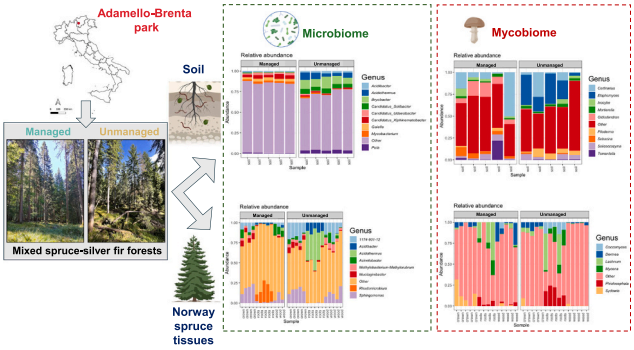
HIGHLIGHTS

- Soil and endophytic microorganisms have a key role in forest health and resilience.
- A managed (thinning) and an unmanaged stand were selected in a Norway spruce forest.
- Fungal and bacterial microbiota were analyzed in soil and plant tissues.
- Specific taxa and metabolic pathways featured the stand and its forest management.
- Specific biomarkers of disturbance can be revealed by this analysis.

GRAPHICAL ABSTRACT



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ABSTRACT

Assessing biodiversity and ecosystem services of forests is pivotal to implement effective climate adaptation strategies, especially in unmanaged forests, which, according to the Nature Restoration Law (Reg. EU 2024/1991), are considered as reference systems to restore biodiversity-rich ecosystems. Forest biodiversity includes also the microbiota which can enhance tree ability to adapt to a broad spectrum of environmental stimuli, including anthropogenic disturbances like silviculture. The aim of this work was to compare the mycobiome and microbiome biodiversity, in both soil and plant compartments, between managed and unmanaged alpine Norway spruce forest stands, assessing the variations driven by forestry. Branches, wood, roots, and bulk soil samples were collected from mature spruce trees for bacterial and fungal metabarcoding in parallel with stand structure and soil proprieties. The effect of stand structure was evident in soil and root microbiota, especially for fungi, even if wood and crown compartments also showed peculiar features. Results showed a more favorable soil nutrient cycling in soil-root compartment of managed stand compared to the unmanaged one but also a higher

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presence of pathotrophic fungal guilds. On the contrary, in the unmanaged stand there was a higher coordination of microorganisms but the presence of potential pathogens in plant compartments. In conclusion, some specific taxa featured the study areas and thus the forest management suggesting the possibility to individuate biomarkers of anthropogenic disturbances that could help in the implementation of sustainable management strategies and the long-term monitoring of forest systems within the framework of climate adaptation and biodiversity restoration policies.

1. Introduction

Microorganisms, both bacteria and fungi, play several key roles in the ecology and physiology of plants, influencing their growth and survival performances through several positive functions, such as the increase of nutrient availability and stress tolerance (Dastogeer et al., 2020). In fact, the synergic interaction between plant functional traits and microbial functional diversity is now referred to as an "extended genome" (Turner et al., 2013; Hawkes et al., 2021). This forest–microbiome holobiont may be crucial in counteracting the challenges posed by climate changes (Nizamani et al., 2024; Baldrian et al., 2023) since can mediate plant response to different environments through the regulation, for example, of carbon and nitrogen biogeochemical cycles (Li et al., 2023; Anthony et al., 2024). Therefore, improving our knowledge on tree microbiomes may help in preserving forest ecosystem functionality, particularly in view of the expected harsh climatic conditions. Thinning is a widely used silvicultural practice aimed at reducing competition for resources among trees maximizing the production of quality wood (Fernandez et al., 2024). The silvicultural treatments modify forest structure and, in turn, plant biodiversity and resilience at both stand and landscape scales (e.g., Chianucci et al., 2024), as well as the biodiversity and abundance of forest associated microorganisms (Tomao et al., 2020; Kim et al., 2021). The understanding of how microbial communities can be shaped by different forest naturalness levels and/or silvicultural treatments is pivotal for the implementation of integrated strategies in sustainable forest management (Xue et al., 2020). Indeed, the comparison among forest stands with similar conditions (i.e., site condition and species composition) but different management histories have provided deeper insights into soil microbial biodiversity evolution (Hartmann et al., 2012; Li et al., 2024). Soil microbiome diversity is affected by several parameters starting from forest stand structure and tree species composition (Baldrian, 2017). The reduction of stand density was found to modulate the soil nutrient content increasing bacterial diversity and thereby stabilizing the ecosystem structure (Xu et al., 2025), while the maintenance of scattered trees has been shown to guarantee key hotspots to preserve fungal diversity (Scali et al., 2025). Even the type of forest treatment has been demonstrated to influence the soil microorganism dynamics: for example, intensive thinning operations (bringing to low stand tree density) led to lower microbial community abundance and diversity but higher microbial activity in the soil compared to conventional practices, favoring the mineralization of organic matter (Fernandez et al., 2024). Usually, unmanaged forests are considered as an important reserve of fungal diversity, especially considering ectomycorrhizal and wood-inhabiting species. On the other hand, a source of disturbance has been shown to potentially provide new opportunities of colonization for different fungal taxa, for example some wood-decaying species with a preference for tree stumps are favored by thinning (Tomao et al., 2020). Several studies have focused on the effect of silviculture on soil microorganisms while fewer information is available on the perturbations caused by forest management on tree endophytes (i.e., Li et al., 2024), a topic more investigated in relation to climate change. Forest management was found to enhance tree growth and survival under environmental stress promoting the microbial diversity and stability in phyllosphere (Li et al., 2024). Information regarding microbial communities of tree phyllosphere, particularly of fungi, may be useful for biomonitoring, providing information about tree health and plant water status in forest ecosystems (Cambon et al., 2023). Würth et al. (2019) demonstrated that environmental parameters had an important effect on needle mycobiome biodiversity in *Picea glauca* while tree genetic variations had a negligible effect. Moreover, among other factors, the plant organ type contributes to shape the associated microbiome (Baldrian, 2017; Dastogeer et al., 2020). Since the forest–microbiome holobiont has a key role in forest functioning and the effects of silvicultural practices on these bacterial and fungal communities are still partly unknown, this work was aimed at evaluating the differences in bacterial and fungal microbiota induced by silvicultural practices in soil and endophytic (root, wood and crown of Norway spruce) biospheres. Soil properties, microbiome and mycobiome diversity and endophytic communities were evaluated in a mountainous conifer forest of the Alpine region, in Italy, in two parcels characterized by different forest management and stand structure.

2. Materials and methods

2.1. Study area and sampling design

This study has been conducted in Val Brenta (southeastern Italian Alps, Trento province) on two forest stands belonging to the Regole di Spinale e Manez district, which represents a form of collective property managed by local community. Over time, such systems have evolved into long-lasting and well-structured forms of social and institutional organization, aimed at ensuring the sustainable management of local forest resources. The area is also included in the Natura 2000 network (ZSC IT3120177 'Dolomiti del Brenta' and, partially, in the ZPS IT3120159 'Brenta'). Both stands refer to mountainous mixed spruce-silver fir forest type (EEA, 2006) and they are located between 1200 and 1500 m.a.s.l. and characterized by a different management history: stand n° 49 (46.1118 N, 10.5048 E), defined as unmanaged (U), and stand n° 77 (46.1210 N, 10.4952 E), defined as managed (M). The unmanaged stand is dominated by Norway spruce (*Picea abies* L., 70 %) and European beech (*Fagus sylvatica* L., 15 %) with silver fir (*Abies alba* Mill., 9 %) and larch (*Larix decidua* mill., 6 %). A vertical structure is typical of a single-storied forest, with large trees even on boulders. The soil is characterized by blocky debris on dolomite with low substrate and high level of organic residues in decomposition. The stand has been excluded by silvicultural treatments since 1987. The managed stand is characterized by a mixed multi-storied forest of Norway spruce (45 %), European beech (33 %), silver fir (15 %), and larch (7 %). The stand has been subjected to selective thinning during March 2022 with removal of dominated trees and openings of small gaps to favorite the regeneration of beech and silver fir seedlings. The two stands are located 2 km apart.

Within each forest stand (M and U), three sampling units (circular plots, $r = 15$ m) were selected as reference units for stand data collection, ensuring a minimum distance of 50 m among plots belonging to the same plot. In both M and U forest stands, five mature Norway spruce trees were selected with similar size and sociological position for plant and soil sampling for DNA extraction and soil sampling for the physical chemical characterization.

2.2. Forest stand data collection

Within each forest plot, all standing woody elements (i.e., living, dead trees and snags with a diameter at breast height, DBH $\geq$ 5 cm,

stumps with diameter at 50 cm-height from the ground ≥10 cm and height < 1.30 m), were identified at species level. Their DBH and height were recorded with the use of a tree caliper and an electronic hypsometer (Vertex IV-360 and Transponder T3). For stumps, two diameters (base and top) were recorded. To assess the number and volume of logs (larger diameter ≥ 10 cm), the Line Intersect Method (Van Wagner, 1968; Marshall et al., 2000) was applied. Main stand structure parameters (number of trees per hectare, mean diameter, mean basal area and volume per hectare) were calculated for living trees and deadwood, and this latter was divided into standing (snag, dead trees, and stumps) and lying components (logs). Diameter variability was calculated through the Gini coefficient (Lexerød and Eid, 2006). The diversity of living tree species was calculated for the most common indices based on the proportional abundances of species (Shannon, Simpson and Pielou, see Magurran, 2013).

2.3. Plant and soil tissue sampling, DNA extraction and metabarcoding sequencing

From each tree, small twigs including needles (collectively defined as "crown"), wood, roots, and a bulk representative soil sample (obtained by mixing 3 soil samples sampled at a distance of 50 cm around the plant, 0–10 cm depth) were collected at the end of June 2022 - three months after the felling in M stand - for the metabarcoding of the 16S and ITS regions to evaluate bacterial and fungal diversity, respectively. Wood cores were sampled using an increment bore previously surface-sterilized with 100 % ethanol before each sampling, removing the bark before the wood coring. Once in the lab, wood and soil samples were stored at −80 °C while root samples were washed to remove the soil and other debris, then roots and crown samples were surface-sterilized by immersion in ethanol (100 %, 30 s), NaClO (5 %, with a few Tween drops, 5 min), and then washed 3 times with sterile MQ-H_2O and frozen. About 60 mg of each sample were ground in liquid nitrogen with a sterile mortar and pestle. Total eDNA was extracted from grinded samples using the NucleoSpin Plant II kit (MACHEREY-NAGEL GmbH & Co., Dueren, Germany) for plant tissues and the FastDNA™ SPIN Kit for Soil (MP Biomedicals, Irvine, CA, USA). The DNA quality was checked by a 1 % agarose gel electrophoresis and nanodrop reads at 230 and 280 nm. The soil eDNA was purified using 1.5× reaction volume with Ampure XP beads (MAGBIO Genomics Wembley, WA, Australia) and then eDNA was quantified with Qubit dsDNA BR assay kit using a Qubit 4.0 (Thermo Fisher Scientific, Waltham, MA, USA). The hypervariable V3-V4 region of the bacterial 16S gene and the fungal ITS2–5.8S region were amplified with target-specific primers (Table 1) including the Illumina overhang adapter sequences for subsequent sample indexing via a second round of PCR. All the bacterial and fungal amplicons were sequenced with the Illumina Sequencing platform MiSeq™ (Caporaso et al., 2012) to generate paired-end raw reads of 300 bp length by an external service (Genartis SRL, Verona, Italy), obtaining a total of ~18 raw million fragments for the 16S region and ~8 raw million fragments for the ITS region (BioProject accession number: PRJNA1249437).

Table 1
Target specific primers and Illumina overhang sequences for V3-V4 and ITS2-5.8S regions.

Primer	Sequence	Reference
V3-V4 F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-CCTACGGGNGGCWGCAG	Thijs et al., 2017
V3-V4 R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-GACTACHVGGGTATCTAATCC	
ITS2-5.8S F	TCGTCGGCAGCGTCAGATGTGTATAAGAGAC-AGGAACGCAGCRAAIIGYGA	Venice et al., 2021
ITS2-5.8S R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-TCCTCCGCTTATTGATATGC	

2.4. Bioinformatic analysis

The bioinformatic analysis was carried out using a QIIME2 analysis workflow based on QIIME2 official documentation (https://docs.qiime2.org/). Demultiplexing analysis was conducted on both forward and reverse sequencings using the software bcl2fastq provided by Illumina documentation (https://support.illumina.com/content/dam/illumina-support/documents/documentation/software_documentation/bcl2fastq). For the 16S region 357,193 fragments per sample on average were produced, whereas for the ITS region the production per sample was on average 156,401 fragments. Read pairs trimmed for 16S and ITS primers ranged from 229,007 to 457,608 and from 93,226 to 416,150. Afterwards reads were trimmed according to their quality, which varied through reads length. For the 16S region, the 3′ ends of the forward reads were trimmed at base position 238, whereas the reverse reads were trimmed at position 176. Regarding the ITS, forward and reverse reads were trimmed at position 243 and 180, respectively. 16S reads passing the denoising pipeline were cleaned from plant chloroplast and mitochondria sequences before the classification process. Reads from 16S and ITS regions were then classified using the Naïve-Bayes classifiers trained on Silva_v138.1_99_16S and Unite-v9-99_ITS databases respectively. A total of 7544 and 15,358 Amplicon Sequence Variants (ASVs) were found for ITS and 16S regions, respectively. Before the rarefaction, one root samples for ITS marker and one crown and two wood samples for 16S marker were excluded for their low number of reads. Then, samples were rarefied and used for statistical analyses. Subsequent analyses were performed in the R environment using QIIME2 exported files, using a set of functions implemented in several packages including Phyloseq (McMurdie and Holmes, 2013), Microbiomeutilities (Shetty and Lahti, 2022), MicroViz (Barnett et al., 2021), MicrobiomeSeq (Ssekagiri et al., 2017), MicrobiomeMarker (Cao et al., 2022), Vegan (Oksanen et al., 2024), DeSeq2 (Love et al., 2014), ggClusterNet (Wen et al., 2022), Metacoder (Foster et al., 2017). FUNGuild (Nguyen et al., 2016) was employed to predict the functional profiles of fungal community according to authors' instructions, after ITS dataset rarefaction (57,077 total ASV); only "highly probable" or "probable" annotations were considered. Fungi were categorized into different guilds according to their nutritional mode across various clusters and health status of the three. Tax4fun (Aßhauer et al., 2015) was used to predict the functional capabilities of microbial communities based on 16S datasets.

2.5. Soil analysis

Topsoil bulk samples of 3 subsamples collected around each tree (0–30 cm deep) were sampled near the roots, as also done for soil samples used for metabarcoding analysis. Soil samples were air dried and then sieved to collect the 2 mm fraction. Soil texture was analyzed by a Mastersizer 2000 (Malvern Panalytical Ltd., Malver, UK) determining silt, sand, and loam fractions. Total organic carbon (TOC) and total nitrogen (TN) were determined by dry combustion using a FlashSmart NC Soil elemental analyzer (Thermo Fisher Scientific, Waltham, MA, USA), previously treating samples with HCl:H_2O (1:1 v:v) before TOC measurement. Soil pH and electrical conductivity (EC) were measured in water extracts 1:2.5 and 1:2, using specific electrodes (ASA-SSSA, 1996). Soil samples were digested by a microwave (ETHOS 900, FKV, Torre Boldone, BG, Italy) using a HNO_3:H_2O_2 mixture (5:2 v:v) following the EPA Method 3051a (EPA, 1995). The total concentration of K, Ca, Mg, Fe, Mn, Na, Zn, Cu, and Cr was determined using inductively coupled plasma spectrometry (ICP-OES 5900 Agilent, Santa Clara, CA, USA) and expressed as mg kg^{-1}. The available concentration of macronutrients (Ca, K, Mg) for plants were also measured by ICP-OES after extraction with 1 M H_3COONH_4 at pH 7. Moreover, available P was calorimetrically determined using the molybdenum blue method following the procedure reported by Watanabe and Olsen (ASA-SSSA, 1996) after an extraction with 0.5 M $NaHCO_3$ at pH 8.

2.6. Statistical analyses

Non-parametric two-samples Wilcoxon rank test (Oyeka and Ebuh, 2012) was used to compare means among the two stands for not normally distributed forest structure data; unpaired two-samples *t*-test were used for normally distributed data of stand structure and soil physical chemical proprieties (Eisinga et al., 2017). Descriptive statistics (means and standard deviation) were performed using R statistical software (R Core Team, 2021); diversity indices were calculated through the "forestmangr" package (Braga et al., 2020), Gini index through "reldis" (Handcock, 2023). Living trees and dead trees volume was calculated through the "ForIT" package (Puletti et al., 2017). The "ggpubr" (Kassambara and Kassambara, 2020) and "ggstatsplot" (Patil, 2021) packages were used to test and visualize data distribution, as well as to compare means between groups. Statistical significance of the differences was set at $P < 0.05$. A permutational multivariate analysis of variance (PERMANOVA) was conducted in R environment to assess the significance of the variable management and sample type for both mycobiome and microbiome datasets. The analysis (999 permutations) was conducted using the adonis2 function of the package Vegan: adonis2 is developed for the analysis and partitioning sums of squares using dissimilarities and is based on the principles of McArdle and Anderson (2001). The Bray-Curtis non phylogenetic dissimilarity index was used to assess β-diversity.

3. Results

3.1. Forest structure

Both stands were dominated by Norway spruce (basal area per hectare >50 %, for all plots), with beech and silver fir as secondary species, respectively in managed (M) and unmanaged (U) stands (Table S1 for detail at plot level). Diameter distribution (Supplementary Fig. S1) confirmed differences in the vertical arrangement of tree layer between U (i.e., a temporary single-storied phase, still influenced by the last intensive silvicultural treatment in 1987) and M (i.e., a multi-storied phase due to the type of silvicultural approach applied) stands. The Gini coefficient showed higher variability of stem diameters for the M plots, even if the difference was marginally significant ($P = 0.08$, Supplementary Table S2). As expected, mean diameter, basal area per hectare, and total volume per hectare had higher values in the U stand. The mean total deadwood volume per hectare was clearly higher in U stand (66.6 ± 115.4 m^3 ha^{-1}) compared to the M one (7.4 ± 9.7 m^3 ha^{-1}), but only the standing element components (dead trees and snags) significantly differed among the two stands (Supplementary Table S2). The tree species diversity indices showed no clear differences among stands (data not shown).

3.2. Soil traits

Soil texture analyses highlighted a sandy soil in the U site while a loamy sandy texture in the M site. Soil pH was acid in the U site while it was neutral in the M site while EC indicated a medium salinity in both study areas. The concentration of TOC and macro and micronutrients in soil are reported in Table 2. The TOC concentration was high in both study areas, especially in the U stand. The TN concentration was also higher in U site even if it was particularly elevated in both areas. The C/N ratio was also particularly high in the U site (20, on average). The other macro and micronutrients were generally higher in the M site compared to the U one, except for total and available K, total Na, and Olsen P that had similar concentrations in the two stands.

3.3. Soil and endophytic fungi

Both management and sample type were significant in determining the mycobiome biodiversity as highlighted through the PERMANOVA analysis (Supplementary Table S3). Concerning the effect of forest management, α-diversity indexes were calculated to analyze the heterogeneity of microbial communities within a sample: observed diversity, Shannon's index, to emphasize the richness component of diversity, Simpson's index, to underline the evenness component, and Chao1 index, more specific for microbial community to highlight rare species. The α-diversity measures (Supplementary Fig. S2) were significantly higher in Unmanaged stand only in wood (observed, Chao1, and Shannon's indexes) and crown (Simpson's index) compartments. The β-diversity measure highlighted a significant effect of management in all analyzed sample types as evident from the statistically significant divergence between sample clustering of the two forest stands (Fig. 1).

Table 2
Soil chemical parameters in the managed and unmanaged stands of Adamello-Brenta park. *t*-Test *P*-values are reported in the table (ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). <LQ = below the limit of quantification.

Parameter	Unit	Managed	Unmanaged	*P*-value
TOC	%	17.9 ± 0.7	43.4 ± 2.4	***
TN	g kg^{-1} DW	13.3 ± 0.6	21.2 ± 0.8	***
Ca	g kg^{-1} DW	23.9 ± 2.6	5.5 ± 0.6	***
K	g kg^{-1} DW	1.24 ± 0.63	0.70 ± 0.22	ns
Mg	g kg^{-1} DW	6.3 ± 0.4	0.99 ± 0.50	***
Fe	g kg^{-1} DW	14.3 ± 1.4	2.95 ± 1.12	***
Mn	mg kg^{-1} DW	660 ± 58	58 ± 21	***
Zn	mg kg^{-1} DW	94 ± 12	77 ± 8	*
Na	mg kg^{-1} DW	75 ± 10	61 ± 9	ns
Cr	mg kg^{-1} DW	19.3 ± 5.1	<LQ	–
Cu	mg kg^{-1} DW	15.6 ± 1.6	11.6 ± 1.6	**
Available Ca	g kg^{-1} DW	4.9 ± 0.3	2.68 ± 0.23	***
Available K	g kg^{-1} DW	0.27 ± 0.04	0.23 ± 0.12	ns
Available Mg	g kg^{-1} DW	1.07 ± 0.11	0.53 ± 0.17	***
Available P	mg kg^{-1} DW	38 ± 10	43 ± 3	ns

The compositional bar plots (Fig. 2) highlight the relative abundances of the 10 most represented genera in soil and plant tissues that correspond to the 46 and 31 % the ASV assigned at genus level, respectively (Supplementary file F1). Fungi belonging to the genus *Elaphomyces* were more represented in the soil of the U study area while M soil samples were enriched in the genus *Oidiodendron*. Indeed, *Elaphomyces* was the dominant taxon in 3 of 5 soil samples of the U site while *Oidiodendron* and *Cortinarius* were both the dominant taxa in 2 of 5 soil samples in the M site. Considering the fungal orders, *Atheliales* and *Eurotiales* were statistically more abundant in soil samples of U study area (Table 3) while, on the contrary, samples from the M forest stand were more enriched in *Hypocreales* and *Sebacinales*. Moving to the endophytic fungal genus bar plots, the greater differences were found in *Coccomyces*, *Dermea*, *Lachnum*, *Mycena*, *Phialocephala*, and *Sydowia*. Considering only the 12 most abundant fungal orders, the taxa significantly enriched in the U site were *Helotiales* and *Xylariales* in crown samples, *Capnodiales*, *Lecanorales*, and *Pleosporales* in wood samples, *Rhytismatales* and *Venturiales* in root samples. On the contrary, considering the M site the significantly more abundant orders were *Phaeomoniellales* in crown samples, *Helotiales* in wood samples, *Hymenochaetales*, *Pleosporales*, and *Sebacinales* in root samples. The LDA Effect Size analysis (Fig. 3) showed other taxa significantly differentially enriched in a specific forest compartment not reported in the analysis of the most represented orders. As example, the analysis highlighted an enrichment in the genus *Alatospora* in root samples of M site, in *Phaeomoniella* genus and *Botryosphaeriaceae* and *Teratosphaeriaceae* families in wood samples of U site, in *Brunnipila* and *Cryptodiscus* genus in crown samples of U site. Considering the core mycobiome analysis, the following taxa were reported as the most shared among plant tissues of the U stand: putative *Dermea piceina* (i.e., *Dermea* sp. if considering that ITS region provides insufficient resolution for species level taxonomic assignment, Nilsson et al., 2019), *Cladophialophora* sp., and other two ASVs taxonomically assigned to beyond the order or phylum level, respectively. On the contrary, for the M study area the analysis of core mycobiome revealed

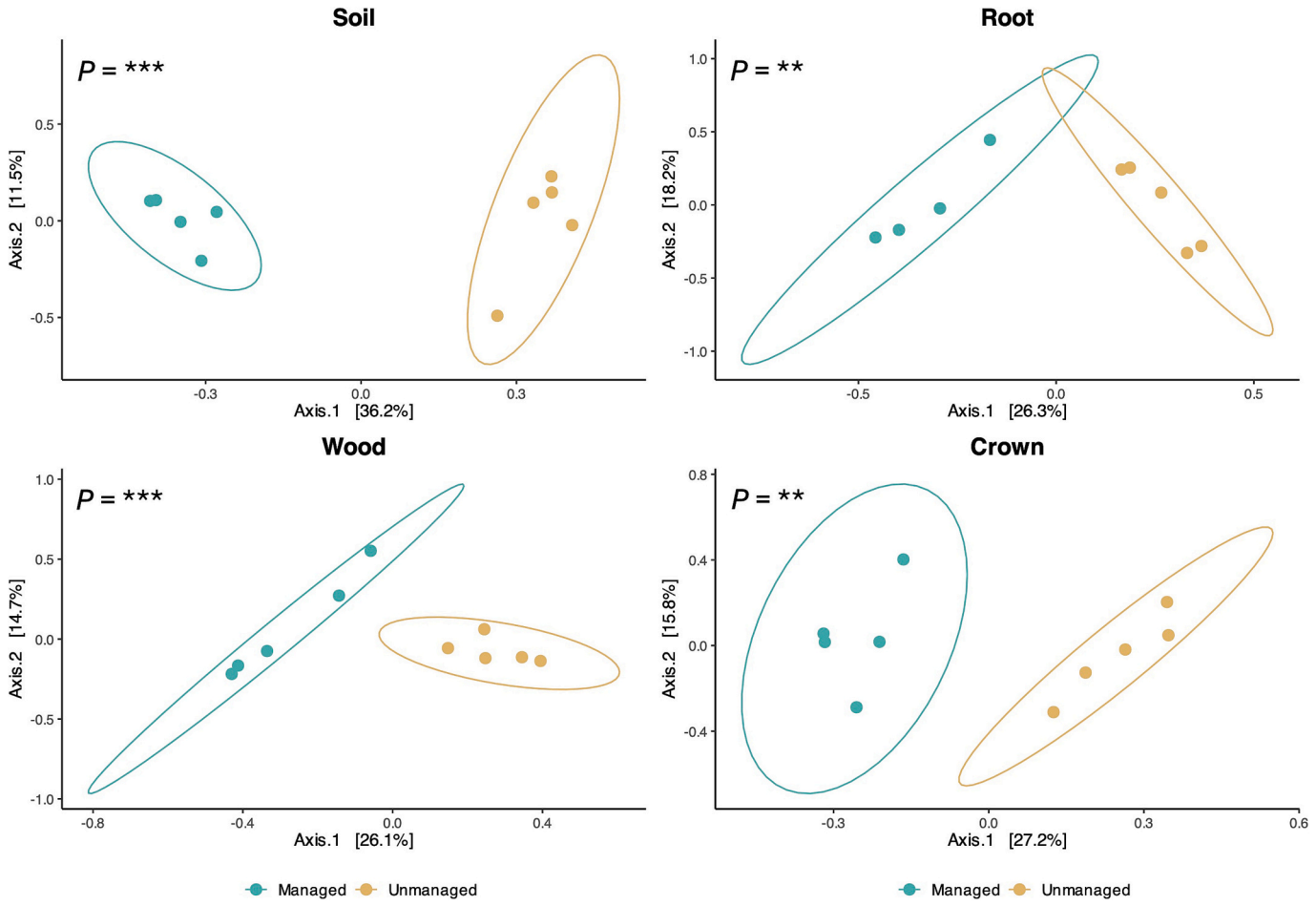

Fig. 1. Bray Curtis β-diversity for fungi in soil, root, wood, and crown of managed and unmanaged stands of Adamello-Brenta park. PERMANOVA *P*-values considering the effect of management are reported in each panel (**, $P < 0.01$; ***, $P < 0.001$).

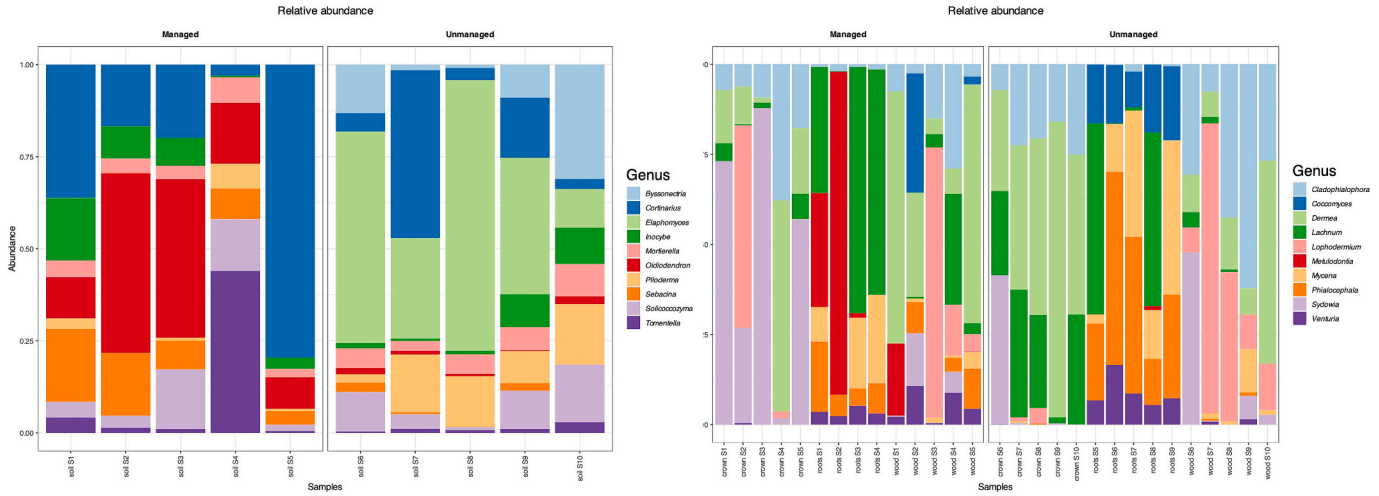

Fig. 2. Fungal genus bar plots for soil (left panel) and root, wood, and crown (right panel) in managed and unmanaged stands of Adamello-Brenta park. Bars represent relative abundance of 46 and 31 % of the ASV assigned at genus level (Supplementary file F1).

the family *Cladosporiaceae* and an ASV assigned beyond the phylum level as the most representative. A comprehensive overview of the main differences found in soil is visually reported by the differential heat tree analysis (Fig. 4) where the phylogenetic branches more enriched in U or M stands are reported in different colors. Specifically, a differential heat tree is a visual representation of taxonomic data that highlights differences in abundance or presence of taxa between sample groups. Regarding the fungal soil functionality associated to the different taxa, the relative abundances of each fungal guilds are reported in Fig. 5. The M study area was found to be enriched in soil in fungal guilds with pathotrophic function, animal pathogens, lichen parasite, and epiphyte. The roots had higher relative abundance of endophyte and plant

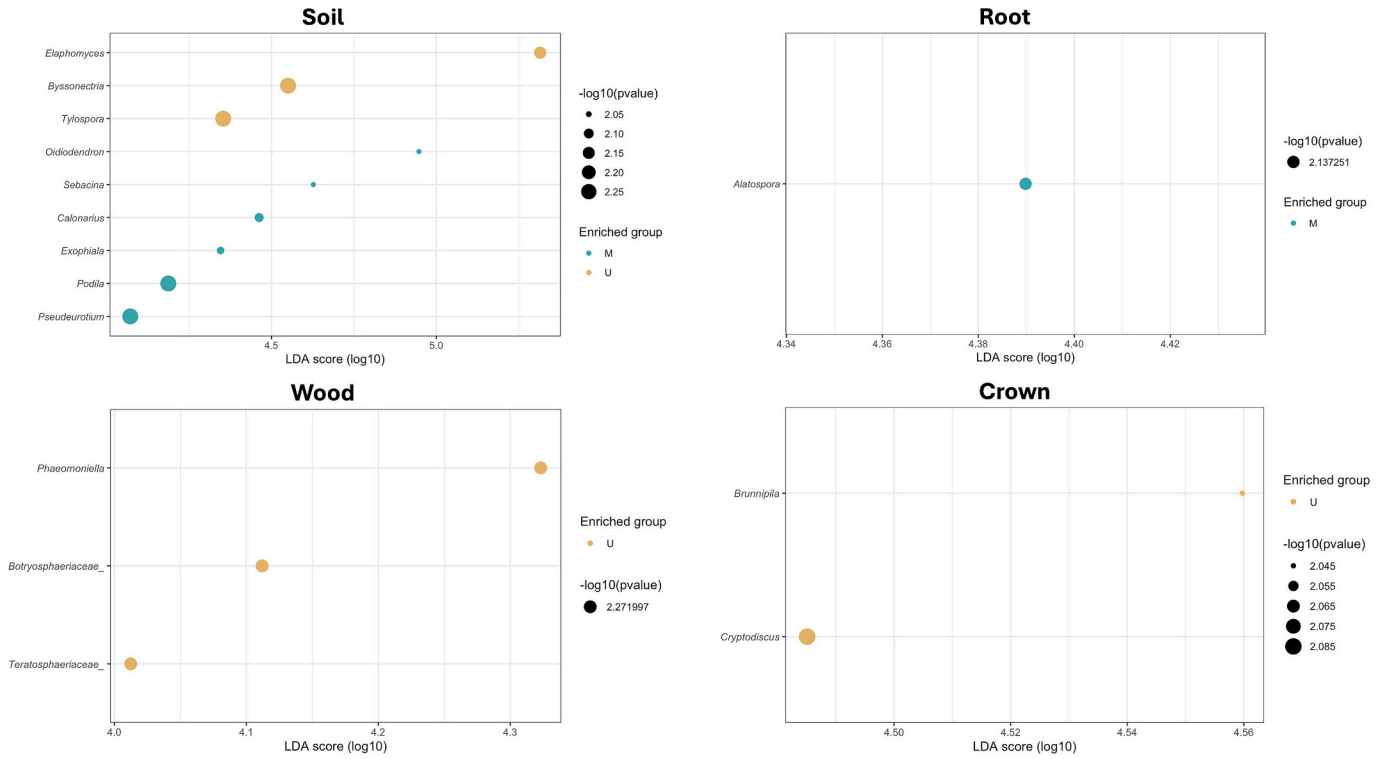

Fig. 3. LDA Effect Size analysis of fungal ASVs in managed (M) and unmanaged (U) stands of Adamello-Brenta park.

pathogen fungal guilds in the U study area. The wood was enriched in symbiotrophic and lichenized guilds in U parcel while in dung saprotrophic in M parcel. Crown samples had more abundant animal pathogen guilds in the M study area.

3.4. Soil and endophytic bacteria

Management and sample type were also both significant in determining the biodiversity of microbiome as highlighted by PERMANOVA analysis (Supplementary Table S4). The α -diversity indexes (Supplementary Fig. S3) did not highlight any significant difference in both soil and plant tissues of M and U parcels. The β -diversity analysis (Fig. 6) highlighted a significant divergence due to the management between M and U stands for all the analyzed sample types as highlighted by PERMANOVA results, even if a lower clustering was found in crown and wood compartments.

The compositional bar plots (Fig. 7) report the relative abundances of the 10 most represented genus in soil and plant tissues that correspond to the 12 and 39 % of the ASV assigned at genus level, respectively (Supplementary file F1). It is evident as some bacterial genera were more represented in the U parcel such as *Acidothermus*, *Bryobacter*, *Candidatus_Solibacter*, *Acidibacter*, and *Puia* while *Candidatus_Udaeobacter*, *Candidatus_Xiphinematobacter*, *Gaiella*, and *Mycobacterium* were more abundant in soil samples of M forest stand. Regarding the dominant taxon, *Vicinamibacterales* and *Acidobacteriae_Subgroup_2* were prevailing in all soil samples of the M and U forest stand, respectively. Considering the bacterial orders, *Acidobacteriales*, *Bryobacterales*, *Frankiales*, *Pedosphaerales*, *Puia*, *Acidobacteriae_Subgroup 2*, *WD2101*, and *WD260* were statistically more abundant in soil samples of U forest stand (Table 4) while, on the contrary, soil samples of the M site were more enriched in *Burkholderiales*, *Chitinopagales*, *Chthoniobacterales*, *Gaiellales*, *Rhizobiales*, *Vicinamibacterales*. Regarding the endophytic genera, the compositional bar plots (Fig. 7) highlight the greater differences in root samples, particularly *Rhodomicrobium* in samples of M stand and *Acidobacter* and *Acidothermus* in samples of U parcel. Moreover, the genus *1174-901-12* of *Beijerinckiaceae* was more represented in crown samples of the U study area. Considering the 12 most abundant orders (Table 3), the taxa significantly more enriched in the U site were *Caulobacterales* and *Rhizobiales* in crown samples, *Acidobacteriales*, *Frankiales*, and *WD260* in root samples. On the contrary, considering the M site the significantly more abundant orders were *Chitinophagales* in crown samples, *Hymenochaetales*, *Corynebacteriales*, *Micromonosporales*, *Rhizobiales*, *Streptomycetales*, and *Xanthomonadales* in root samples. No significant difference was found between wood samples considering the 12 most abundant orders. The LDA Effect Size analysis (Fig. 8) highlighted also other taxa differentially enriched in soil or root samples of specific forest compartments such as in soil *Puia*, *Roseiarcus*, and *Granulicella* genera and *Micropepsacaeae* and *Acetobacteracea* families in the U parcel while *Gaiella*, *Pedomicrobium*, *TRA3-20*, *Mycobacterium*, *KD4-96*, *MB-A2-108*, and *Flavobacterium* genera and *Microscillaceae* and *Saprospiraceae* families in the M parcel. In root, the LDA Effect Size analysis revealed an enrichment in *Acidothermus*, *Acidibacter*, *Puia*, *Bryobacter*, and *Granulicella* genera and *Caulobacteraceae* family in the U study area while *Rhodomicrobium*, *Streptomyces*, *Cryptosporangium*, *Frankia*, *Kineosporia*, *Pedomicrobium*, *Dongia*, and *Rhodoplanes* genera and *Micromonosporaceae* family were more abundant in the M study area. The core microbiome analysis reported some taxa as the most shared among plant tissue samples i.e., *Cutibacterium*, *Sphingomonas*, *Methylobacterium-Methylorubrum*, *Jatrophihabitans*, *WD260*, *Staphylococcus*, and *Terriglobus* for the U parcel while *Rhizobium*, *Cutibacterium*, *1174-901-12*, *Staphylococcus*, and *Bacillus* for the M parcel.

The differential heat tree analysis of soil samples (Fig. 9) showed that bacteria belonging to the same families were split in different genus branches and some phyla were more representative of U or M study area (e.g., *Patescibacteria* for U forest stand or *Bacteroidota*, *Chloroflexi*, and *Actinobacteriota* for M forest stand).

The analysis of bacterial functionalities showed some differences in soil and root samples of U and M study areas while any difference was found in wood and crown functionalities (Fig. 10). In root samples, the more abundant functionalities related to the M study area were mostly related to metabolic activities while those related to the U study area were mostly involved in bacterial communication and environmental

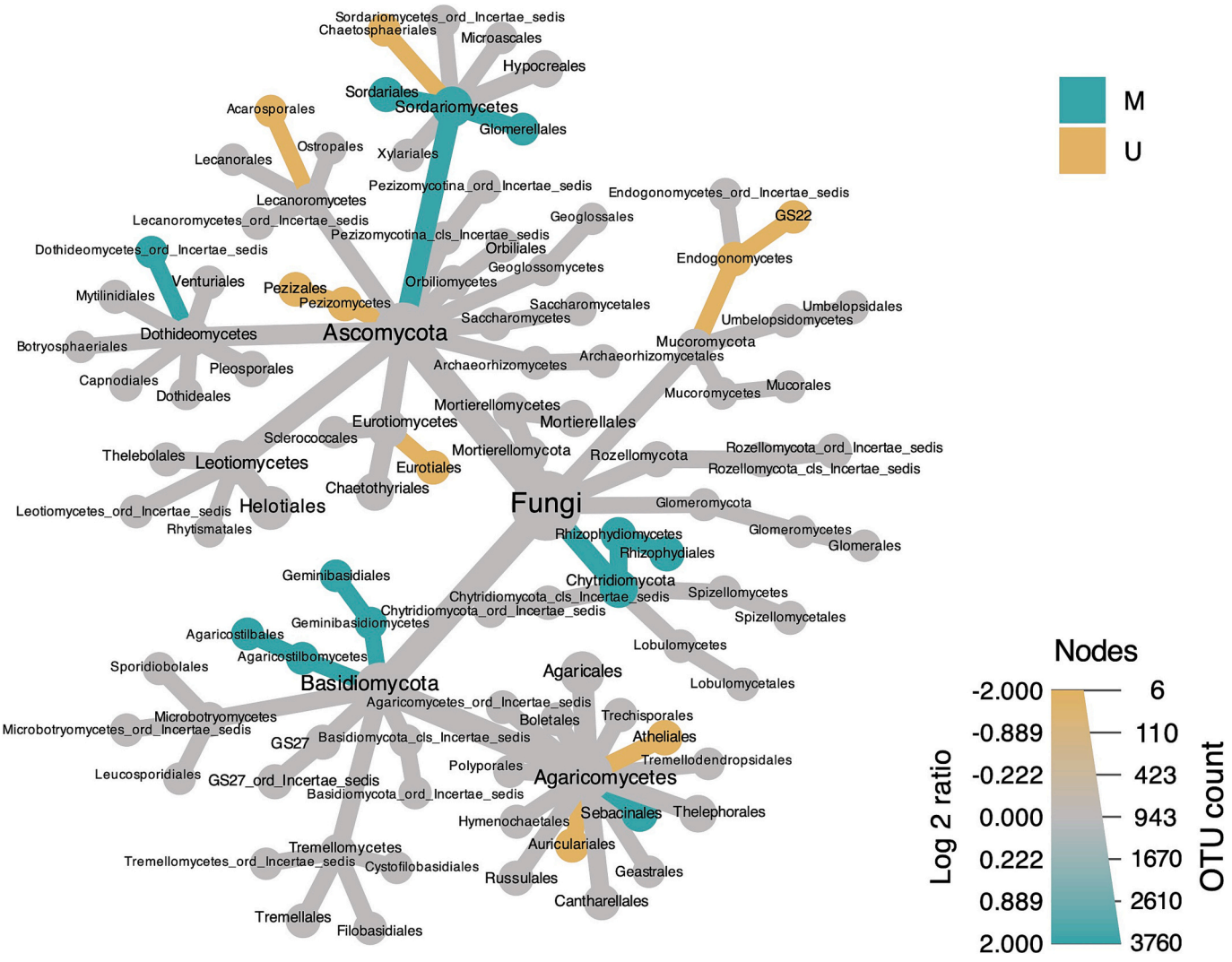

Fig. 4. Differential heat tree analysis of fungal orders in soil samples from managed (M) and unmanaged (U) forest stands in the Adamello-Brenta Park ($P < 0.05$). The thickness/diameter of branches and nodes is proportional to the abundance of ASVs assigned to each taxonomic group (OTU counts). Gray-colored branches indicate taxonomic groups with no statistically significant differences in relative abundance between M and U stands, based on the $\log_2$ ratio of their frequencies (Wilcoxon rank-sum test, with multiple testing correction using the false discovery rate [FDR], Benjamini–Hochberg method).

interactions. Considering the differences among plant tissues in the same forest management, any significant difference was found among endophyte's functionalities of the M study area while several functionalities were found to be predominant in a specific tissue in the U site (Supplementary Fig. S4).

4. Discussion

In the last decade, research has focused on the relationship between forest stand structure and biodiversity, in terms of taxonomic diversity and, more recently, functional diversity (e.g., Terhonen et al., 2019). Efforts are particularly aimed at understanding the effect of forest management and disturbances on ecological resilience and microbial biodiversity in soil (e.g., Bowd et al., 2022; Choma et al., 2021; Kim et al., 2021; Klavina et al., 2022), since a lively debate is underway on the importance of incrementing the percentage of old-growth forest area at European level (Gilhen-Baker et al., 2022; see also EC, 2020). In montane old-growth forests, Zeng et al. (2023) highlighted, as an example, the functional power of soil fungal communities, particularly regarding the organic matter decomposition. However, less attention has been paid to the alterations caused in both soil and plant environments and the relation among different plant compartments (i.e., crown, wood, root) despite a relation between thinning and phyllosphere microbiota has been already found in temperate forests (Liu et al., 2024). Our analysis of mycobiome and microbiome diversity in mixed spruce-silver fir forests confirmed the effect of management in microorganism relative abundance and taxa composition. Multi-layered vegetation, deadwood abundance and distribution, as well as disturbance history are known to determine the variability in forest spatial structure, influencing stand-level spatial heterogeneity of soil chemistry and microbial biomass (Baldrian, 2017). In our study, this effect was particularly evident in soil but also in root compartment, particularly for fungal taxa, even if wood and crown compartments also showed peculiar features as reported as follows. Indeed, it is well known that disturbances may have different effects on bacterial and fungal taxa. As example, logging-associated soil compaction have different effects on soil microbiota, both in terms of kingdom abundance, i.e., decrease in cyanobacteria and N-fixers while increase in fungi (Adelizzi et al., 2022), and taxa composition, i.e., increase in anaerobic bacteria and saprobic and parasitic fungi while decrease in ectomycorrhizal species (Hartmann et al., 2014). Moreover, thinning has also showed a different effect on phyllosphere microbiota highlighting a higher diversity and co-occurrence networks in endophytic fungi while a lower co-occurrence networks in endophytic bacteria (Liu et al., 2024).

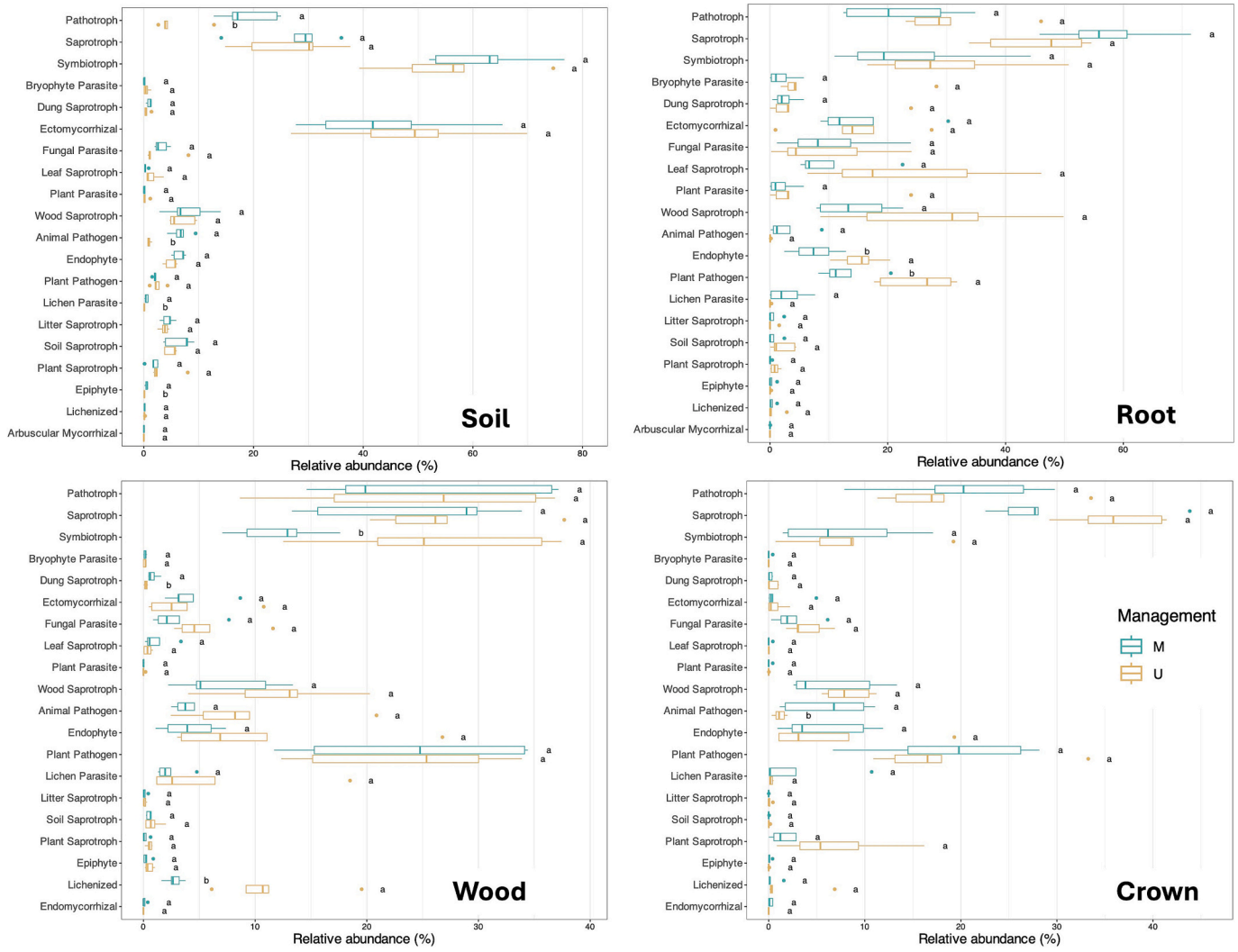

Fig. 5. Mycobiome functional guild annotation in soil, root, wood, and crown of managed (M) and unmanaged (U) stands of Adamello-Brenta park. Pairwise comparisons between M and U stands are reported in the figures for every guild (different letters indicate significant differences).

Table 3
Fungal orders differentially abundant in managed or unmanaged forest stands of the Adamello-Brenta Park. Only fungal orders that showed statistically significant differences and were among the 12 most abundant taxa in each sample type are reported. (*t*-Test *P*-values are reported in the table, *, $P < 0.05$; **, $P < 0.01$.)

Order	Type	More abundant	*P*-value
Atheliales	Soil	Unmanaged	**
Eurotiales	Soil	Unmanaged	**
Hypocreales	Soil	Managed	*
Sebacinales	Soil	Managed	**
Helotiales	Crown	Unmanaged	**
Phaeomoniellales	Crown	Managed	**
Xylariales	Crown	Unmanaged	*
Capnodiales	Wood	Unmanaged	**
Helotiales	Wood	Managed	*
Lecanorales	Wood	Unmanaged	*
Pleosporales	Wood	Unmanaged	**
Auriculariales	Root	Unmanaged	**
Hymenochaetales	Root	Managed	*
Rhytismatales	Root	Unmanaged	*
Pleosporales	Root	Managed	*
Sebacinales	Root	Managed	*
Venturiales	Root	Unmanaged	*

4.1. Soil and endophytic fungi

The analysis revealed several differently abundant fungal taxa in soil, root, wood, and crown compartments. Soil mycobiome is crucial for forest ecosystem particularly regarding mycorrhizal networks, improving nutrient uptake and signaling among plants in turn enhancing tree growth and biogeochemical cycles (Li et al., 2023). The U stand had a higher percentage of snags and dead trees as well as TOC and TN, all key aspects in driving the fungal soil diversity, particularly after disturbances (Mayer et al., 2022). Despite this finding, the soil α-diversity was not significantly different between the study areas possibly due to the generally low nutrient availability in conifer forest soil characterized by recalcitrant litter and high C/N ratio (i.e., 13 and 20 in M and U stands, respectively) that may not favor the fungal diversity (Xie and Yin, 2022). However, the statistically more abundant orders *Atheliales* and *Eurotiales* found in soil of the U stand have been also found among the 20 most common OTUs in Norway spruce log samples (Ottosson et al., 2015) suggesting an effect of the higher percentage of deadwood in the U stand. On the other hand, ASVs belonging to the order *Sebacinales* were statistically more abundant in soil of M stand as well as in root samples; *Sebacinales* are involved in highly diverse interactions with plants acting as root endophytes with also saprotrophic abilities, as well as obligate biotrophs forming mycorrhizal associations that can improve mineral nutrient acquisition and provide other positive

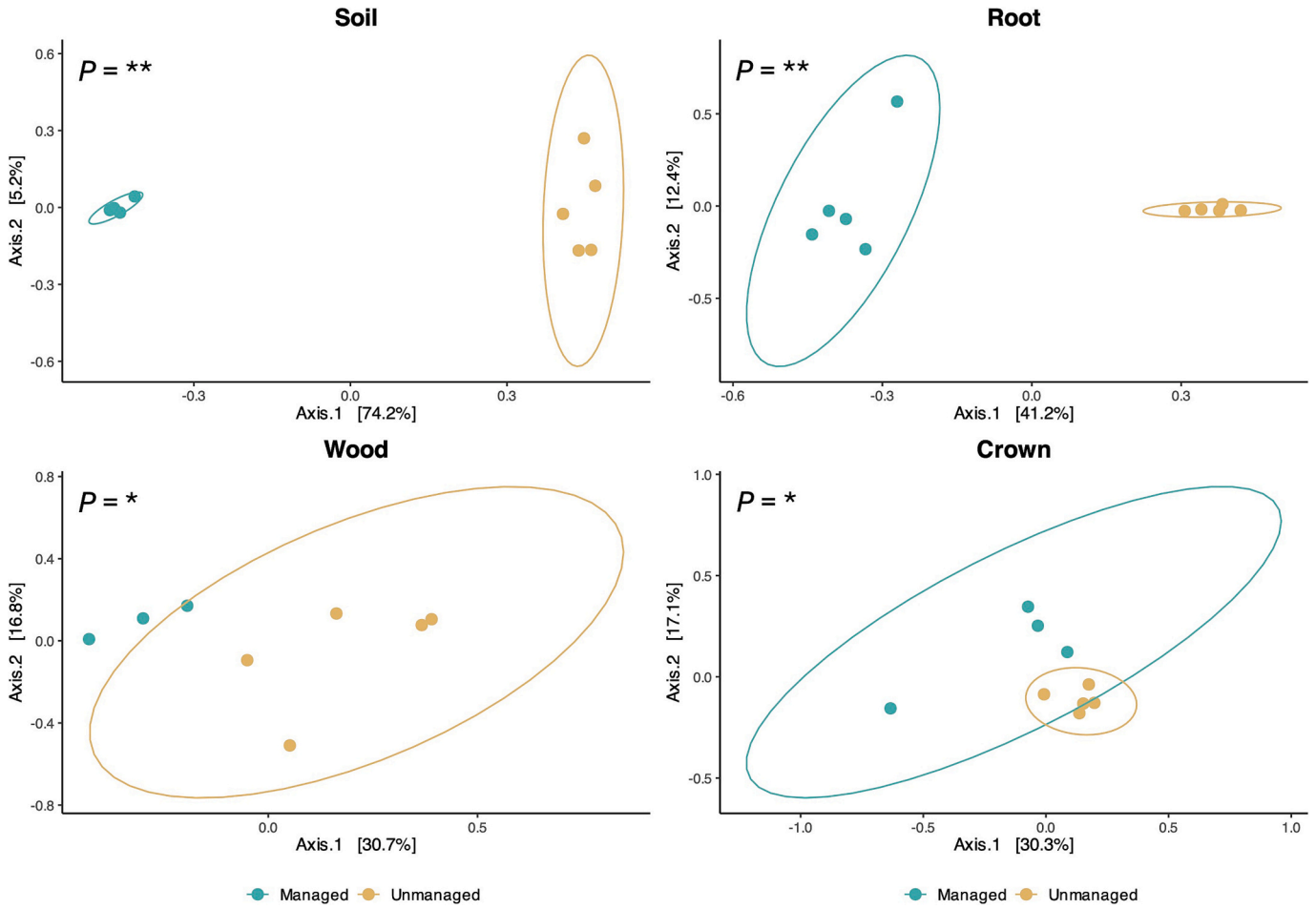

Fig. 6. Bray Curtis β-diversity for bacteria in soil, root, wood, and crown of managed and unmanaged stands of Adamello-Brenta park. PERMANOVA *P*-values considering the effect of management are reported in each panel (*, $P < 0.05$; **, $P < 0.01$).

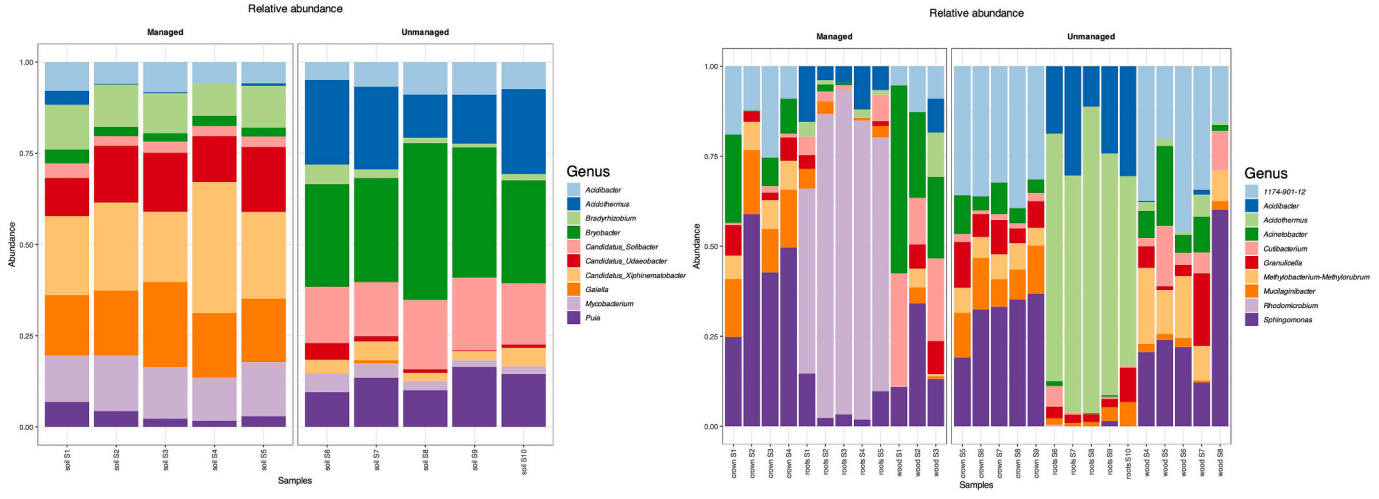

Fig. 7. Bacterial genus bar plots for soil (left panel) and root, wood, and crown (right panel) in managed and unmanaged stands of Adamello-Brenta park. Bars represent relative abundance of 12 and 39 % of the ASV assigned at genus level (Supplementary file F1).

activities (Weiß et al., 2016). The LDA Effect Size analysis showed that *Elaphomyces* was significantly more abundant in U soil. The genus *Elaphomyces* (also known as deer truffles) is one of the most important ectomycorrhizal fungal genera in temperate forest ecosystems (Paz et al., 2017). This genus, especially abundant in acidic soils, produces hypogeous ascomata, that, for their size, constitute a considerable fruiting biomass in the soils of mountain old-growth coniferous forests, in accordance with our results, that see this genus as more abundant in the less perturbed and acid soil of U stand. There was another ectomycorrhizal genus as indicator of U stands soil, *Tylospora*, a basidiomycete often associated with spruce (Eberhardt et al., 1999). In the M stand soil, other mycorrhizal genera were found by the LDA Effect Size analysis:

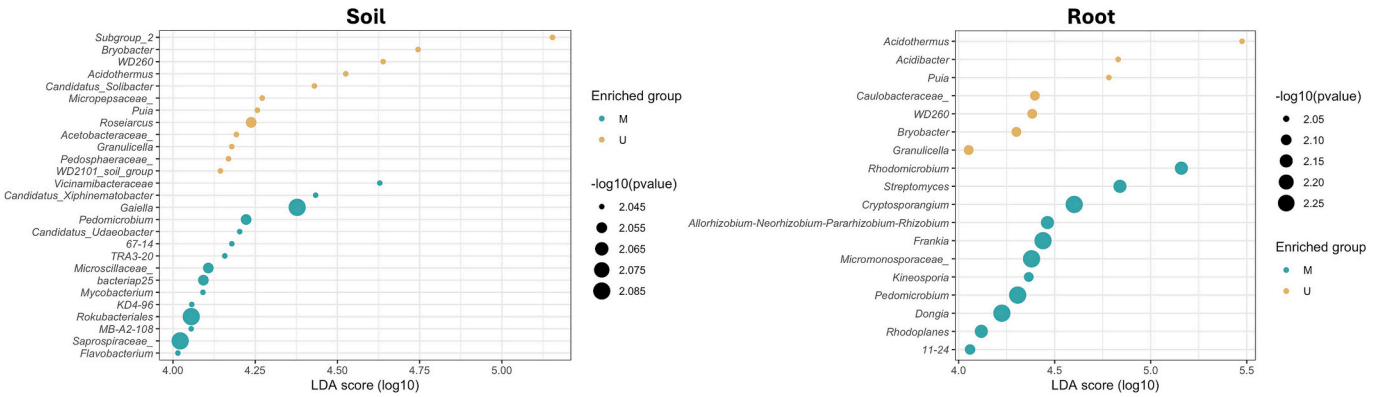

Fig. 8. LDA Effect Size analysis of bacterial ASVs in managed (M) and unmanaged (U) stands of Adamello-Brenta park (no ASVs were found for crown and wood compartments).

Table 4
Bacterial orders differentially abundant in managed or unmanaged forest stands of the Adamello-Brenta Park. Only bacterial orders that showed statistically significant differences and were among the 12 most abundant taxa in each sample type are reported. (*t*-Test *P*-values are reported in the table, *, $P < 0.05$; **, $P < 0.01$.)

Order	Type	More abundant	*P*-value
Acidobacteriales	Soil	Unmanaged	**
Bryobacterales	Soil	Unmanaged	**
Burkholderiales	Soil	Managed	**
Chthoniobacterales	Soil	Managed	**
Frankiales	Soil	Unmanaged	**
Gaiellales	Soil	Managed	**
Pedosphaerales	Soil	Unmanaged	**
Rhizobiales	Soil	Managed	**
Acidobacteriae_Subgroup_2	Soil	Unmanaged	**
Vicinamibacterales	Soil	Managed	**
WD260	Soil	Unmanaged	**
Caulobacterales	Crown	Unmanaged	*
Chitinophagales	Crown	Managed	*
Rhizobiales	Crown	Unmanaged	*
Acidobacteriales	Root	Unmanaged	*
Corynebacteriales	Root	Managed	*
Frankiales	Root	Unmanaged	**
Micromonosporales	Root	Managed	**
Rhizobiales	Root	Managed	**
Streptomycetales	Root	Managed	**
WD260	Root	Unmanaged	*
Xanthomonadales	Root	Managed	*

Oidiodendron, an ascomycetous ericoid mycorrhiza (Wei et al., 2016); *Sebacina*, a basidiomycete that can form a broad spectrum of mycorrhizal types (Ray and Craven, 2016) as already reported above at order level, and *Calonarius*, also a basidiomycete forming ectomycorrhizal associations with members of the *Pinaceae* family, including the *Picea* genus (Liimatainen et al., 2022). Other genera found associated with M soils included *Exophiala*, a black ascomycetuous yeast that, a part from its role as a pathogen in animals and humans, can also live saprophytically in bulk soil and rhizosphere (Maciá-Vicente et al., 2016); and *Podila*, a member of the family *Mortierellaceae*, generally isolated from forest soils (Telagathoti et al., 2022), playing a key role in the microbiota of alpine and subalpine habitats (Telagathoti et al., 2021).

Regarding the endophytes, the higher diversity in fungal taxa was found in wood samples, as already reported in Norway spruce by Marčiulynas et al. (2022) and Durodola et al. (2023). Indeed, in woody plants the stem had a richer fungal endophyte assemblage than the leaves (Harrison and Griffin, 2020). Among the most represented taxa, the endophytes significantly enriched in the U stands were the genus *Phaemoniella*, especially known for its most famous member *P. chlamidospora*, one of the causal agents of the esca disease in grapevine, but which also hosts other endophyte species living in plants of the *Pinaceae* family (Sanz-Ros et al., 2015; Alonso et al., 2011); *Botryosphaeriaceae*, an ascomycete family of sac fungi, containing 26 genera, among which there are notable plant pathogens (Kirk et al., 2008) and showing interesting endophytic to saprotrophic/pathogenic lifestyle in response to plant stress (Slippers and Wingfield, 2007); a further family, *Teratosphaeriaceae*, belonging to the *Mycosphaerellales* order, counting numerous genera, including many pathogens causing leaf diseases and stem cankers (Pérez et al., 2009; Crous et al., 2009). As indicator of the crown endophytes of the U stand the genus *Cryptodiscus* was found. Most *Cryptodiscus* species are saprotrophs, except for some that are lichenicolous (Pino-Bodas et al., 2017), and some species were isolated previously from decorticated branches of *Picea abies* (Fernández-Brime et al., 2018).

Despite the different ASV composition, fungal guilds showed similar functionalities between M and U forests, suggesting an ecological redundancy as also reported by Zeng et al. (2023) comparing soil communities in old-growth montane forests. This could be due to the evolutionary phase of the U stand following the cessation of treatments, where the signal of the past silvicultural treatments is still observable. Indeed, even if we can observe an amount of deadwood that is typical of a stand in transition to an Alpine old-growth forest (Sitzia et al., 2012; Motta et al., 2015), the tree layer arrangement of the forest is still near to an even-aged vertical structure (caused by the relatively recent last cutting intervention, i.e., about 40 years ago). This could result in a lower microhabitat heterogeneity respect to old-growth forests (e.g., Šamonil et al., 2010), which also show greater variability of nutrients distribution. Moreover, Zeng et al. (2023) also suggested that the ecological functional redundancy, in presence of different taxa, is related to a process of niche adaptation that allows different taxa to evolve similar functions, including genes related to the organic matter degradation. Interestingly, among others the M stand had more fungal guilds with pathotrophic functions, including animal pathogens and lichen parasite in soil, while the U study area had more plant pathogen guilds in roots. This suggests a higher presence of potential "negative functions" guilds in plant tissues of U stand and in soil of M stand. Indeed, some potential plant pathogenic taxa were found more abundant in the wood of plants of U stand, such as those belonging to the already described *Teratosphaeriaceae* and *Botryosphaeriaceae* families, the latter also specifically identified as Norway spruce pathogen by Aiello et al. (2023), or the putative core microbiome species *Dermea piceina*, found as a conifer pathogenic species by Smerlis (1969). In agreement, some taxa with plant pathogenic functions were found in soil of M study area, such as the genus *Oidiodendron*, which is being investigated as a possible agent of spruce trunk canker (Shabunin et al., 2024). The increase in parasitic fungi was also found in forest soils following disturbances related to logging-associated compaction (Hartmann et al., 2014).

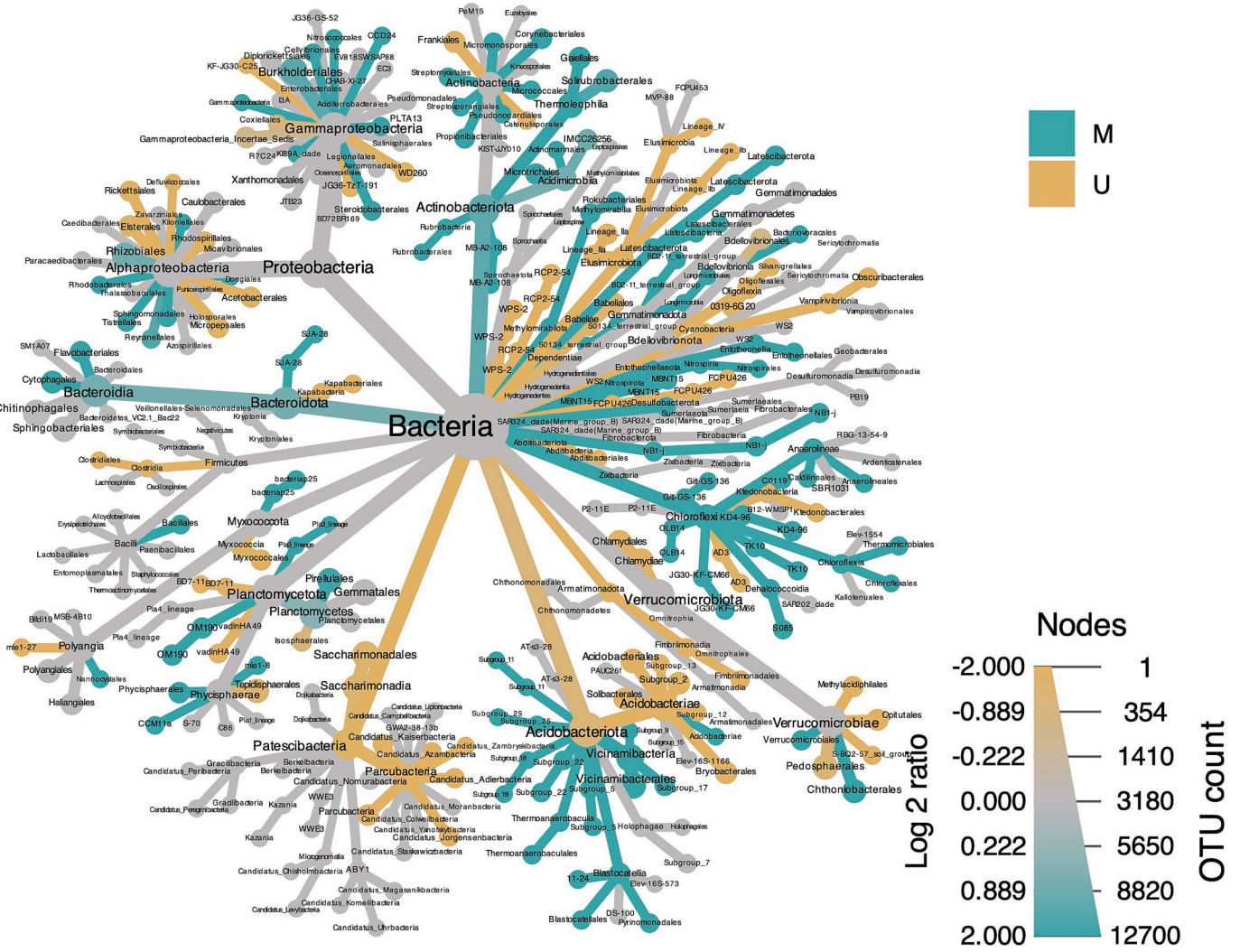

Fig. 9. Differential heat tree analysis of bacterial orders in soil samples of managed (M) and unmanaged (U) soil stands of Adamello-Brenta park ($p < 0.05$). Thickness/diameter of branches/nodes are proportion to the abundance of ASV belonging to that taxonomic group (OUT count); the gray color indicates that no statistically significant differences resulted from the comparison of log2 ratio of taxonomic data of M vs. U forest stand (Wilcoxon rank-sum test, correct for multiple testing using FDR, Benjamini-Hochberg).

4.2. Soil and endophytic bacteria

The most significant differences in bacterial taxa were found in the soil and root compartments, both in terms of diversity (number of different of taxa) and functions. The order *Acidobacteriales*, which includes bacteria able to stimulate plant growth recovery (Kielak et al., 2016), was more abundant in soil samples from the U study area; this result is consistent with a comparative study on disturbed and undisturbed boreal forests, where permafrost thaw was considered as disturbance (Seitz et al., 2022). Notably, a higher presence of *Acidobacteriales* was also observed in root samples of U stands. The higher presence of this taxa in the U stand was probably also driven by the different soil pH levels found in the two study areas: acidophilic bacterial groups as the *Acidobacteriales* were indeed more abundant at low pH as also observed in a *Picea abies* plantation with a different pH gradient (Cruz-Paredes et al., 2021). Moreover, the order *Frankiales* was also more abundant in U stand in both soil and root compartments in agreement with the presence of this taxon in forests enriched in fallen, decaying trees and its ability to form nitrogen-fixing nodules also in wood substrate of conifer stands (Li et al., 1997). The genus *Frankia* has also a well-recognized role in plant growth promotion displaying genes encoding hormones, siderophores, nitrogenases, and other proteins involved in environmental stress responses (Nouioui et al., 2019). On the contrary, the M stand was enriched in both soil and root compartments in bacteria belonging to the nitrogen-fixing orders of *Rhizobiales* and *Burkholderiales* (Walker et al., 2015). Besides their ability to symbiotic nitrogen fixation in legumes, rhizobia also exhibit a range of plant growth-promoting traits, including auxin production, phosphate solubilization, and siderophore release, having potential benefits even in non-legume cropping systems (Jaiswal et al., 2021). Similarly, *Burkholderia* has been demonstrated to have also plant growth promotion abilities boosting nutrient availability, acting against plant pathogens and improving abiotic stress resistance (Pal et al., 2022).

Our analysis did not highlight any difference in the microbiota of the wood compartment of the two sites, while only a few taxa were identified as more abundant in crown compartment of a specific study area. Specifically, *Caulobacterales* and *Rhizobiales* were more abundant in U stand while *Chitinophagales* in M stand. Interestingly *Rhizobiales* seemed to prefer a different plant compartment in U stand compared to the M stand where they were found in roots. Even if this bacterial family is usually found in soil and roots, some authors have already reported its presence in conifer phyllosphere, but its function in this plant compartment has yet to be clarified (Addison et al., 2023). Instead, any information is available regarding the role of *Caulobacterales* and

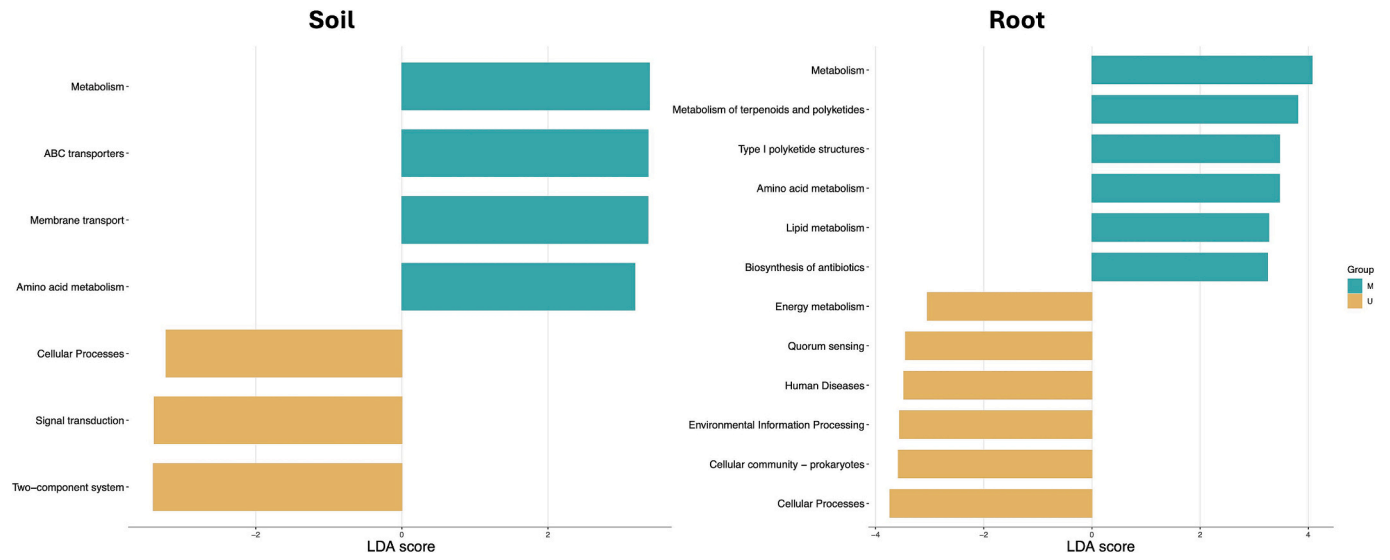

Fig. 10. LDA Effect Size analysis of bacterial functionalities found differentially represented in soil and root compartments in response to management: (M) managed stand and (U) unmanaged stand of Adamello-Brenta park (no statistically significant differences were found in wood and crown samples).

Chitinophagales in forest ecosystems; thus, it might be interesting to assess their role in crown and if they might be possible proxies of tree physiological conditions.

Regarding the bacterial activities, some site-specific peculiar functions were identified only in soil and roots while any difference was found in wood and crown compartments. This result is not surprising considering the lower differences in taxa composition found in these compartments and the high functional redundancy in microbic systems that makes every function be encoded by different taxa (Louca et al., 2018). However, in soil and root samples the bacterial functions were found to be enriched in different metabolic pathways. Specifically, soil and root compartments of M stand were characterized by bacterial functions related to general or specific metabolism (i.e., lipid, amino-acidic, terpenoids, polyketides, as well as membrane and ABC transporters) while soil and root compartments of U stand were enriched in communication functions (i.e., two-component system, quorum sensing, cellular transduction, prokaryote community, environmental information processing). The decomposition of dead plant biomass by bacteria is the crucial process regulating C flow in soil systems influencing the ratio between C immobilization and mineralization electing microorganism as key actors of C cycling, particularly considering that forest comprise two-thirds of global C (Lladó et al., 2017). The forest management applied in the M stand favored these metabolic functions in bacteria suggesting a more efficient and effective C nutrient cycling. Bacterial communication signaling may help microorganisms in coordinating the extracellular enzyme production to achieve an efficient C and nutrient acquisition from litter (Strickland et al., 2013; McBride and Strickland, 2019). Therefore, the increase in bacterial signaling functions in the U stand may suggest the development of coordination process in the U stand characterized by a more recalcitrant litter and a lower availability of other nutrients than the M stand. This higher coordination among bacterial taxa in the U stand was also highlighted by the presence of statistically different functionalities among plant compartments, not found in trees of M study area.

5. Conclusions

Ecosystem-level processes are deeply influenced by disturbances of various intensity and duration (Baldrian, 2017). Among the anthropogenic ones, silvicultural operations, followed by forest stand recovery after disturbance, cannot be properly understood without considering different biotic components of the ecosystems, including the microbiota. Our study reveals the possibility to detect some specific taxa featuring the study areas and thus the different forest management. Further studies might be important to assess the presence of these microorganisms in similar conditions to confirm these taxa as biomarkers of anthropic disturbance. Indeed, the identification of specific targets might allow the use of high throughout and cheap techniques as on-site nanopore sequencing or ultrarapid mobile qPCR (Cambon et al., 2023). The identification of some specific taxa and metabolic functionalities in the soil-root compartment of M stand allowed to highlight a more favorable soil nutrient cycling of this stand compared to the U study area but also a higher presence of fungal guilds with pathotrophic function. Nonetheless, the development of signaling process in the soil-root compartment of U stand might suggest a higher coordination of microorganisms in this study area but the presence of potential pathogens in plant compartments.

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CRediT authorship contribution statement

Silvia Traversari: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Lidia Nicola:** Writing – original draft, Investigation, Data curation, Conceptualization. **Alessio Giovannelli:** Writing – review & editing, Funding acquisition, Data curation, Conceptualization. **Sara Barberini:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Giovanni Trentanovi:** Writing – original draft, Methodology, Formal analysis, Data curation. **Solveig Tosi:** Writing – review & editing, Funding acquisition, Conceptualization. **Maria Laura Traversi:** Writing – review & editing, Data curation. **Giovanni Emiliani:** Writing – original draft, Visualization, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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