

Short-term machinery impact on microbial activity and diversity in a compacted forest soil

Agnese Bellabarba^{a,b}, Laura Giagnoni^c, Alessandra Adessi^{a,*}, Elena Marra^d, Andrea Laschi^e, Francesco Neri^a, Giovanni Mastrolonardo^a

^a Department of Agriculture, Food, Environment and Forestry (DAGRI), University of Florence, I-50144, Florence, Italy

^b Genexpress Laboratory, Department of Agriculture, Food, Environment and Forestry (DAGRI), University of Florence, I-50019 Sesto Fiorentino, Italy

^c Department Civil, Environmental, Architectural Engineering and Mathematics (DICATAM), University of Brescia, I-25123 Brescia, Italy

^d Institute of Research on Terrestrial Ecosystems (IRET), National Research Council of Italy (CNR), 50019 Sesto Fiorentino, (FI), Italy

^e Department of Agricultural, Food and Forest Sciences (SAAF), University of Palermo, I-90128, Palermo, Italy

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ABSTRACT

Forest soils are complex ecosystems including a huge biodiversity including different microbial communities that are responsible for soil nutrient cycling and organic matter decomposition. One of the main threats to forest soil health is soil compaction caused by forest exploitation activities and, in particular, by wood extraction operations. These latter may change the physical, chemical and, in turn, biological properties of soil, eventually impacting tree growth and regeneration. However, there is a significant lack of knowledge in understanding the response of soil microbial communities to soil compaction. Furthermore, most of previous studies did not properly frame the short-term response of soil microbiota to compaction, which could serve as an early indicator of soil health. This study aims to investigate and monitor the short-term response of forest soil to severe compaction stress integrating soil physico-chemical analysis with biological and biochemical analysis. To investigate the early response of soil microbial communities to compaction over time, forest soil was analyzed 8 and 12 months after the repeated passage of a tractor pulling some logs simulating skidding operations in a heavily trafficked area for wood extraction. Despite the initial strong increase in bulk density (up to 42 %), the soil almost recovered from compaction after just one year. Most of the soil chemical parameters (i.e. pH, C, and N content) were not affected by soil compaction, while the analysis of enzymatic activity showed a change of some functions upon soil compaction in the first 8 months, followed by a substantial recovery after one year. The microbial communities showed different responses to compaction, highlighting a greater bacterial community resilience in the short term compared to the fungal community, which showed persistent and significant shifts between compacted and not-compacted soil. We also identified some indicator species that may be useful for monitoring early changes in microbial communities and activities following soil compaction.

1. Introduction

Soil is a fundamental component of forest ecosystems, playing a pivotal role in supporting forest health and providing essential ecosystem services (Vanermen et al., 2021). This complex ecosystem harbors an incredible diversity of microbial communities playing a crucial role in soil nutrient cycling and organic matter decomposition (Dominati et al., 2010; Hartmann et al., 2014; Nielsen and Ball, 2015), directly impacting on forest productivity (Lewandowski et al., 2019). One of the main threats to forest soils and their huge biodiversity is soil

compaction (Vanermen et al., 2021). Indeed, forest operations imply an unavoidable impact on forest soils, particularly during timber extraction (Picchio et al., 2020). Rutting, alteration to soil plasticity, and soil compaction are all possible negative effects of intensive logging activities having an adverse impact on soil (Cambì et al., 2015; Marra et al., 2021). The impact degree of logging operations on soil compaction depends on soil properties such as texture, water content, and initial bulk density (Cambì et al., 2015) as well as on the wood extraction system (Ampoorter et al., 2012), and work organization, e.g. machine load and weight and number of machine passes (Marra et al., 2018;

* Corresponding author.

E-mail address: alessandra.adessi@unifi.it (A. Adessi).

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Picchio et al., 2020).

Ground-based timber extraction systems can impact soil physical properties by decreasing porosity, increasing bulk density and soil penetration resistance (D'Acqui et al., 2020; Lee et al., 2020). The decrease in soil porosity, hydraulic conductivity, and water infiltration can significantly affect soil microbial communities (Hartmann et al., 2014; Jensen et al., 1996). Indeed, these changes of soil structure alter the habitat of microorganisms, for instance favouring a microbial community centered on species capable of withstanding anoxic conditions (Frey et al., 2009; Hartmann et al., 2014). Consequently, changes in the physical, chemical, and biological properties of soil can lead to changes in tree growth and regeneration, further influencing the structure and function of microbial community (Mariotti et al., 2020). However, there is still a significant lack of knowledge in understanding the response of soil microbial communities to soil compaction (Hartmann et al., 2014). Studies investigating how soil microorganisms respond to ground-based forest logging, through measurements of microbial biomass and activity or by using newer techniques like next-generation sequencing, report a range of effects. These effects vary from significant impacts to no change in the soil microbial communities (Busse et al., 2006; Frey et al., 2009; Hartmann and Niklaus, 2012; Hartmann et al., 2014; Jennings et al., 2012; Jordan et al., 2003; Mariani et al., 2006; Schnurr-Pütz et al., 2006; Tan et al., 2008; Wilhelm et al., 2017). However, most of these studies were conducted in harvested forest areas, where the impacts of logging operations cannot be fully separated from those caused by the reduction of canopy cover and biomass removal (Hartmann et al., 2014; Latterini et al., 2023).

Understanding the response of microbial communities to soil compaction is crucial for predicting the sustainability of forest management. This issue is particularly relevant in Europe, where there has been a gradual intensification in the use of vehicles that are more powerful and efficient, but also heavier, hence leading to greater impacts on soil (Horn et al., 2007; Vossbrink and Horn, 2004). Recognizing the importance of soil health, European Commission launched the EU Soil Strategy for 2030. Aligned with the Biodiversity Strategy for 2030 (COM/2020/380), this initiative, titled "A Soil Deal for Europe," aims to promote sustainable soil management in agriculture and forestry sectors, facilitating the transition towards healthy soils (Königer et al., 2022; Panagos et al., 2022). These policies, as well as a sustainable forest and soil management, should also rely on the assessment and monitoring of the influence of soil disturbance on the composition, functions and activity of soil microbial communities (Wilhelm et al., 2017), as the latter play a crucial role in key soil processes. Such an assessment can even provide an early warning signal of soil and forest health decline because of forest management practices, estimating these impacts before they become irreversible (Hartmann et al., 2014). Indeed, some studies highlighted the early impacts of several types of disturbance (such as logging or burning) on forest soil bacterial and fungal communities (Ammitzboll et al., 2021, 2022). However, several of the most recent studies, investigating the effect of different disturbances on forest microbial communities with high-throughput DNA sequencing, were carried out through long-term surveys that failed to capture the dynamics of these communities in the short-term.

This study aims to investigate the short-term response of forest soil to severe compaction stress by integrating soil physico-chemical with biological and biochemical analysis (microbial community composition, diversity, and enzyme activities), hypothesising the latter allowing to get an insights into the short-term response of soil to compaction, potentially serving as early indicators of soil health decline resulting from forest management practices. With this aim, a soil in Vallombrosa forest (Tuscany, Italy) was analyzed 8 and 12 months after a compaction event caused by repeated skidding, for monitoring the evolution of the early response of soil microbial communities to compaction.

2. Materials and methods

2.1. Study area

The study was conducted in central Italy, in the Vallombrosa Biogenetic Reserve (44°44'12.87" N, 11°32'45.73" E), which is in the municipality of Reggello (Florence Province). The study area was included in a forest parcel of 5.78 ha between 920 and 980 m a.s.l. and was characterized by moderate steep terrain (mean slope = 20 %). The climate is characterized by a mean annual air temperature of 11.7 °C and a mean annual precipitation of 1037 mm. The soil developed on sandstone material and was classified as Dystric Cambisol based on the World Reference Base for Soil Resources (Schad, 2016). The forest vegetation is an artificial and pure plantation of silver fir (*Abies alba* Mill.) 63 years old. The forest management in this area was applied since the XIV century by Vallombrosa's monks. The aim of their silviculture was to produce high quality timber to be used also for buildings, through pure silver fir stands with a rotation period of almost 100 years, applying thinning and a final clear cut, followed by the next artificial renovation (plantation) (Bottalico et al., 2014). No silvicultural or logging operation has been conducted in the study area, where no vehicle has had access for at least the last 40 years. The experimental design employed the use of a tractor New Holland T6050 equipped with a winch Pro Forst SWE 8500. Its unloaded mass was 6190 kg and was equipped with two 480 mm wide tires (480/65 R28 136D TL MULTIBIB) and two 600 mm wide tires (600/65 R38 153D TL MULTIBIB). The study simulated a real trees extraction operation by skidding, except that trees were not actually cut in the designated area. In fact, four logs of silver fir extracted from a different area in the same forest were used during skidding operations in the study area. On September 2020, the skidding extraction was carried out by the described tractor skidding the four logs. It began the skidding operations with a first passage, creating the temporary trail used for all subsequent passes, for a total of 30. The tractor moved uphill in the direction of the slope (20 %) pulling the logs creating the same disturbance caused by real skidding operations in a heavily trafficked area.

2.2. Soil sampling and analysis

The sampling scheme is reported in Fig. S1. Along the skid trail, one rectangular plot (length 8 m, width 2 m) was marked out on the ground. To determine soil compaction three intact cores of soil were collected after the compaction event within the rectangular plot, one sample each meter, inside both left and right wheel ruts (six samples in total), and six samples were collected between the wheel ruts. At the same time, to determine the bulk density (BD) of the undisturbed soil, i.e. not compacted, six intact cores of soil were collected 8 m away, outside the tractor trail in a contiguous undisturbed surface, hereafter termed as "off-trail". All soil samples were collected from the top 10 cm of the mineral soil layer using a steel cylinder (7.5 cm inner diameter and 10 cm height). The same sampling procedure was repeated eight months and one year after to determine the trend of physical soil characteristics in the compacted soil. The soil samples were oven-dried until constant weight at 105 °C and then weighted for determining the BD knowing the volume of the steel cylinder used for sampling.

For evaluating the dynamics of biochemical properties and the microbial communities of the compacted soil, the same sampling design for BD determination was used, but changing sampling method and number of replicates. A total of nine soil samples were taken by a shovel from the top 10 cm of mineral soil after removing any litter material: six along the trail, three inside the ruts (combining together sub-samples from left and right rut) and three between the ruts, taking one sample each meter both inside and between the ruts, and three samples 8 m away outside the trail, one sample each meter, for comparison, as off-trail control. The sampling was repeated twice, during late spring (T1, beginning of May 2021), at the beginning of vegetative growth period, and one year after

disturbance, at the end of vegetative growth period (T2, September 2021). Hence, this study did not consider the soon-after compaction period (T0) for the biological and biochemical analysis, as the primary focus was not on evaluating the direct disturbance effect of compaction on soil microbial communities. Additionally, although microbial activity might be promptly affected by disturbed soil conditions, the slow-growing fraction of the microbial community could take more time to experience structural changes (Hartmann et al., 2014). Lastly, cold mountain temperatures slow down microbial processes and capturing the specificity of microbial winter activity was beyond the scope of this study; therefore, the winter period was not considered.

At the same time of soil sampling (T1 and T2), soil respiration was measured by an EGM-1 PP Systems portable gas analyzer (Hitchin, UK) equipped with an SRC-1 closed air-circulation chamber (1.17 dm³ in volume). CO₂ efflux was measured between 9:00 and 11:00 a.m. by applying 12 PVC collars along the trail, each collar at a one-meter distance from each other, four inside and between the ruts and four 8 m outside the trail.

The soil samples were handled in the laboratory within 24 h after sampling and sieved at 2 mm at field moisture. Then, they were divided into three homogenous aliquots: one aliquot was stored at 4 °C for enzyme activities assay, one aliquot was air-dried for physico-chemical analysis, and the third was stored at -80 °C for microbial community analysis. The pH was measured in a suspension in distilled water (1:2.5) with an XS pH-metre model PC8. Total organic C and N contents were measured on 5–15 mg of finely ground and oven-dried (60 °C overnight) samples by dry flash combustion using a Carlo Erba NA 1500 CNS analyzer (Carlo Erba Instruments, Milan, Italy). The measured C was assumed to be all in organic form since the moderately acidic soil pH is incompatible with the presence of carbonates. Soil texture was determined by the hydrometer method (Gee and Bauder, 1986), on two composite soil samples obtained by bulking together the samples used to determine BD. Available P was determined by Olsen method (Olsen and Dean, 1965), and the P concentrations were determined by spectrophotometry at 880 nm with the sulfo-molybdic acid reagent (Murphy and Riley, 1962). The soil samples, stored at 4 °C, were used to measure soil enzyme activities related to the main biogeochemical (C, P, N, and S) cycles: the acid and alkaline phosphomonoesterase activities (Tabatabai and Bremner, 1969) for P cycle, the protease activity (Ladd and Butler, 1972) for N and C cycle, and the β -glucosidase activity (Tabatabai, 1982) as the major glycosyl-hydrolases member and the arylsulfatase activity for S cycle (Tabatabai and Bremner, 1970).

2.3. Soil DNA extraction, sequencing, and bioinformatics processing

Total genomic DNA was extracted from 0.5 g of sieved soil samples, stored at -80 °C, using the GenElute™ Soil DNA Isolation Kit (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's instructions. DNA purity and quantity were assessed by electrophoresis on 0.8 % agarose gel and using an ND-1000 Spectrophotometer (NanoDrop Technologies, Labtech, Ringmer, UK). DNA quantification was performed using Qubit™ 4 Fluorometer and Qubit™ ssDNA Assay Kit (Thermo Fisher Scientific), then standardized to a concentration of 10 ng/ μ L. Amplicons preparation and sequencing were carried out at IGATech (Udine, Italy). In particular, the bacterial V3-V4 hypervariable regions of 16S rRNA and the Internal Transcribed spacer (ITS) regions were PCR-amplified with primers 341F (5'- CCTACGGGNGBCASCAG -3') - 805R (5'- GACTACNVGGGTATCTAATCC -3') (Takahashi et al., 2014) and with ITS1F (5'-TCCGTAGGTGAACCTGCGG -3') - ITS4R (5'-TCCTCGCTTATTGATATGC-3') (White et al., 1990), respectively. Libraries were prepared using a custom Illumina 16S Metagenomic Sequencing Library Preparation protocol and were sequenced on a MiSeq instrument (Illumina, San Diego, CA) using 300-bp paired-end mode. To process the Illumina reads of bacterial and fungal soil communities, the DADA2 pipeline (Callahan et al., 2016) (version 1.22.0) was used in RStudio software 4.1.2 (R Core Team, 2022). For 16S rRNA

reads, filtering and trimming parameters were maxEE = c(1,1), truncLen = c(280,250), and trimLeft = c(17,21). Sample inference, the merging of paired reads, and the removal of the chimera were performed with default parameters to obtain the full denoised sequences. The taxonomic assignment was carried out by comparing our 16S rRNA sequences against the SILVA database v.138.1 (Pruesse et al., 2007) (confidence 80 %). For ITS sequences, the packages Biostrings (v. 2.62.0) (Pagès et al., 2022) and ShortRead (v. 1.52.0) (Morgan et al., 2009) were used to identify and count the primers present on raw fastq files. Instead, Cutadapt (v. 2.8) (Martin, 2011) was used to remove ITS primers. Then, the filtering and trimming step was performed with the following settings: maxEE = c(2,2), maxN = 0. Dereplication, sample inference, merging of paired reads, and the removal of the chimera were performed with default parameters according to the ITS workflow of DADA2 (Callahan et al., 2016). The UNITE ITS database (v. 8.3) (Nilsson et al., 2018) was used to align and classify ITS sequences.

2.4. Bioinformatic and statistical analysis

Before each statistical analysis, the Shapiro–Wilk test was applied to evaluate data distribution and the Levene's test was applied to evaluate homogeneity of variance. Enzymatic activities were analyzed by one way ANOVA and the Tukey's post hoc test (p value < 0.05) was used after. BD and soil chemical variables were analyzed by two way ANOVA using compaction conditions (off-trail, inside, and between the ruts) and time after compaction (0, 8 and 12 months, T0, T1 and T2, respectively; T0 was included only in the analysis of BD data) as fixed factors. The above cited statistical analyses were performed using SPSS, version 29 (IBM Corp., Armonk, NY, USA).

Annotated Amplicon sequence variants (ASVs) bacterial and fungal datasets were processed in RStudio environment (version 4.1.2) (R Core Team, 2022) mostly with functions of the vegan package (v. 2.5–6) (Oksanen, 2010). ASVs with a relative abundance lower than 0.01 % in all the samples were removed from both datasets. Rarefied bacterial and fungal datasets were generated, with subsample sizes of 10,000 and 8000 sequences per sample respectively, using the function *rarefy* in the vegan package (v. 2.5–6). Rarefaction curves were generated with the function *rarefied* in the vegan package (v. 2.5–6). For alpha diversity, the richness (Sobs), Pielou's index (J), and the Shannon diversity index were estimated using the *estimateR* and *diversity* functions in the vegan package. Index plots were carried out with the ggplot2 package (v. 3.3.6) (Wickham, 2016). Differences in α -diversity indices were detected performing the Kruskal-Wallis test with *in.stats* (v. 4.1.2) package, followed by the post-hoc Dunn Test, with the Benjamini–Hochberg correction for multiple comparisons, using the FSA (v. 0.9.3) (Ogle et al., 2021) and *rcompanion* (v. 2.4.18) packages (Mangiafico, 2020). The tests were performed i) among different levels of soil compaction (inside the ruts, between the ruts and off-trail) at each sampling time (T1 and T2) and ii) between different sampling times (T1 and T2) for each level of soil compaction. Hellinger transformation of rarefied ASV abundances was performed before ordinations and statistical analysis of Beta diversity. Non-metric multidimensional scaling (NMDS) ordination of Bray–Curtis distances was carried out using the function *ordinate* of the phyloseq R packages (v. 1.38.0) (McMurdie and Holmes, 2013). Differences in community structure were investigated between off- and in-trail samples, and among different levels of soil compaction (inside the ruts, between the ruts and off-trail) at T1 and T2 sampling times (respectively 8 and 12 months after soil compaction). Permutational multivariate analysis of variance (PERMANOVA) of Bray–Curtis dissimilarity metrics was executed on Hellinger transformed rarefied ASVs abundances with the *adonis2* function in vegan package (v. 2.5–6). Since PERMANOVA could be affected by non-homogeneous within-group dispersion of data (Anderson, 2001), the Analysis of multivariate homogeneity (PERMDISP) was also fulfilled to test if the average within-groups dispersion was the same in all groups (Anderson, 2006) investigated through the functions *betadisper* and *permutest* implemented in

the vegan package (v. 2.5–6). Moreover, the analysis of similarity test (ANOSIM) was carried out as confirmation of PERMANOVA outputs by using *anosim* function. The number of permutations was set to 99,999 for all analyses, and a $P < 0.05$ was considered as significant. In relative abundance analysis, bar plots of average relative abundances (%) at the order level were created with the *ggplot2* package (v. 3.3.6). Only orders with an average relative abundance of at least 3 % were reported for both datasets. Only genera with a relative abundance of at least 1 % in at least one sample were considered for each condition. Statistical differences in relative abundances of bacterial and fungal taxa, at order and genus level, were determined by performing Kruskal–Wallis among different conditions (off-trail, inside, and between the ruts) at T1 and T2 sampling times, respectively 8 and 12 months after the compaction. Then post hoc Dunn Test, with the Benjamini–Hochberg correction for multiple comparisons, was performed i) between different degrees of soil compaction at the two sampling times and ii) between different sampling times for each condition. Distance-based redundancy analysis was performed using the *dbRda* function (McArdle and Anderson, 2001) in the vegan package (v. 2.5–6). To fit environmental vectors significantly correlated with microbial community structure into db-RDA ordination, the function *envfit* implemented in the vegan package was used with 9999 permutations. The enzymatic activities were considered significant for $p < 0.05$ after Benjamini–Hochberg correction for multiple comparisons. Db-RDA biplots were generated with the *scores* function of vegan package and the *ggplot* function in the *ggplot2* package (v. 3.3.6). Indicator species analysis was conducted for each degree of soil compaction (inside the ruts, between the ruts, and off-trail) at different sampling times with the function *multipatt* implemented in *indicspecies* package (De Caceres et al., 2016). Rarefied ASVs abundances were used as the community data matrix. The Indicator Value indices (Dufrene and Legendre, 1997) were measured for ASVs-soil

compaction association analyses and the number of permutations was set to 99,999.

3. Results

3.1. Soil physicochemical properties and enzymatic activities

The studied soil had a silty loam texture (34 % of sand and 10.5 % of clay) and an initial BD of 0.76 g cm^{-3} . The results of the two-way ANOVA analysis on BD data showed a significant difference among different level of soil compaction ($P < 0.01$) and time after compaction ($P = 0.02$), but a non-significant interaction effect between the two factors ($P = 0.08$). Repeated skidding caused an immediate significant soil compaction as the increase of soil BD was 42 % inside the ruts (1.07 g cm^{-3}) and 26 % between the ruts (0.96 g cm^{-3}) in comparison to off-trail samples (undisturbed soil) (Fig. 1). At T2 the soil compaction level was reduced by 50 % inside the ruts (0.92 g cm^{-3} , 21 % higher than the pre-compaction conditions), and by 35 % between the ruts (0.89 g cm^{-3} , 17 % higher than the pre-compaction conditions).

The two-way ANOVA revealed a non-significant interaction effect between different level of soil compaction (off-trail, inside, and between the ruts) and time after compaction (T1 and T2) also on soil chemical properties. Soil compaction did not significantly affect pH, C and N content and soil respiration at both the sampling times, while a significant main effect of both time and compaction condition was found on available P (Table 1). Available P was significantly lower at T2 compared to T1 and in the off-trail compared to the compacted soil conditions.

At T1, the arylsulfatase activity was significantly lower between the ruts than in the off-trail soil, while acid and alkaline phosphomonoesterase activities were significantly lower inside the ruts compared to

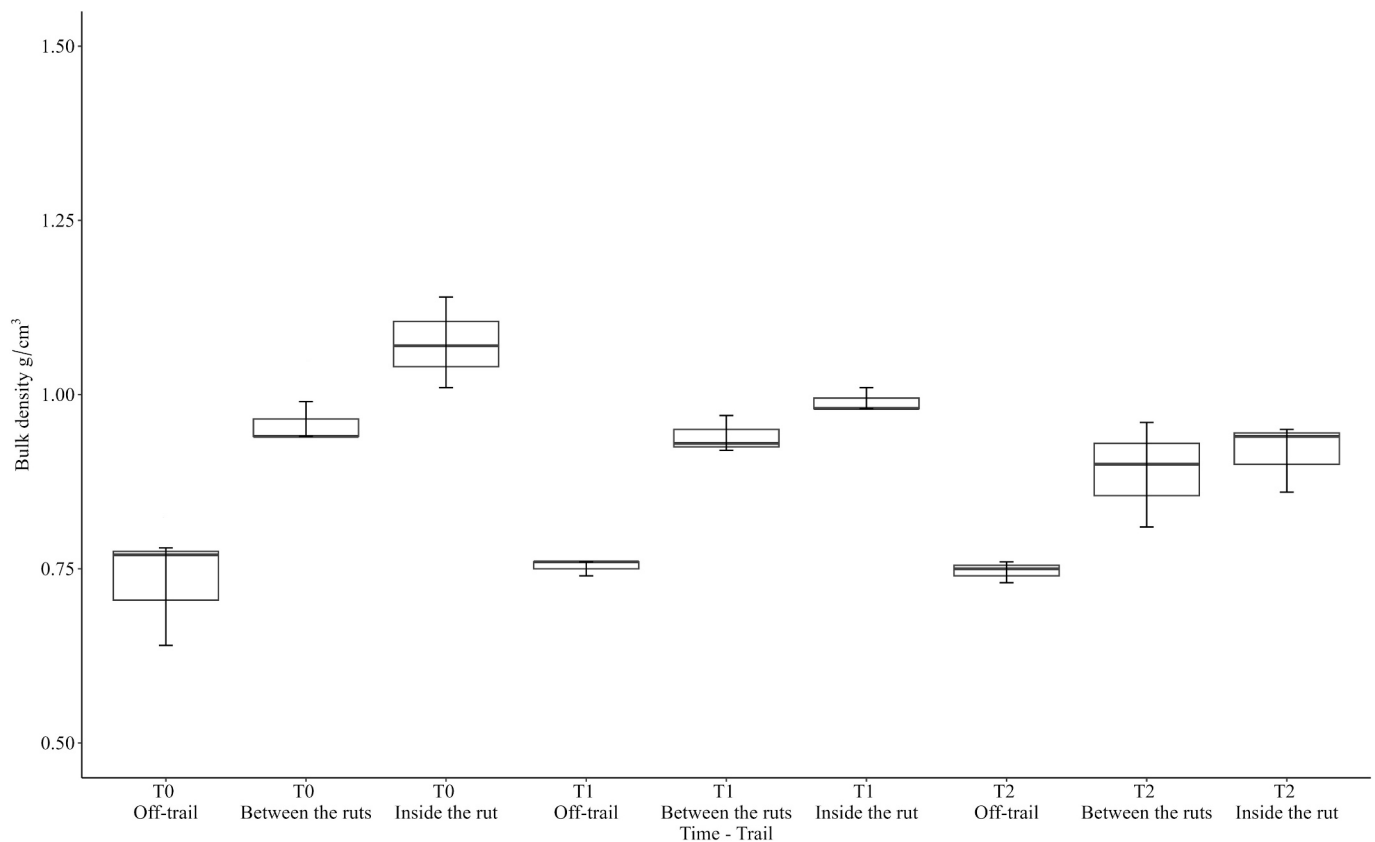


Fig. 1. Soil BD at T0 (soon after compaction), T1 (8 months after compaction) and T2 (12 months after compaction) at different level of soil compaction (in the off-trail samples, in inside the ruts and between the ruts samples). The results of the two-way ANOVA analysis show a significant difference among different level of soil compaction ($P < 0.01$) and time after compaction ($P = 0.02$), and a non-significant interaction effect between the two factors ($P = 0.08$).

Table 1

Soil pH, total C and N, available P, and respiration at T1 (8 months after compaction) and T2 (12 months after compaction) in the off-trail samples, in inside the ruts and between the ruts samples. Reported values are means \pm standard deviations of 3 replicates, apart from soil respiration for which the number of replicates is 4.

Time	Levels of soil compaction	pH	TOC (g kg ⁻¹)	N (g kg ⁻¹)	C/N	P Olsen (mg 100 g ⁻¹)	Soil respiration (g CO ₂ m ⁻² h ⁻¹)
T1	Between the ruts	5.44 \pm 0.11	4.37 \pm 0.85	0.26 \pm 0.03	16.7	6.11 \pm 1.85	0.53 \pm 0.17
	Inside the ruts	5.39 \pm 0.15	5.35 \pm 1.11	0.31 \pm 0.10	17.9	3.68 \pm 0.54	0.54 \pm 0.22
	Off-trail	5.35 \pm 0.10	4.84 \pm 0.42	0.30 \pm 0.02	16.1	7.20 \pm 2.40	0.51 \pm 0.20
T2	Between the ruts	5.48 \pm 0.06	3.73 \pm 2.39	0.19 \pm 0.09	18.5	1.78 \pm 0.92	0.55 \pm 0.28
	Inside the ruts	5.49 \pm 0.06	3.80 \pm 2.29	0.21 \pm 0.12	17.6	1.53 \pm 0.67	0.30 \pm 0.22
	Off-trail	5.56 \pm 0.17	5.05 \pm 0.56	0.31 \pm 0.04	16.2	6.16 \pm 1.60	0.54 \pm 0.44
Statistics^a	Levels of soil compaction	<i>P</i> = 0.950	<i>P</i> = 0.528	<i>P</i> = 0.193	–	<i>P</i> = 0.049	<i>P</i> = 0.129
	Time	<i>P</i> = 0.060	<i>P</i> = 0.324	<i>P</i> = 0.157	–	<i>P</i> = 0.002	<i>P</i> = 0.930
	Levels of soil compaction *Time	<i>P</i> = 0.444	<i>P</i> = 0.679	<i>P</i> = 0.559	–	<i>P</i> = 0.569	<i>P</i> = 0.213

^a The results of the two-way analysis of variance using compaction conditions (inside the ruts; between the ruts; and off-trail) and time after compaction (T1 and T2) as fixed factors are shown. Values in bold highlight level of significance of *P* < 0.05.

between the ruts (Fig. 2). Also, the protease activity was significantly lower inside the ruts compared to both off-trail and between the ruts soils (Fig. 2). At T2 no significant differences were observed in the alkaline phosphomonoesterase, arylsulfatase and protease activities, while the acid phosphomonoesterase activity was significantly higher inside the ruts than off-trail and between the ruts, showing contrasting results compared with the results at T1 (Fig. 2). The β -glucosidase activity inside and between the ruts was significantly lower than the off-trail at both sampling times (Fig. 2).

3.2. Taxonomy of bacterial and fungal communities

A total of 2,737,524 16S rRNA and 2,582,064 fungal ITS high-quality sequences were obtained, with an average of 152,085 \pm 35,847 of 16S

rRNA sequences and an average of 143,448 \pm 33,811 ITS sequences per sample. After quality filtering, denoising, and removal of chimeras, approximately 297,113 high-quality 16S rRNA sequences, and 70,185 ITS sequences remained for the following analysis. The sequencing depths were enough to accurately describe the biodiversity within the bacterial and fungal communities (Fig. S2). As a result, the 16S rRNA sequences obtained were classified into 4675 ASVs assigned to both Archaea and Bacteria Kingdoms, 37 phyla, 81 classes, 163 orders, 209 families, and 287 genera. Instead, ITS sequences resulted in a total of 1422 ASVs, 14 phyla, 32 classes, 63 orders, 88 families, and 135 genera. A complete list of the detected bacterial and fungal ASVs, including the related taxonomic assignment, is provided (File S1).

In all soil samples analyzed, the predominant bacterial phyla were Acidobacteriota, with an average relative abundance between 20.7 %

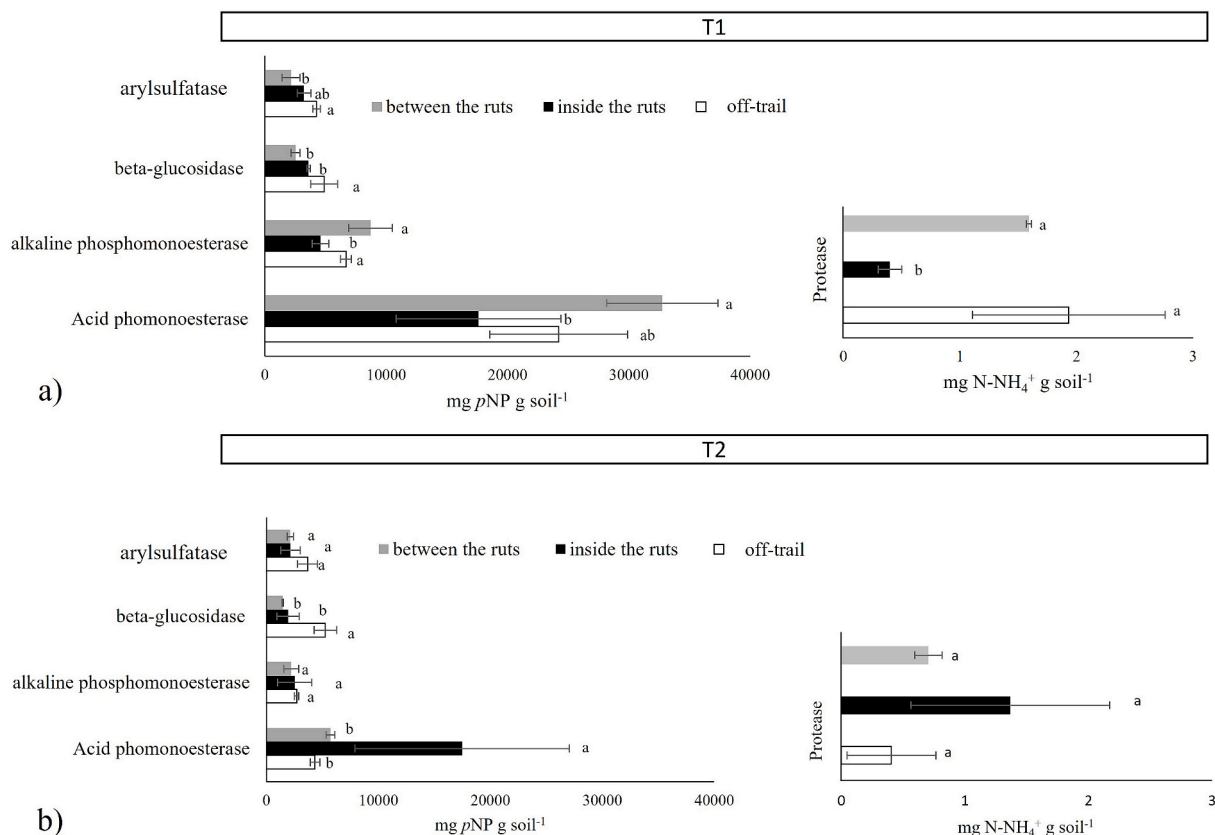


Fig. 2. Soil enzyme activities (arylsulfatase, beta-glucosidase, alkaline and acid phosphomonoesterase, and protease activities) in off-trail samples (blank bar), inside the ruts samples (black bar), and between the ruts samples (grey bar), at T1 (a) and at T2 (b). The error bars indicate the standard deviation. For each enzymatic activity, different letters indicate statistical differences among different levels of soil compaction at each sampling time based on the Tukey test (*P* < 0.05).

and 35.3 %, Proteobacteria (25 % to 35 %), Actinobacteriota (5 % – 12 %), Verrucomicrobiota (6 % – 10 %), Bacteroidota (6 % – 10 %), Planctomycetota (4 % – 6 %), Chloroflexi (1 % – 3 %) and Firmicutes (2 % – 3 %) (Fig. S2 A). Alphaproteobacteria was the most abundant class of Proteobacteria with an average relative abundance between ~16 % and ~21 %, followed by Gammaproteobacteria, which accounted for a range of mean relative abundance from 9 % to 14 %.

At order level, a core microbiota (i.e. taxonomic groups shared by all conditions) was composed of seven orders with an average relative abundance higher than 3 % (Fig. 3A). These “core” taxa were composed by Acidobacteriales (~16 % – ~11 %), Burkholderiales (~3 % – ~6 %), Chitinophagales (~4 % – ~5 %), Chthoniobacteriales (~5 % – ~8 %), Rhizobiales (~8 % – ~15 %), Solibacteriales (~3 % – ~5 %) and Subgroup 2 (~4 % – ~11 %) (Fig. 3A). Significant differences in bacterial relative abundances across different conditions were investigated both at order and genus level at each sampling time and entirely reported in File S2. In particular, at sampling time T1 shifts of the order relative abundances among inside and between the ruts samples were observed. The orders Acidobacteriales, Subgroup 2, and WD260 were significantly more abundant inside the ruts compared to between the ruts samples (Fig. 3A, File S2). Differently, Sphingomonadales and Vicinamibacteriales were significantly less abundant inside the ruts than between the ruts samples (Fig. 3A, File S2). Additionally, the order Saccharimonadales were significantly more present in compacted soils (between and inside the ruts samples) than in the undisturbed soils (off-trail samples) (File S2). Moreover, the post hoc comparisons performed at sampling time T1 showed that genera *Gemmatimonas* and *Sphingomonas* were significantly less abundant in inside the ruts than in between the ruts samples (Fig. 3C and File S2).

Regarding the bacterial genera, 15 genera showed an average relative abundance of 1 %, or higher, in at least one group of soil conditions tested (Fig. 3C, File S2): the most abundant genera were *Candidatus Solibacter* (average relative abundance ranged from 3 to 6 % approximately), *Candidatus Udaebacter* (~3 % – ~6 %), *Bryobacter* (~3 % – ~6 %), *Puia* (~1 % – ~3 %), *Acidothermus* (~1 % – ~2 %) and *Streptococcus* (~1 % – ~2 %) (Fig. 3C, File S2). The bacterial genera profile in inside the ruts was deeply different than in between the ruts and in off-trail samples (Fig. 3C). In Inside the ruts samples, the genera *Candidatus Udaebacter*, *Candidatus Solibacter*, *Granulicella*, and *Bryobacter* were more abundant than off-trail and between the ruts samples (Fig. 3C).

Overall, the fungal community was dominated by the phylum Ascomycota with a range of mean relative abundance from 67 % (inside the ruts samples at T2) to 82 % (inside the ruts at T1 and off-trail samples at T1), together with Basidiomycota (6 % – 20 %), Rozellomycota (<1 % – 5 %), Zoopagomycota (<1 % – 2 %) and Olpidiomyota only detected in between the ruts samples at T1 with a maximum relative abundance of 2.24 % (Fig. S3 B). At the order level, the orders Helotiales and Chaetothyriales showed a relative abundance higher than 3 % (range of ~18 % – ~30 %) in all soil conditions (Fig. 3B, File S2). At T1 and T2 the relative abundances of some taxa significantly varied between levels of soil compaction reflecting the diversity in structure of fungal communities. Indeed, at T1 the order Tremellales was significantly less abundant in not-compacted than compacted soil samples (Fig. 3B, File S2), while Capnodiales and the order GS37 showed the opposite trend, being significantly enriched in not-compacted soil samples (Fig. 3B and File S2). At T2, the order Trichosporonales were significantly less abundant in the compacted soil conditions (File S2).

Concerning the fungal communities, thirty-one genera displayed an average relative abundance of 1 %, or higher, in at least one group (Fig. 3D, File S2). Among these, the genera characterized by a high relative abundance were *Cenococcum*, with a range of mean relative abundance equal to ~3 % – ~12 %, *Ciliophora* (~2 % – 6 %), *Cryptosporiopsis* (~0.8 % – ~7 %), *Hygrophorus* (<1 % – ~10 %), *Inocybe* (<1 % – ~14 %), *Lambertella* (<1 % – ~7 %), *Oidiodendron* (~1.1 % – ~3 %) and *Saitozyma* (~2 % – ~5 %) (Fig. 3D). Following the Dunn Test post hoc, at T1 the relative abundance of genus *Saitozyma* resulted

significantly higher in the compacted soil conditions (Fig. 3D and File S2).

3.3. Overall diversity of bacterial and fungal communities

Alpha diversity values showed that all samples were characterized by high species richness (S_{obs}) in both the fungal and bacterial communities (Fig. 4A & B). At each sampling time, the S_{obs} did not differ significantly in any soil condition (between and inside the ruts, and off-trail) (Fig. 4A), indicating that soil compaction did not reduce species richness. Moreover, in the bacterial community, S_{obs} remained largely unvaried among the two sampling times. In general, for bacteria the Evenness Pielou's index (J') was consistently high in all the soil conditions examined and considerably uniform among sampling times T1 and T2 (Fig. 4A). However, at T1 the Evenness Pielou's index (J') was significantly lower inside the ruts than between ruts, hinting that higher soil compaction slightly amplifies the dominance of some bacterial species (Fig. 4A). Similar to bacteria, at T1 the fungal dataset displayed constant values of S_{obs} (i.e. fungal richness) among different soil compaction levels (Fig. 4B). Instead, the fungal communities in all the soil conditions had a lower even distribution of ASVs compared to the bacteria dataset (Fig. 4B). Moreover, the Evenness Pielou's index (J') significantly increased in between the ruts samples with sampling times, suggesting a time-effect on ASVs distribution at this degree of soil compaction (Fig. 4B). Bacterial and fungal communities did not differ in relation to Shannon index, showing H values relatively high and stable across all the soil conditions tested (Fig. 4A, Fig. 4B).

For bacterial communities, the NMDS ordination plots illustrated a progressive split between the off-trail and in-trail samples (both inside and between the ruts samples) over time (Fig. 5A–B). The most remarkable clustering was visible at sampling time T1, in which the inside the ruts samples clustered separately from the off-trail and between the ruts samples (Fig. 5A). Indeed, at T1, significant differences in community structure at different degrees of soil compaction were observed, as strongly confirmed by PERMANOVA results (Fig. 5A), as well as by ANOSIM statistics (Fig. 5A) and corroborated by PERMDISP test (Fig. 5A). In agreement with the NMDS plot, the range of rank dissimilarity was significantly lower in inside the ruts than between the ruts and the off-trail samples (Fig. S4), hinting that the bacterial community inside the ruts samples truly differs from the other two groups. Although the NMDS plot showed an evident separation between not-compacted and compacted soil samples, no significant differences in community structure were observed between these groups at T2 (Fig. 5B). Concerning the fungal dataset, the clustering analysis showed a consistent grouping of samples between compacted and not-compacted soil (Fig. 5C–D). At sampling time T1, according to the statistical analysis soil compaction significantly altered the fungal community structure, leading to a significant differentiation of communities among different levels of compaction (inside the ruts, off-trail, and between the ruts) and between compacted and not-compacted soil conditions (Fig. 5C). Furthermore, soil compaction effects were most pronounced at T2 as persistent and significant shifts were recorded here between compacted and not-compacted soil samples as hinted by the ordination analysis (Fig. 5D).

3.4. Relationship between enzymatic activities and community structure

Distance-based redundancy analysis (db-RDA) coupled with multiple regression analysis was used to evaluate the relationship between both microbial community structure and soil enzyme activities. The first two axes together explained 36.07 % and 48.65 % of the variance in the bacterial communities, respectively at T1 and T2 (Fig. 6A–B). Concerning the fungal communities, the total variance explained was 30.28 % and 37.44 % at T1 and T2, respectively (Fig. 6C). Db-RDA plots showed the relationships among enzyme activities and the structure of both the microbial communities, and their activities growth trends along

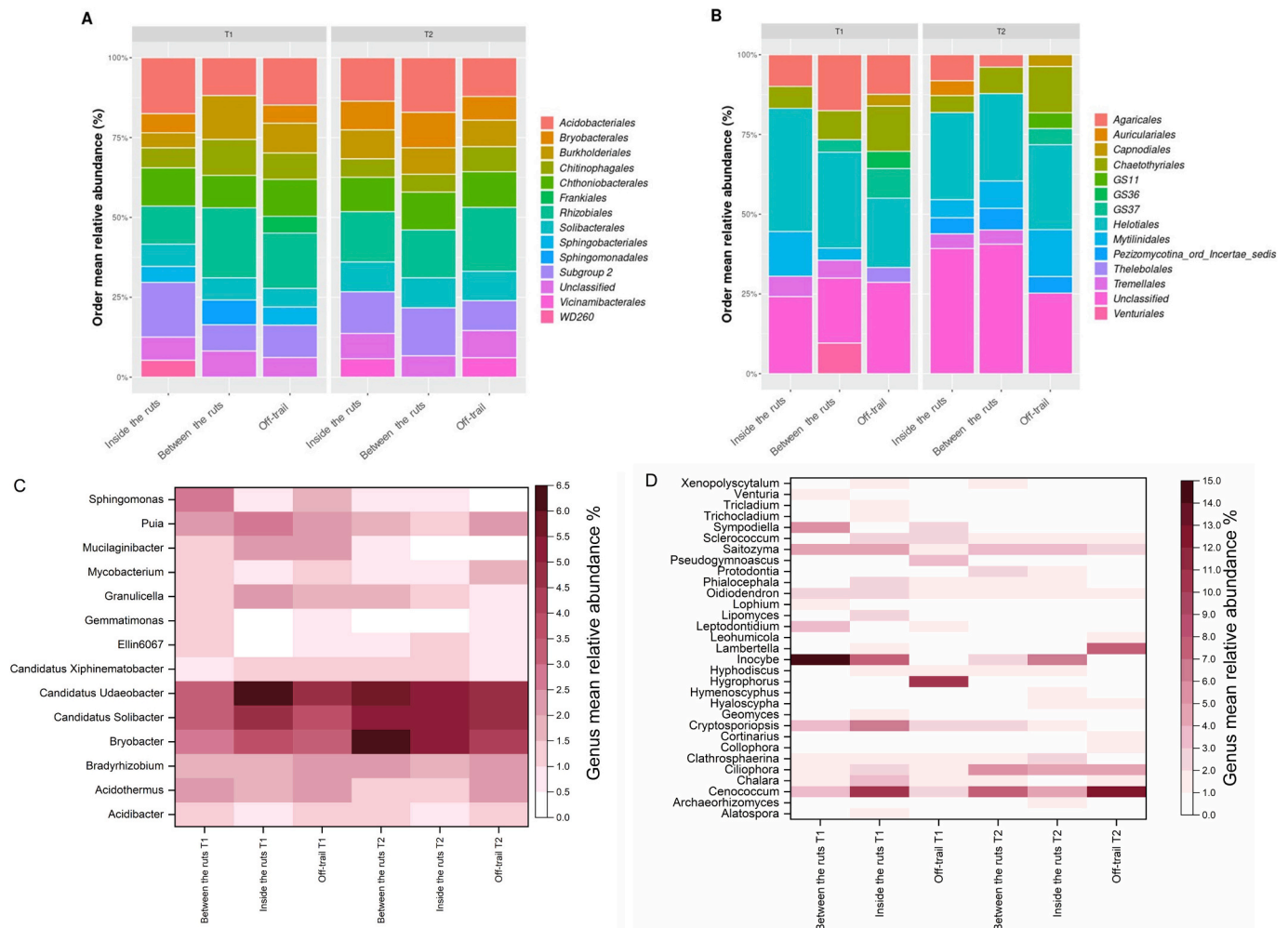


Fig. 3. Taxonomic composition of bacterial microbial communities (orders in A, genera in C) and fungal communities (orders in B, genera in D) enriched in different conditions (off-trail, between and inside the ruts) at T1 and T2 sampling times. In panels A) and B) average abundances (%) at order level are reported for each tested condition. Only orders with an average relative abundance of at least 3 % (or higher) are reported. In panels C) and D), heatmaps with mean relative abundances (%) at genus level of bacterial and fungal communities, respectively, in different conditions. Only the genera with a mean relative abundance of at least 1 % in at least one sample are shown.

with the changes of soil microbial communities in different soil conditions. (Fig. 6 and File S3).

At T1, the enzyme activities associated with the bacterial structure were arylsulfatase, protease, and alkaline phosphatase activity (Fig. 6A, File S3). Consistently with enzyme activity results (Fig. 2), the regression analysis showed that the arylsulfatase activity was positively associated with changes in bacterial structure in the undisturbed soil (off-trail samples), while the protease and alkaline phosphatase activities progressively increased along with changes in bacterial structure in medium compacted soil (between ruts samples) (Fig. 6A). Moreover, major increases in these enzymatic activities did not appear to be connected with the structure of the bacterial community in heavily compacted soil (inside ruts samples) (Fig. 6A).

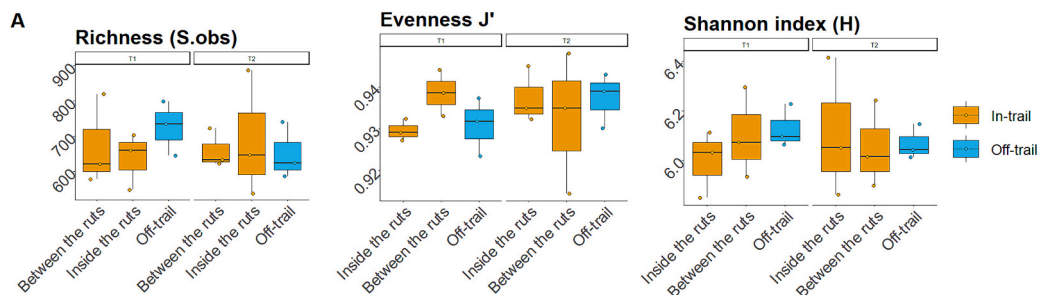
At T2, the enzyme activities significantly associated to bacterial community structure changed compared to T1 as acid phosphomonoesterase and β -glucosidase emerged as newly related enzymes, while protease was no longer significant (Fig. 6B; File S3). In particular, the increment of acid phosphatase activity was connected to changes in bacterial community composition of compacted soils (inside ruts samples), while other activities (β -glucosidase, alkaline phosphatase, and arylsulfatase activity) were more associated with undisturbed soils (off-trail samples) (Fig. 6B), although they showed lower activity (Fig. 2).

For fungi, a lower number of relationships between soil functionality

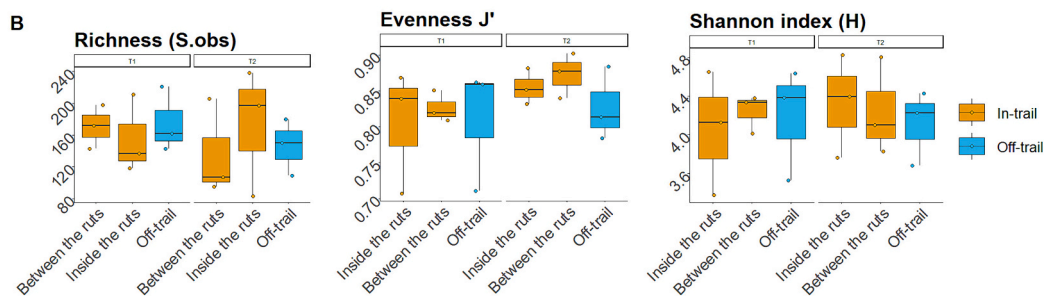
and community structure were observed at T1 (Fig. 6C; File S3). Similarly to bacteria, the arylsulfatase activity increased along with changes in fungal community composition in undisturbed soils (off-trail samples), while the protease activity increased along with changes in fungal community composition in medium disturbed soils (between ruts samples) (Fig. 6C; File S3). Also, the structure of the fungal community in compacted soils (inside ruts samples) was not associated with these two activities. At T2, no enzymatic activity was significantly related to changes in fungal community structure in both compacted and non-compacted soils (File S3).

3.5. Bacterial and fungal indicator species of soil compaction

We additionally conducted an indicator species analysis to uncover taxonomic groups that were significantly (Indicator Value index $P < 0.05$) associated with compacted (both inside and between the ruts samples) or off-trail soils (undisturbed soils). For the bacterial dataset, at T1 22 ASVs, 7 ASVs, and 5 AVSs, respectively for inside the ruts, between the ruts and off-trail samples, were identified as indicator species, as significant differences in bacterial community structure were observed (Fig. S5A, Table S4). More precisely, the association analysis revealed ASVs-soil compaction association patterns that were specific and exclusive for each condition tested (Fig. S5A, Table S4). Bacterial



	<i>Bacteria</i>					
	S.obs		J'		H	
	T1	T2	T1	T2	T1	T2
Off-trail	725 ^{A,a}	649 ^{A,a}	0.93 ^{AB,a}	0.94 ^{A,a}	6.13 ^{A,a}	6.07 ^{A,a}
Between the ruts	674 ^{A,a}	659 ^{A,a}	0.94 ^{A,a}	0.93 ^{A,a}	6.10 ^{A,a}	6.05 ^{A,a}
Inside the ruts	639 ^{A,a}	688 ^{A,a}	0.93 ^{B,a}	0.94 ^{A,a}	6.00 ^{A,a}	6.11 ^{A,a}



	<i>Fungi</i>					
	S.obs		J'		H	
	T1	T2	T1	T2	T1	T2
Off-trail	175 ^{A,a}	146 ^{A,a}	0.81 ^{A,a}	0.83 ^{A,a}	4.18 ^{A,a}	4.11 ^{A,a}
Between the ruts	171 ^{A,a}	136 ^{A,a}	0.83 ^{A,a}	0.87 ^{A,b}	4.24 ^{A,a}	4.24 ^{A,a}
Inside the ruts	155 ^{A,a}	173 ^{A,a}	0.80 ^{A,a}	0.86 ^{A,a}	4.05 ^{A,a}	4.33 ^{A,a}

Fig. 4. Effect of soil compaction and time on α -diversity of bacterial (A) and fungal (B) communities in differently compacted soils (off-trail, between and inside ruts) at T1 and T2 sampling times. Values of richness (S_{obs}), the Evenness Pielou's index (J), and Shannon (H) index are reported on the y axis. Horizontal lines of boxes represent the median, whereas the whiskers represent the maximal and minimal values. In Tables, mean values of observed richness (S_{obs}), the Evenness Pielou's index (J) and Shannon index (H) are reported for each community. Different superscript capital letters indicate results of Dunn test's post-hoc among different levels of soil compaction (between the ruts, inside the ruts and off-trail) at each sampling time. Different superscript small letters indicate results of Wilcoxon test for each level of soil compaction among different sampling times ($P < 0.05$).

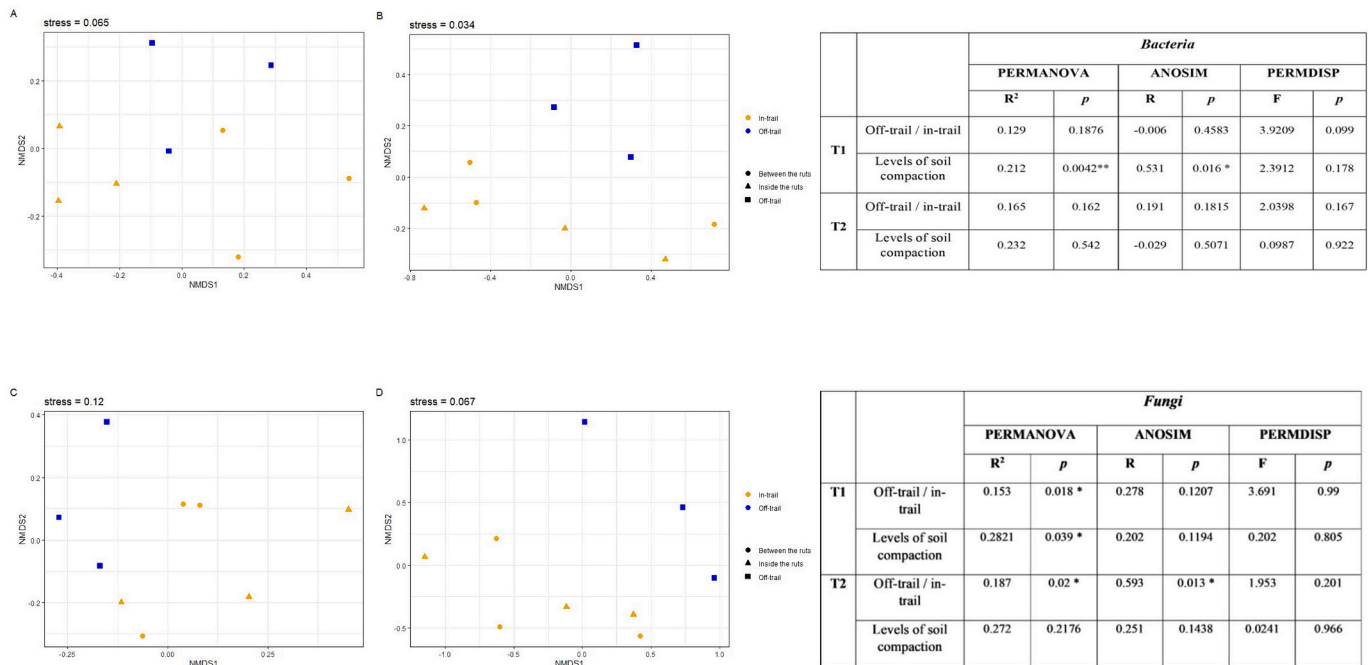


Fig. 5. Effect of soil compaction on β -diversity of bacterial (panels A and B) and fungal (panels C and D) communities at T1 (A and C) and T2 (B and D) the sampling time. Non-metric multidimensional scaling plot is based on Bray–Curtis distance on Hellinger transformed ASVs abundance of bacterial and fungal community structures. Different colours indicate the split in off-trail samples and in-trail samples (between the ruts and inside the ruts samples); shapes refer to different degrees of soil compaction (off-trail, between the ruts and inside the ruts samples). Stress values are reported for each NMDS. Statistical differences with permutational multivariate analysis of variance (PERMANOVA), analysis of similarities (ANOSIM), and Analysis of multivariate homogeneity (PERMDISP) between off- and in-trail samples, and among different levels of soil compaction (between the ruts, inside the ruts and off-trail) at different sampling time (T1 and T2) are reported in tables. F-ratio (F) for PERMDISP, the estimation of the variance component (R²) for PERMANOVA and the Global R for ANOSIM are reported together with the level of significance (ns, not significant; * P < 0.05; ** P < 0.01).

taxa that were significantly associated with inside the ruts samples were assigned to taxonomic groups at the class level as Alphaproteobacteria, Acidobacteria, Gammaproteobacteria, Verrucomicrobiae, Kapabacteria, Bacteroidia, Vampirivibrionia and Chthonomonadetes (Fig. S5A, File S1, Table S4). Consistent with the obtained results, most of the indicator ASVs found, associated with inside the ruts samples, were affiliated with orders belonging to Subgroup 2, Acidobacteriales (genera *Occallatibacter*, WD260, and Chitinophagales (genera *Puia* ASV_379, and ASV_680) (Fig. S5A, File S1, Table S4). Other significant indicators ASVs, for inside the ruts samples, were attributed to Rhizobiales A0839, Pedosphaerales, Kapabacteriales, Solibacterales (specifically *Candidatus Solibacter*), Obscuribacterales, Chthonomonadales (genera *Chthonomonas*), *Caulobacterales* (Fig. S5A, File S1, Table S4).

As soil compaction significantly altered the fungal community structure at T1 and T2, fungal indicator species were also investigated for fungal datasets (Fig. S5B–E). At T1, no ASVs were detected as significantly associated with inside the ruts samples (Fig. S5B, File S1, Table S4), while for off-trail and between the ruts groups some indicator species were identified (Fig. S5B–E, File S1, Table S4). The genera significantly associated with between the ruts samples were *Ciliophora*, *Phacidium*, *Sanchytrium*, *Xenopolyscytalum*, whereas *Sclerococcum ahtii*, *Capnobotryella* and ASVs attributed to the class of Lecanoromycetes were pinpointed as good indicators for off-trail soils, at T1 (Fig. S5B and C, File S1, Table S4). At T2 the indicator species significantly associated with between the ruts and off-trail samples changed compared to T1. Indeed, the only ASVs detected was ASV_274 (phylum Ascomycota) for the between the ruts group, and ASV_40 for both between and inside the ruts groups (i.e. in-trail) (phylum Ascomycota) (Fig. S5D–E, File S1, Table S4). For the not-compacted soil samples, the ASVs detected were related to *Vanrija albida*, *Ciliophora*, *Collophora parla* and an ASV attributed to the Sordariomycetes (ASV_71) (Fig. S5D–E, File S1, Table S4). Moreover, single indicator ASVs were associated with specific

conditions, such as ASV_181 (class Eurotiomycetes) for inside the ruts samples and ASV_40 (class Leotiomycetes) for compacted soil samples (both inside and between the ruts) (Fig. S5D–E, File S1, Table S4).

4. Discussion

4.1. Soil physicochemical properties and enzymatic activities

In our study area, after 30 repeated passes by a tractor simulating skidding operations in a heavily trafficked area for wood extraction, the soil was severely compacted. Indeed, the BD increased by 42 % inside the ruts, a result higher than the average values reported in two published meta-analyses about the impacts on soil after forest logging (Ampoorter et al., 2012; Latterini et al., 2023). This condition was due to the high wheel traffic intensity (i.e. number of passes), although it is commonly reported that the first passes have the highest relative impact, thus already resulting in a high compaction degree (Ampoorter et al., 2012; Han et al., 2006). Furthermore, Vallombrosa forest soil showed a very low initial (undisturbed) soil BD (0.76 g cm⁻³) and therefore the soil was characterized by high porosity and was prone to compaction (Ampoorter et al., 2012). Arguably, compaction should have had a certain impact on soil structure and, consequently, on air fluxes and hydraulic conductivity (Hartmann et al., 2014). This compaction condition, however, was transient as the BD rapidly decreased after 1 year from disturbance (T2) in the compacted soil, in the same manner both between and inside the ruts, as showed by the lack of significant interaction effect by the two-way ANOVA analysis between the different levels of compaction and time after compaction. Many studies report soil recovery periods longer than 5 years (Croke et al., 2001; Hartmann et al., 2014; Marchi et al., 2016). However, our data suggests a still incomplete soil recovery at T2. Bulk density (BD) values remained 15 % above those of the undisturbed soil, a threshold reported to be still

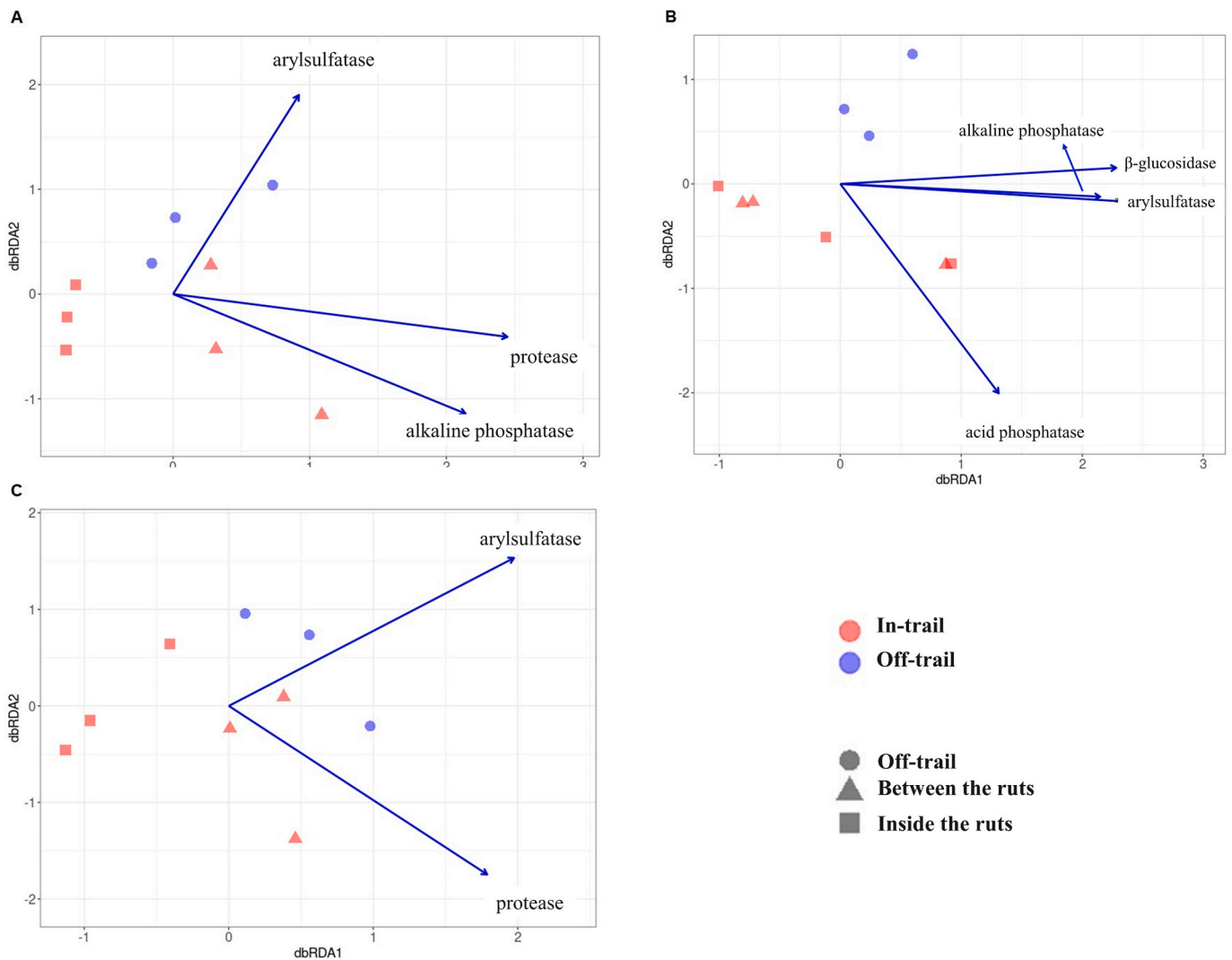


Fig. 6. Distance-based redundancy analysis (RDA) plots between enzymatic activities and bacterial (A and B) and fungal community structures (C). Different colours indicate the split in off-trail and in-trail samples (between the ruts and inside the ruts samples); shapes refer to different degrees of soil compaction (off-trail, between the ruts and inside the ruts samples). Vectors indicate enzymatic activities significantly correlated with microbial community structures (ENVFIT analysis).

detrimental for soil functions (Lacey and Ryan, 2000).

Compaction did not significantly affect C and N content and just a slight decreasing trend of these two elements may be noticed in our compacted soil after 1 year from disturbance. Conversely, a general decrease of SOM (soil organic matter) in compacted conditions, even in the short term (1–3 years), has been observed by a meta-analysis of studies carried out in several Italian broadleaf's forests (Latterini et al., 2023). However, the analyzed enzyme activities related to soil biogeochemical cycles (beta-glucosidase and protease activities for SOM dynamics or acid and alkaline phosphomonoesterase activities for P cycle), showed a significant decrease inside the ruts in comparison to undisturbed soil (off-trail) at T1, indicating a potential slowing down of biotic SOM decomposition due to soil compaction. In this regard, soil enzyme activities can be considered as a soil quality indicator, allowing to highlight early soil stress or soil functionality recovery (Dick et al., 1996).

The analysis of soil enzymatic activities may provide a valid link between the metabolic response of soils and changes in microbial communities to soil disturbance (Frąc et al., 2011; Nannipieri et al., 2012; Tan et al., 2008). Soil functionality and biodiversity are strictly related to soil physicochemical conditions, such as nutrient availability, water and air fluxes, porosity, and soil structure. Soil compaction reduces the microenvironments with optimal conditions for soil microbial

growth and could lead to lower activity of specific groups (Kihara et al., 2012; Lu et al., 2019). Indeed, after 8 months from tractor passage, enzyme activities related to biogeochemical cycles were reduced by soil disturbance showing a short-time soil functionality change as previously reported by Tan et al. (2008). The db-RDA analysis showing a negative association between enzyme activities and soil microbial communities (bacteria and fungi) confirmed the negative impact in soil functionality and communities structure induced by soil disturbance.

After 1 year since compaction (T2), the negative effect of soil disturbance was no longer observable. In fact, most of the enzyme activities, except betaglucosidase activity, did not show significant lower values of compacted soils compared to the undisturbed soil, indicating a general fast recovery of soil functionality, as observed in previous experiments in agricultural soils (Kwiatkowska and Joniec, 2022). In particular, the increase of acid phosphomonoesterase activity inside of the ruts, observed at T2, can be related to a microbial restoration, confirming that the phosphomonoesterase activities can be considered a suitable indicator of soil health (Acosta-Martinez et al., 2018). In addition, the db-RDA analysis confirmed the positive association between bacterial community composition and acid phosphomonoesterase. The metabolic re-activation of soil organisms may result by the increase of soil porosity due to the decrease of soil compaction, as previously reported (Xue et al., 2018).

4.2. Diversity of bacterial and fungal communities

The microbial community structures can be used as an indicator for soil health due to their sensitivity to disturbances, and their structural variation can be strictly associated with changes in soil ecosystem processes (Allison and Martiny, 2008). Our investigation on microbial communities was conducted in a relatively short period compared to previous works (García-Carmona et al., 2021; Hartmann et al., 2014; Lewandowski et al., 2016), challenging the idea that short-term studies are insufficient to assess the significant effects of forest logging operations on soil microbial communities. This remark is particularly true for the bacterial community, which in our work was provisionally altered in its structure and composition by soil compaction. Indeed, soil compaction considerably affected the bacterial beta diversity only after eight months from soil disturbance, thus exerting a temporary effect on the bacterial community composition, to promptly return to the initial state showing good resilience to the disturbance introduced in the longer term. Although the common idea is that a complete recovery of bacterial communities from soil compaction may take place in years, this is not always the case. Ammitzboll et al. (2022), for instance, found that the bacterial community structures in a post-logging compacted soil showed a similar temporal trend to our findings, confirming the great abilities of bacteria to restore the initial community composition on a monthly scale. In general, increasing trends of bacterial alpha diversity were recorded along with soil disturbances such as compaction (García-Carmona et al., 2021; Hartmann et al., 2014; Longepierre et al., 2022) or salvage-logging operations and wildfire (Ammitzboll et al., 2022; Bowd et al., 2022). Conversely, in our soils, bacterial alpha diversity was not affected by compaction. In particular, species richness remained consistently high over time for all compaction levels. Similarly, previous studies related to other disturbances, such as wildfire-affected soils (Weber et al., 2014) and severe organic matter removal treatments (Wilhelm et al., 2017), did not reveal any significant bacterial alpha-diversity trends, as well as no significant impact of soil compaction on richness expressed as microbial biomass carbon MBC at surface-medium soil depth (Busse et al., 2006; Nazari et al., 2021; Shestak and Busse, 2005). However, the decrease of the Pielou index in compacted soil suggested changes in bacterial communities possibly linked to the temporary leading of dominant species more tolerant and resistant to disturbance (Sun et al., 2017; Tanentzap et al., 2013) who may benefit from post-compaction soil changes. Overall, considering that microbial diversity guarantees the “multifunctionality” of soil ecosystems (Delgado-Baquerizo et al., 2016; Tardy et al., 2014), a high starting richness and diversity of the bacterial community may ensure greater plasticity and inherent capacity to adapt to soil compaction (Shade et al., 2012).

Modified soil conditions due to soil compaction significantly altered fungal community structures, leading to a clear differentiation between in- and off-trail samples at the two times observed. Indeed, the variation of beta-diversity over time between compacted and non-compacted soil samples revealed the scarce resilience ability of fungal communities to restore the initial community structure. The higher sensitivity to compaction of fungal communities compared to bacteria is consistent with previous findings pointing out this lack of resilience of total fungal community to disturbance (Hartmann and Niklaus, 2012; Hartmann et al., 2014).

Fungal community species richness was not affected by soil compaction, similarly to what was observed for bacterial species richness. However, the slightly compacted soil (between the ruts samples) underwent a more even distribution of detected ASVs, indicating that mild soil compaction did not translate into a greater dominance of fungal species resistant to perturbation, but rather into an increase of biodiversity in the short-term. Highly diverse and even microbial communities, in which several species are functionally equivalent, may show higher functional stability under environmental disturbance ensuring good functionality in stress conditions (Wittebolle et al., 2009). In this

perspective, the significant increase in fungal evenness can be interpreted as an improved ability of the soil ecosystem to carry out overlapping functions in the longer term. The greater resilience ability of bacteria compared to fungi can be due to the higher bacterial growth rates and turnover in soil habitats (Reischke et al., 2014; Rousk and Bååth, 2011) and the major use of readily available C sources, preferred to the degradation of more complex carbon substrates, which is a prerogative of the fungal community (Hicks et al., 2022; Reischke et al., 2014).

The shifts recorded in the bacterial community structures are in accordance with relative abundance data. Indeed, eight months following the compaction event, different degrees of soil compaction led to distinct enrichment of bacterial relative abundances. Oligotrophic highly metabolically versatile microorganisms of Acidobacteriales (Ho et al., 2017) and Subgroup 2 (class Acidobacteriae) were significantly more abundant in the most compacted soil, probably due to their capability to tolerate fluctuations in water availability (Ward et al., 2009) coupled with their ability to grow across different oxygen gradients (Eichorst et al., 2018), especially in low oxygen tension environments as compacted forest soils (Bruce et al., 2010). Indeed, peat microbiota in anoxic microcosms was persistently and highly composed of Subgroups 1, 2, and 3 (Hausmann et al., 2018). For fungi, significant variations in the relative abundances of two orders reflected changes in community structures. The orders Tremellales with species known for their saprotrophic lifestyle was significantly more abundant in compacted soils, as *Saitozyma podzolica* (formerly *Cryptococcus podzolicus*) a typical soil-borne yeast that frequently occurred in different forest soils (França et al., 2016; Mašíňová et al., 2016; Middelhoven, 2006; Yurkov et al., 2016). *S. podzolica* is considered a good indicator in acids and well-drained soils (Yurkov, 2018) but is frequently present in disturbed post-mining (Detheridge et al., 2018; Monteiro Moreira and Martins do Vale, 2020) and fire-affected soils (Orumaa et al., 2022), hinting its key role in the recovery of degraded lands. Conversely, not-compacted soils were significantly characterized by species of order Capnodiales, whose greater abundance was previously correlated with phosphorus availability in forest soil (Mason et al., 2021), in accordance with our data showing a higher P availability in the undisturbed soils.

After one year, the fungal community compositions of high- and medium-compacted soils (ruts and between the ruts, respectively) showed similar abundance profiles and different from the abundance profile of undisturbed soil. Multiple studies have reported that post-disturbance fungal communities mainly undergo changes in different ratios between saprotrophic and symbiotrophic organisms in disturbed soils (Ammitzboll et al., 2022; García-Carmona et al., 2021; Hartmann and Niklaus, 2012; Hartmann et al., 2014; Sun et al., 2017). Specifically, logging management frequently shifted fungal community composition promoting the increase of saprophytes to the detriment of mycorrhizal (Ammitzboll et al., 2022; Hartmann and Niklaus, 2012; Hartmann et al., 2014). Saprophytes are usually fungi highly tolerant to environmental stresses (Bastida et al., 2017) involved in soil organic matter decomposition, contributing to the soil nutrient enrichment process and thus to the recovery of post-disturbance soils (Bowd et al., 2022; García-Carmona et al., 2021). The analyzed soil samples were rich in mycorrhizal genera (such as *Cenocccum*, *Hygrophorus*, *Inocybe*, *Lambertella*, *Oidiodendron*), therefore clear differences between disturbed and undisturbed soils in terms of mycorrhizal abundance were not recorded. Nonetheless, in our study the abundance data and related statistics hinted a slightly increasing trend of saprophytic fungi in disturbed soils, confirming previous findings.

4.3. Bacterial and fungal indicator species

Indicator species analysis provided key information on ASVs associated with different levels of compaction, and that can be useful in soil monitoring and in predicting the possible long-term evolution of soil conditions. Microorganisms belonging to bacterial taxa

Chitinophagaceae, as the genus *Puia*, play an important role in organic matter decomposition and C cycling. In this case, the association of genus *Puia* identified as indicator species in inside the ruts soil samples, may be due to a decrease of C mineralization in more compacted soil (De Neve and Hofman, 2000). The finding of denitrifying bacteria *Candidatus Solibacter*, often found in compacted forest soil (Hartmann and Niklaus, 2012; Hartmann et al., 2014), as a genus associated with soil compaction is consistent with the reduction trend of N content recorded in our soil in the short-term and is coherent with the increase of denitrification processes in wheel-traffic-induced soil compaction (Longepierre et al., 2022; Wolkowski, 1990). The indicator species analysis also confirmed that the class Acidobacteriae was significantly associated with soil compaction, as for members of genus *Occallatibacter*, which were previously found in restored peatlands, in which compaction increased during mining process (Kaupper et al., 2021). The uncultured bacteria of orders WD260 (*Gammaproteobacteria*) are common inhabitants of forest soils and peatlands (Sabrekov et al., 2021; Seitz et al., 2022), but no information is available about their ecological and functional role. Similarly, the genus *Chthonomonas* (phyla Armatimonadota) has few cultured and poorly characterized representatives.

Fewer indicator species of fungal communities were associated with compaction compared to bacteria, and only to mildly compacted soil samples (between the ruts). The orders Leotiomyces (i.e. *Phacidium*) were related to weakly disturbed soils, as previously observed in soils slightly burnt (Ammitzboll et al., 2021), or compacted by grazing (Wu et al., 2022). Also, the hemicellulolytic fungi *Xenopolyscytalum* (Leotiomyces) were associated with light compaction changes, and previously shown as taxa affected by timber harvesting (Leung et al., 2016).

5. Conclusion

The present study described the short-term response of a forest soil after a severe compaction stress induced by repeated skidding mimicking logging activities for wood extraction operations. Despite the initial strong increase in bulk density, the soil compaction degree was reduced by 50 % in the most compacted part of the trail after just one year, highlighting a fast soil recovery. The chemical parameters of soil analyzed were not affected by soil compaction, with the only exception of available phosphorus, suggesting that a meaningful evaluation of a complex system like soil cannot be limited to those parameters, particularly in the short-term. Analysis of enzymatic activity reveals a sensitivity (decrease) of some functions to compaction, which are in turn related to the structure of the microbial community analyzed. In our microbiota analysis, the microbial communities showed different responses to compaction, highlighting a greater bacterial community resilience in the short term compared to the fungal community. Nonetheless, in soil particularly rich in species, such as our forest soil, considerable differences in terms of relative abundances were not observed. In this regard, the high degree of functional redundancy and complementarity that characterized bacterial communities, together with high observed biodiversity, may function as an “ecological preventive measure” for the soil ecosystem lessening the functional shifts induced by environmental disturbances. In this context, the indicator species identified in our study, such as the genera *Candidatus Solibacter* and *Puia*, may be useful for monitoring early changes in microbial communities and activities following soil compaction. Our study's findings contribute to the knowledge of the impact of soil compaction on microbial communities in forest soils and provide insights into the short-term microbial response to soil compaction, which could prove to be early indicators useful for predicting the long-term sustainability of forest management.

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CRedit authorship contribution statement

Agnes Bellabarba: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. **Laura Giagnoni:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Alessandra Adessi:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Elena Marra:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Andrea Laschi:** Writing – review & editing, Conceptualization. **Francesco Neri:** Writing – review & editing, Funding acquisition, Conceptualization. **Giovanni Mastrolonardo:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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.

Data availability

Data will be made available on request.

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