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The combined role of plant cover and fire occurrence on soil properties reveals response to wildfire in the Mediterranean basin

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ABSTRACT

Climate change has strongly increased the fire frequency in Mediterranean forests causing changes in soil bacterial, fungal and microarthropod communities. Fire impacts on soil properties depend on vegetation covers. In this framework, the aim of this research was to evaluate the effects of fire on bacterial, fungal communities and microarthropod community in soils under trees and shrubs in a Mediterranean area. Surface soil cores were sampled in unburnt and burnt (three years since fire occurrence) patches in the Vesuvius National Park trees (*Quercus ilex* L. and *Pinus nigra* L.) and shrubs (*Ginesta* sp., *Myrtus communis* L., *Laurus nobilis* L.). Samples were analyzed for abiotic (pH, water content and concentrations of C, N, C_{org}, P, NO_2^-, NH_4^+ and P_{avail}) and biotic (bacterial and fungal biomasses, and density, taxa richness, diversity, evenness and QBS-ar of microarthropods) properties. Results showed that, three years since fire, the abiotic properties were recovered in shrub stands but not yet in tree stands. Fire stimulated the development of bacteria only in shrub stands; no effects were observed for the fungal community in both shrub and tree stands; the amount and the taxa richness of microarthropods recovered to the values of the pre-fire conditions in both the stands. In conclusion, in the investigated area, fire differently impacted the vegetation covers, making soils under trees more similar to shrubs with the consequence to reduce the differences between the vegetation covers.

1. Introduction

In the Mediterranean Basin, fires are among the main disturbance factors of forest stability [1,2], causing serious ecological, economic and social problems [3]. In the last decades, climate change (*i.e.*, low humidity, high temperature and wind speed) together with high fuel availability have strongly increased both fire frequency and burnt area extension [4]. Several studies have highlighted the impacts of fire on soil properties but scarce are the information in the Mediterranean area. Moreover, the current knowledge about the role of different plant covers in controlling the fire effects on soil biota are poor.

The main impacts of fire on soil abiotic properties are linked to heat, runoff, water repellence [5], and organic matter reduction [6,7]. In addition, also organic phosphorous decreases and is quickly mineralised [8]; instead, production of nitrite and nitrate is scarce [9]. Fire, changing the soil abiotic properties, likely influences the edaphic community [10,11]. Bacteria and fungi are key organisms of post-fire soil recovery, as the biomass of bacteria increases in the short term since fire [12,13] and contributes to the formation of stable aggregates and to the decay of organic matter [14]. Instead, fungi play crucial roles in terms of nutrient addition, decay of recalcitrant matter, carbon pool, soil formation, and symbiotic links with plants [15,16]. Moreover, fungal

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mycelia contribute to soil stabilization, increasing the soil water-holding capacity [17]. However, the results from individual studies are controversial, therefore it is difficult to provide a general conclusion about the fire effects on soil bacterial and fungal communities. As a consequence, location-based studies are necessary to better understand this topic [18].

As bacteria and fungi, also microarthropods are involved in several processes that take place in soils. For instance, they actively participate in processes of soil formation [19] and contribute to the vertical distribution of dead organic matter along the soil profile [20]. As different taxa of microarthropods have specific behavioural, trophic and ecological traits, they are considered as good bioindicators of soil quality [21–24].

The fire impact on soil microbial communities and their recovery rates might be also mediated by soil properties [25] and by plant-soil interactions [26,27]. In fact, bacteria are stimulated in soils covered by vegetation, that enhances water permeability and organic matter content, as compared to those in bare soils [28], and both symbiotic and saprophytic fungi are impacted by post-fire plant communities [29]. Microarthropod diversity and functionality are affected by amount and quality of litter that falls from plants forming the stands [24,30].

According to future scenarios regarding climate and land-use changes, fire regimes will endure noticeable transformations in semi-arid terrestrial ecosystems, such as those in the Mediterranean Basin. In order to provide fire prevention planning in forests, it is necessary to assess the impact of fire on soil biota that will determine, in great extent, the post-fire recovery of the whole ecosystem [12,31]. Recently, research that integrate the below-ground responses to wildfires and the different vegetation covers are desirable in order to better understand their interactions on forest ecology [32]. In this framework, the main aim of the research was to evaluate the medium-term effects of fire on bacterial and fungal communities, and on microarthropod community in soils under trees and shrubs in a Mediterranean area. It can be hypothesized that levels of vegetation maturity differently impact soil biota and its recovery in burnt areas. In order to achieve the aim, soil cores were sampled in unburnt and burnt (three years after the fire) patches of a Mediterranean forest covered by trees (*Quercus ilex* L. and *Pinus nigra* L.) and shrubs (*Ginesta* sp., *Myrtus communis* L., *Laurus nobilis* L.), and characterized for bacteria, fungi and microarthropod abundances.

2. Materials and methods

2.1. Site description and soil sampling

The Vesuvius National Park, located in the South of Italy, is covered by plant species typical of the Mediterranean maquis: holm oak, pines (*Pinus pinea* L., *Pinus nigra* L.) and various herbs, such as *Myrtus communis* L., *Laurus nobilis* L., *Viburnum tinus* L., *Cistus* sp., *Ginesta* sp.; additionally, few specimens of black locust (*Robinia pseudoacacia* L.) can be found [33].

In June 2017 a wildfire burned approximately the 50% of the Vesuvius National Park extension [26]. After three years, in October, surface (depth: 0–10 cm, core diameter: 10 cm) Lepti-Vitric Andosol [34] were collected (after 15 days without rainfall) at twenty-four stands: twelve at the unburnt area and twelve at the burnt area (Fig. 1), in total three burnt and three unburnt areas for each ecosystem type. At the unburnt areas, the soil sampling was performed after removing litter; whereas, at the burnt areas, after removing ash and the thin layer of burnt litter or accumulated after fire. For each unburnt and burnt area, soils were collected under three stands of holm oak and three stands of pine (namely, trees), and under three stands of herbs and three stands of shrubs (namely, shrubs). For each stand, at both burnt and unburnt area, five soil cores were randomly collected and mixed together to obtain a homogeneous sample to perform, on triplicate, chemical and physical analyses as well as the evaluation of bacterial and fungal communities. Contextually, for each stand, at both burnt and unburnt area, three soil cores were randomly collected and kept separated to perform the analyses of the microarthropod community. The soil samples were put in sterile flasks and transported on ice to the laboratory.

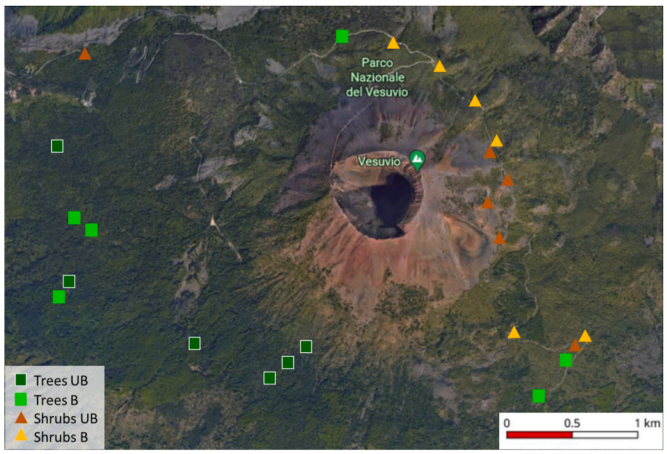


Fig. 1. Map of the sampling field points collected under unburnt (dark green) and burnt trees (light green), and unburnt (dark orange) and burnt shrubs (yellow) inside the Vesuvius National Park. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

2.2. Soil analyses

2.2.1. Chemical analyses

In laboratory, the soil samples were sieved (2 mm mesh size) to perform the analyses. Each soil sample was characterized by pH, water content (WC), total Carbon (C), Nitrogen (N) and Phosphorus (P), and for organic carbon (C_{org}), nitrite (NO_2^-), ammonium (NH_4^+) and P available (P_{avail}) concentrations. pH was measured in a soil:distilled water (1:2.5 = v:v) suspension by an electrometric method. WC was determined gravimetrically drying fresh soil at 105 °C until constant weight. C_{org} was measured by Elemental Analyzer (Thermo Finnigan, CNS Analyzer) in soil samples previously treated with HCl (10%) to exclude carbonates. The total C and N concentrations were evaluated in oven-dried (105 °C, until constant weight) and ground (Fritsch Analysette Spartan 3 Pulverisette 0) soil samples by Elemental Analyzer (Thermo Finnigan, CNS Analyzer).

NH_4^+ and NO_2^- concentrations were measured after extraction of fresh (4 °C) soil samples in a solution of KCl (2 M) according to the Italian Law DM 13/09/99 [35]. Particularly, to determine the NH_4^+ concentration an aliquot of sample was properly diluted and was build up to 10 mL in a plastic tube where 0.4 mL of phenol solution (10% w/v in ethanol), 0.4 mL of sodium nitroprusside solution (0.5% w/v in water) and 1 mL of oxidizing solution based on sodium hypochlorite were added. For color development, the solution was left at room temperature for at least 1 h in the dark. After this time, the absorbance at 640 nm was read to the spectrophotometer UV–Vis (Cary50, Varian) in cells with 1 cm of optical path against a reagent blank treated in the same way of the samples. NH_4^+ concentration was determined against a linear calibration curve built with six standard solutions prepared from stock solution in the range from 0.025 to 0.80 mg/L.

To determine the NO_2^- content an aliquot of sample was properly diluted and was build up to 10 mL in a plastic tube where 0.4 mL of dye solution (p-Aminobenzenesulfonamide, 2-(1-Naphthylamino)ethylamine dihydrochloride in water) was added. After 10 min for the color development, the absorbance at 543 nm was read to the spectrophotometer UV–Vis (Cary50, Varian) in cells with 1 cm of optical path against a reagent blank treated in the same way of the samples. NO_2^- concentrations was determined against a linear calibration curve built with five standard solutions prepared from stock solution in the range

from 0.002 to 0.025 mg/L.

The total P concentration was determined on 0.50 ± 0.01 g of fresh soil (0.2 mm) that were treated for acid digestion with the subsequent addition of 5 mL of H2SO4 (96%, Sigma Aldrich), 3 mL of H_2O_2 (30% wt, Honeywell) and 1 mL of HF (47%, VWR Chemicals). Digestion was completed by placing the solution on a preheated plate at 150 °C for 15 min. After cooling, the solution was brought to a final volume of 50 mL with ultrapure water, homogenized and filtered with Whatman® quantitative filter paper, ashless, Grade 42. An aliquot of 500 μL of sample was made up to 5 mL with ultrapure water, five drops of p-nitrophenol indicator (0.25% in water) were added and then, drop by drop, NaOH solution (5 N, in water) sufficient to change the color of the indicator to yellow. In order to remove the interference due to fluoride ion, 15 mL of boric acid solution (0.8 M) were added. For color development, 8 mL of sulfomolybdic reagent were added and the solution was brought to a final volume of 50 mL with ultrapure water. After 10 min, the absorbance at 882 nm was read to the spectrophotometer UV–Vis (Cary50, Varian) in cells with 1 cm of optical path against a reagent blank treated in the same way as the samples. P concentration was determined against a linear calibration curve built with five standard solutions prepared from stock solution in the range from 0.1 to 2 mg/L.

The P_{avail} concentration was determined on 2.00 ± 0.01 g of fresh soil (0.2 mm) that were transferred to a plastic container where 0.50 g of activated carbon and 40 mL of sodium bicarbonate solution (0.5 M in water) were added. Each solution was stirred for 30 min in a rotary shaker and then was filtered with Whatman® quantitative filter paper, ashless, Grade 42. Successively, an aliquot of 1 mL of sample was made up to 5 mL with ultrapure water, and five drops of p-nitrophenol indicator (0.25% in water) were added and then, drop by drop, H_2SO4 solution (2.5 M, in water) was added sufficient to change the color of the indicator to yellow. For color development, the solution was made up to 25 mL with ultrapure water and 4 mL of sulfomolybdic reagent were added. After 10 min, the absorbance at 882 nm was read to the spectrophotometer UV–Vis (Cary50, Varian) in cells with 1 cm of optical path against a reagent blank treated in the same way as the samples. P_{avail} concentration was determined against a linear calibration curve built with five standard solutions prepared from stock solution in the range from 0.1 to 2 mg/L.

2.2.2. Biological analyses

2.2.2.1. Bacterial and fungal communities. The bacteria and fungi of the soil microbial community were detected by DNA extraction and subsequent amplification of a specific gene by qPCR [36].

Total soil DNA was extracted by the FastDNA™ SPIN Kit for Soil (MP Biomedicals) with the modifications to the manufacturer instructions according to Ceccherini et al. [37]. DNA yield (ng g^{-1}) and purity were quantified by spectrophotometry (Picodrop™); whereas DNA quality was assessed by agarose gel electrophoresis.

Quantitative PCR (qPCR) analyses were used to quantify the total bacterial (16S rDNA) and total fungal (18S rDNA) sequences in each soil DNA sample. In this instance, the unit of measurement is expressed in nanograms of sequence per g of soil (ng g-1), which does not correspond to the number of individuals; it is only a means of monitoring the trend of bacterial and fungal communities [38]. For the total bacterial DNA (16S rDNA) the oligos Eub431f (5′ CCTACG GGAGGCAG 3′) and Eub515r (5′ TACCGCGGC KGCTGGCA 3′) were used for the amplification, respectively [39,40]. Whereas the total fungal DNA (18S rDNA) the oligos FF390 (5′ CGATAACGAACGAGACCT 3′) and FR-1 (5′ A[I] CCATTCAATCGGTA[I]T 3’) were used for the amplification [41,42].

The qPCR was performed using 25 μL of a reaction mixture containing 1X iTAQ UNIVERSYBR GREEN SMX 2500 mix (Bio-Rad Laboratories, CA, USA), 10 μM each forward and reverse primers, 40 ng of template DNA and sterile ddH_2O to reach the appropriate volume. Each sample was assayed by CFX96 Touch Real-Time PCR detection system (Bio-Rad laboratories, CA, USA).

Nanograms of the target sequence were normalized to gram of soil [30] in order to compare the results between bacteria and fungi in unburnt and burnt soils in tree and shrub stands.

2.2.2.2. Microarthropod extraction. The microarthropods were extracted using the MacFadyen method over a one-week period [43] and sorted using a dissecting stereomicroscope. The microarthropods were identified according to the major (class or order) taxonomic groups.

The results of the microarthropod community analyses are reported, for each site, as density (i.e., individual number m^{-2} soil) and taxa richness (i.e., mean taxa number at each site) and the relative abundance of each taxon within the community. For each site, to evaluate the diversity of soil microarthropod taxa and the repartition of taxa abundances inside the community, the diversity and evenness indices were, respectively, calculated. The diversity index [44] was calculated according to the following formula:

$$\text{Diversity index: } H = -\Sigma P_i \ln P_i$$

and the evenness index [45] was calculated according to the following formula:

$$\text{Evenness index: } E = H / \ln(\text{total number of taxa})$$

where P_i percentage of the individuals represented by species *i* on the total number of individuals. High diversity and evenness are indicated by high values of the diversity and evenness indices.

In addition, the soil biological quality index (QBS-ar) was evaluated as reported by Parisi et al. [46]. This QBS-ar index classifies soil microarthropods based on morphological characteristics, assigning to each microarthropod group a different weight, represented by a different score, thereby defining the Ecomorphological indices (EMI) shown in Parisi et al. [46]. The QBS is calculated as the sum of EMI values in each soil [46]. For the groups that have the ranges of EMI, the intermediate value was chosen, as morphological characteristics typical of both surface and soil dwelling species were found.

For each microarthropod taxon, the vertical distribution, expressed as the preference of each taxon to live in soil, litter, or surface (above litter), was attributed using information reported in various literature sources [46–49]. The results were reported as the relative abundances of microarthropods living in soil, litter or surface.

2.3. Statistical analyses

The normality of the distribution of the data sets was assessed by the Shapiro-Wilk test.

The two-way ANOVA test was performed in order to highlight the effects of vegetation cover (trees and shrubs) and fire occurrence (unburnt: UB and burnt: B) and their interactions on soil properties.

A Principal Components Analysis (PCA) was performed on soil abiotic and biotic properties to identify the main abiotic properties associated to the biotic ones, and the distribution of sites according to the soil properties. In addition, the confidence ellipses (for $\alpha = 0.05$) for unburnt and burnt soils and for the different vegetation covers were superimposed to PCA (addEllipses function). Differences in soil properties for unburnt and burnt soils and for the different vegetation covers were tested by permutational multivariate analysis of variance using distance matrices (Permanova analysis, Adonis function).

All the statistical analyses were performed using the R 4.0.3 programming environment with ade4ˆ package. The graphs were created by SigmaPlot12 software (Jandel Scientific, San Rafael, CA, USA).

3. Results

3.1. Effects of vegetation cover, fire occurrence and their interactions on the soil properties

The results of the two-way ANOVA highlighted that soil abiotic and biotic properties were mainly influenced by vegetation cover than by fire occurrence (Table 1, Figs. 2–6). The interactions between vegetation cover and fire occurrence significantly influenced soil pH, microarthropod evenness and the abundance of Symphyla (Table 1). Soil pH was significantly affected by both vegetation cover and fire, and their interactions (Table 1); instead, the microarthropod evenness and the abundance of Symphyla were only affected by the interaction between vegetation cover and fire (Table 1).

3.2. Comparison of the properties of unburnt and burnt soils under trees

Under trees, the mean values of pH were 7.43 in unburnt (UB) and 7.07 in and burnt (B) soils and were significantly higher in UB soils (Fig. 2); water contents were, on average, 26.4% d.w. In UB and 29.1% d.w. In B soils and not significantly differences were detected between UB and B soils (Fig. 2). The mean concentrations of C (UB: 6.20; B: 6.76% d.w.); C_{org} (UB: 4.02; B: 3.74% d.w.); N (UB: 0.26; B: 0.40% d.w.) and P (UB: 0.05; B: 0.05% d.w.) did not significantly differ between UB and B soils (Fig. 2). The mean concentrations of NO_2^- were 0.61 mg Kg^{-1} d.w. In the unburnt (UB) and 1.07 mg Kg^{-1} d.w. In burnt (B) soils and no significantly differences were detected (Fig. 2); the mean concentrations of NH_4^+ were significantly higher in UB (10.3 mg Kg^{-1} d.w.) than B (5.06 mg Kg^{-1} d.w.) soils (Fig. 2), by contrast, the mean concentrations of P_{avail} were significantly lower in UB (17.5 mg Kg^{-1} d.w.) than B (32.6 mg Kg^{-1} d.w.) soils (Fig. 2).

Table 1
Coefficients (F values) of the two-way Anova performed on soil abiotic properties in relationship to vegetation covers (Veg), fire (Fire) and their interactions (Veg x Fire). Asterisks indicate significant impacts of factors and their interactions on soil characteristics.

	Vege	Fire	Veg x Fire
pH	21.7[c]	5.92[a]	6.15[a]
WC	12.3[c]	0.86	0.09
C	3.28[a]	1.06	0.44
N	0.07	2.48	0.09
Corg	2.76	0.13	0.40
NO_2^-	4.86[a]	0.03	3.94
NH_4^+	14.1[c]	7.61[a]	3.66
P_tot	2.22	1.29	1.19
P_avail	0.68	4.94[a]	0.04
Bacteria	1.90	3.47[a]	0.11
Fungi	7.97[a]	0.11	1.17
Density	3.36	<0.01	1.03
Taxa Richness	8.87[b]	0.61	0.02
Diversity index	8.19[b]	1.34	0.71
Evenness index	0.02	1.32	3.68[a]
QBS_ar index	5.16[a]	0.06	0.82
Collembola	2.68	0.20	0.02
Acarina	4.22[a]	0.26	3.08
Diplopoda	1.92	0.08	0.03
Diplura	0.54	0.18	1.45
Diptera Larvae	0.03	0.57	0.74
Pauropoda	3.89[a]	0.45	2.94
Protura	0.06	0.00	0.34
Symphyla	0.48	0.63	9.23[b]
Soil	2.65[a]	0.25	0.08
Litter	2.19[a]	0.31	0.06
Surface	0.32	0.06	0.01
Detritivorous	6.21[a]	0.18	0.10
Herbivorous	2.07	0.00	0.00
Predators	2.31	0.27	0.32

[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$.

The mean amounts of bacteria and fungi were, respectively, 462 and 0.05 ng g^{-1} in UB soils and, respectively, 774 and 0.04 ng g^{-1} in B soils, and they did not significantly vary between UB and B soils (Fig. 3).

The mean values of density (UB: 6640; B: 8842 organisms m^{-2}) and taxa richness (UB: 6.11; B: 5.61 mean taxa number) of microarthropods as well as the indexes of diversity (UB: 0.96; B: 1.04), evenness (UB: 0.73; B: 0.78) and QBS-ar (UB: 69; B: 74) did not significantly differ between UB and B soils (Fig. 4). The relative abundances of the microarthropods taxa in UB and B soils are reported in Fig. 5. The mean percentages of Symphyla and Diplopoda were significantly higher in B than UB soils (Fig. 5). The mean percentages of microarthropods with different vertical distribution are reported in Fig. 6 and they did not significantly differ between UB and B soils (Fig. 6).

3.3. Comparison of the properties of unburnt and burnt soils under shrubs

Under shrubs, the mean values of pH were 6.90 in the unburnt (UB) and 6.91 burnt (B) (Fig. 2), and those of water contents were 9.57% d.w. In UB and 15.0% d.w., in B soils; both pH and water contents did not significantly differ between UB and B soils (Fig. 2). The mean concentrations of C (UB: 2.35; B: 3.71% d.w.), C_{org} (UB: 1.65; B: 2.68% d.w.); N (UB: 0.22; B: 0.49% d.w.), P (UB: 0.06; B: 0.06% d.w.), NO_2^- (UB: 1.69; B: 1.13 mg Kg^{-1} d.w.), NH_4^+ (UB: 3.81; B: 2.85) and P_{avail} (UB: 23.9; B: 36.5 mg Kg^{-1} d.w.) did not significantly differ between UB and B soils (Fig. 2).

The mean of 16S sequences bacteria (UB: 115; B: 561 ng g^{-1}) was significantly higher in B than UB soils; whereas, the mean 18S sequences of fungi were 0.01 ng g^{-1} in UB soils, and 0.02 ng g^{-1} in B soils, and did not significantly vary between UB and B soils (Fig. 3).

The mean values of density (UB: 4950; B: 2924 organisms m^{-2}) and taxa richness (UB: 4.4; B: 4.1 mean taxa number) of microarthropods did not significantly differ between UB and B soils (Fig. 4). Instead, the indexes of diversity (UB: 0.88; B: 0.72), evenness (UB: 0.78; B: 0.67) and QBS-ar (UB: 59; B: 50) were significantly higher in UB than B soils (Fig. 4). The contribution percentages of Symphyla were significantly higher in UB than B soils (Fig. 5). The mean percentages of microarthropods with different vertical soil distribution did not ~~statistically~~ significantly differ between UB and B soils (Fig. 6).

3.4. Comparison of properties of unburnt soils under trees and shrubs

The mean values of pH, water content, and C and NH_4^+ concentrations were significantly higher in soils covered by trees than in those covered by shrubs (Fig. 2); by contrast, NO_2^- concentrations were significantly higher in soils covered by shrubs (Fig. 2). All the other abiotic properties did not significantly differ according to plant covers (Fig. 2).

Bacteria amounts did not statistically differ between soils covered by trees and shrubs (Fig. 3); instead, fungi amounts were significantly higher in soils covered by trees (Fig. 3).

Density of microarthropods as well as the investigated indices did not significantly differ according to plant covers (Fig. 4); whereas, taxa richness was significantly higher in soils under trees (Fig. 4). The contribution percentages of Symphyla were significantly higher under shrubs (Fig. 5). The mean percentages of microarthropods with different vertical soil distribution significantly differed only for those that occupy the deep soil and litter, as the formers were significantly higher under shrubs than under trees, and the latter were significantly higher under trees than under shrubs (Fig. 6).

3.5. Comparison of properties of burnt soils under trees and shrubs

The mean values of water content and NH_4^+ concentrations were significantly higher in soils covered by trees than in those covered by shrubs (Fig. 2); instead, all the other abiotic properties did not

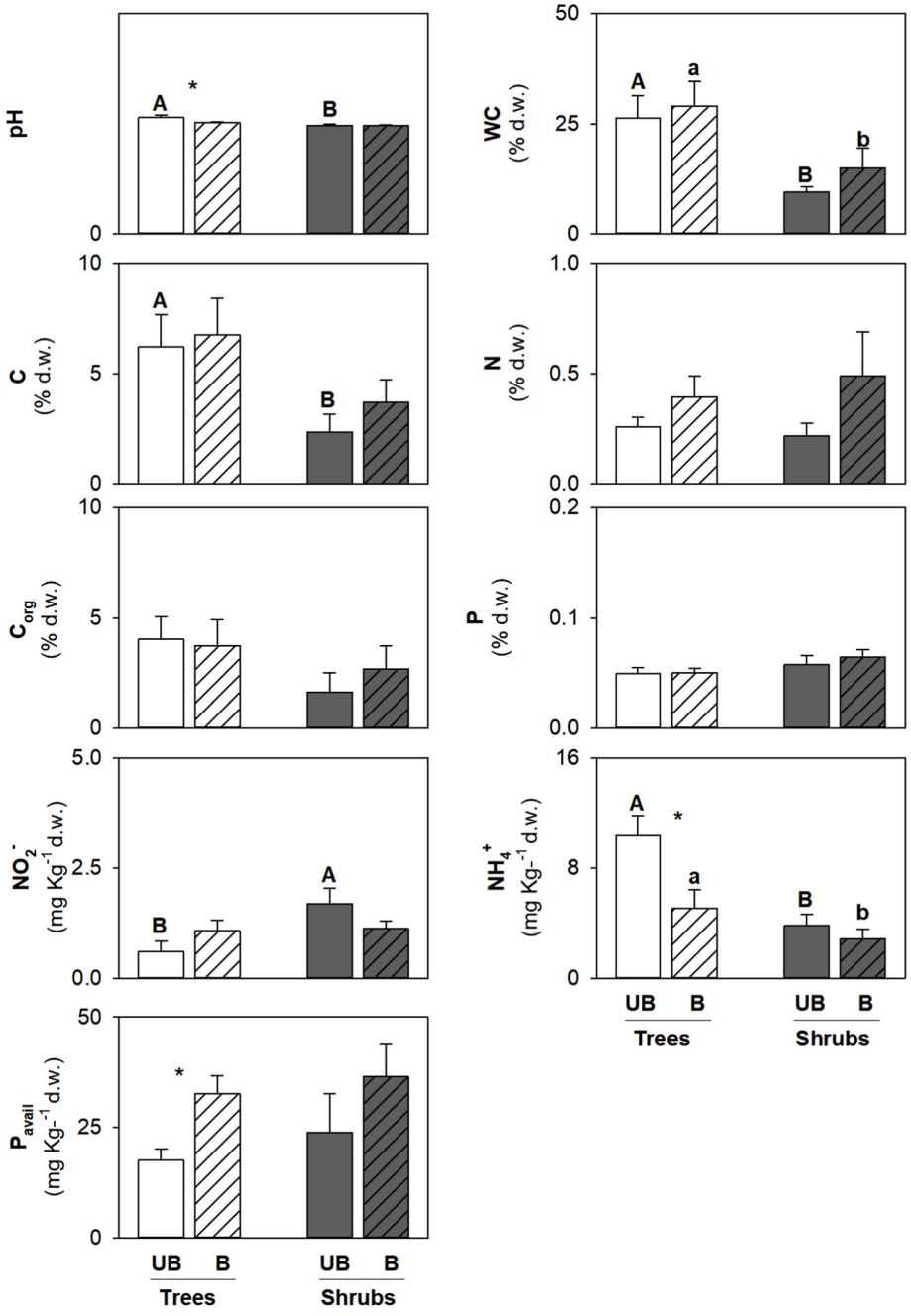


Fig. 2. Mean values (±s.e., n = 24) of pH, water content (WC, expressed as % d.w.), carbon, nitrogen and phosphorus (C, N and P, respectively, expressed as % d.w.), organic carbon (C_{org}, expressed as % d.w.), nitrite (NO_2^-, expressed as mg Kg^{-1} d.w.), ammonium (NH_4^+, expressed as mg Kg^{-1} d.w.) and available phosphorus (P_{avail}, expressed as mg Kg^{-1} d.w.) in unburned (UB, empty bars) and burned (B, dashed bars) soils collected inside the Vesuvius National Park under trees (white bars) and shrubs (grey bars). Different capital and small letters indicate significant differences (at least, $P < 0.05$) in soil properties, respectively, in UB and B soils under different vegetation covers. Asterisks indicate significant differences ($P < 0.05$) between UB and B soils within the same vegetation cover type.

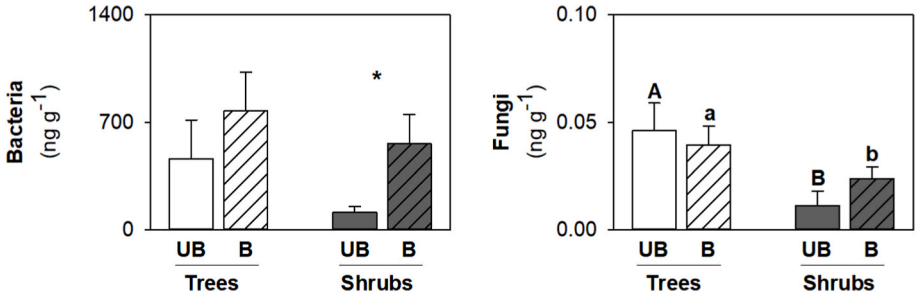


Fig. 3. Mean values (±s.e., n = 24) of bacteria and fungi (expressed as ng g^{-1} d.w.) in unburned (UB, empty bars) and burned (B, coarse bars) soils collected inside the Vesuvius National Park under trees (white bars) and shrubs (grey bars). Different capital and small letters indicate significant differences (at least, <0.05) in soil properties, respectively, in UB and B soils under different vegetation covers. Asterisks indicate significant differences ($P < 0.05$) between UB and B soils within the same vegetation cover type.

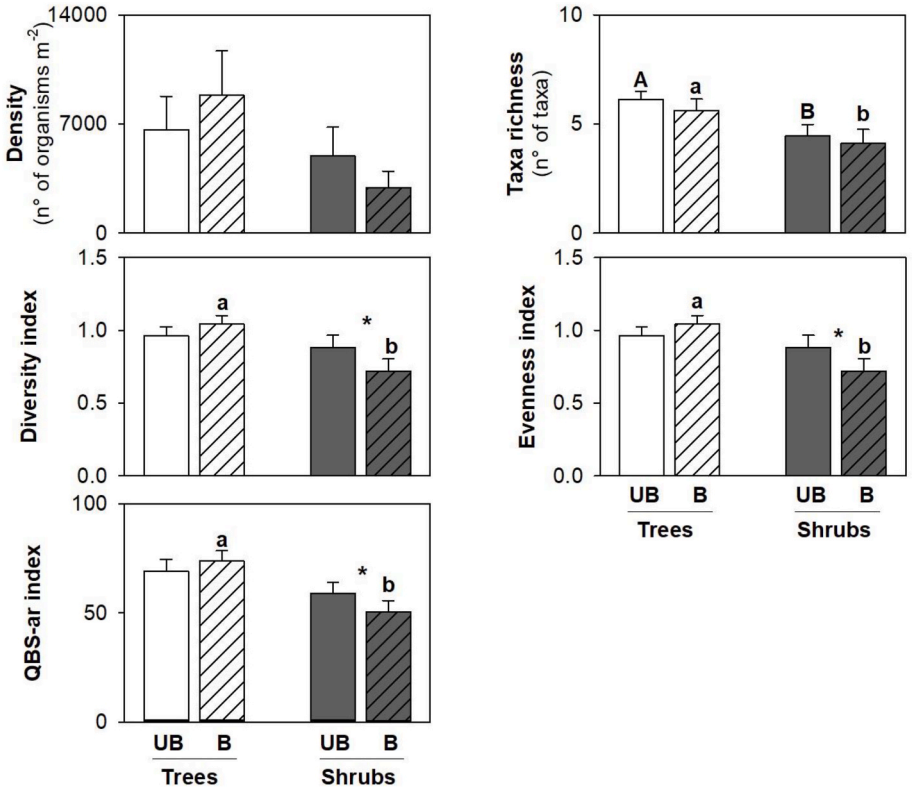
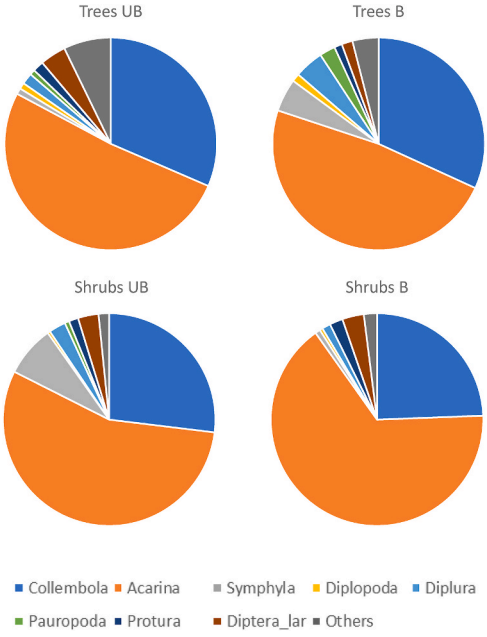


Fig. 4. Mean values (±s.e., n = 24) of density (expressed as n° of organisms m^{-2}), taxa richness (expressed as n° of taxa), diversity, evenness and QBS-ar indices calculated for microarthropod communities in unburned (UB, empty bars) and burned (B, dashed bars) soils collected inside the Vesuvius National Park under trees (white bars) and shrubs (grey bars). Different capital and small letters indicate significant differences (at least, $P < 0.05$) in soil properties, respectively, in UB and B soils under different vegetation covers. Asterisks indicate significant differences ($P < 0.05$) between UB and B soils within the same vegetation cover type.



TAXA	Trees UB	Trees B	Shrubs UB	Shrubs B
Collembola	A	a	A	a
Acarina	A	b	A	a
Symphyla	B*	a	A*	b
Diplopoda	A*	a	A	a
Diplura	A	a	A	a
Pauropoda	A	a	A	b
Protura	A	a	A	a
Diptera_larvae	A	a	A	a
Others	A	a	A	a

Fig. 5. Relative percentage of taxonomical composition of microarthropod communities in unburned (UB) and burned (B) soils collected inside the Vesuvius National Park under trees and shrubs. In the table are reported the significant differences among the taxonomical composition. Different capital and small letters indicate significant differences (at least, $P < 0.05$) in taxonomical composition, respectively, in UB and B soils under different vegetation covers. Asterisks indicate significant differences (at least, $P < 0.05$) between UB and B soils within the same vegetation cover type.

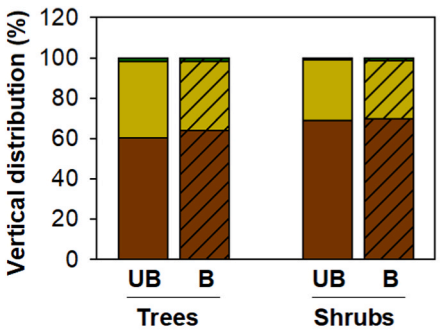
significantly differ according to plant covers (Fig. 2).

Bacteria amounts did not significantly differ between soils covered by trees and shrubs (Fig. 3); instead, fungi amounts were significantly higher in soils covered by trees (Fig. 3).

Density of microarthropods did not significantly differ according to plant covers (Fig. 4). Instead, microarthropod taxa richness as well as the indices of diversity, evenness and QBS-ar were significantly higher in soils under trees than in those under shrubs (Fig. 4). The contribution percentages of Acarina were significantly higher under shrubs, whereas those of Symphyla and Pauropoda were significantly higher under trees (Fig. 5). The mean percentages of microarthropods with different vertical soil distribution did not significantly differ between burnt trees and shrubs (Fig. 6).

3.6. *Relationships between soil biotic and abiotic properties*

The results of the PCA highlighted that the first two axes accounted, respectively, for 38% and 21% of the total variance (Fig. 7). The first axis



Vertical distribution	Trees UB	Trees B	Shrubs UB	Shrubs B
Soil	B	a	A	a
Litter	A	a	B	a
Surface	A	a	A	a

Fig. 6. Relative percentage of vertical distribution (soil: brown, litter: yellow, surface: green) of microarthropod communities sampled in unburnt (UB, no pattern) and burnt (B, dashed pattern) soils under trees and shrubs. In the table significant differences among the taxonomical composition is reported. Different capital and small letters indicate significant differences (at least, $P < 0.05$) in vertical distribution, respectively, in UB and B soils under different vegetation covers. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

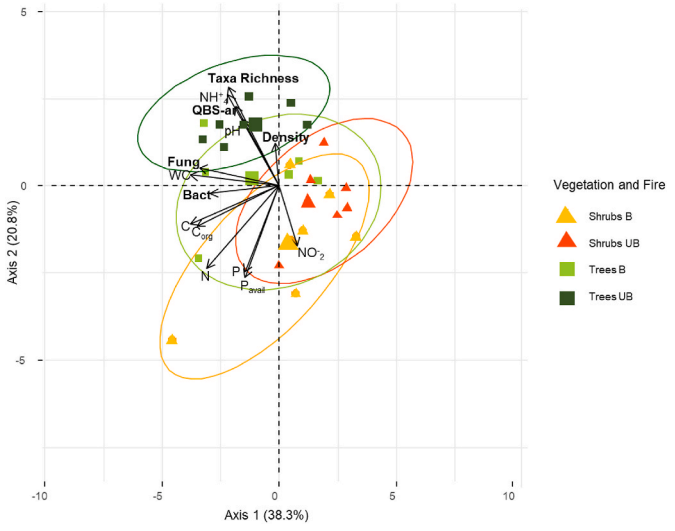


Fig. 7. Graphical display of the first two axes of the Principal Component Analysis (PCA) on the soil abiotic (pH; water content: WC; total concentration of C, N and P, organic carbon: C_{org}, nitrite: NO_2^-, ammonium NH_4^+ and available phosphorus: P_{avail}) and biotic (bacterial and fungal biomasses, microarthropod density, taxa richness and QBS-ar index) properties measured in unburnt (UB, light color) and burnt (B, dark color) soils under trees (square) and shrubs (triangles) collected inside the Vesuvius National Park. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

separated the soils according to the vegetation (Fig. 7); it was negatively correlated to total C, C_{org} and N contents, water content and bacterial and fungal communities (Fig. 7); whereas, the second axis separated the soils according to the fire occurrence (Fig. 7); it was positively correlated to pH, NH_4^+ content, taxa richness and QBS-ar, and negatively to total and P_{avail} concentrations (Fig. 7). According to the investigated soil properties, the unburnt soils under trees significantly (Permanova analysis $P < 0.05$) differed from unburnt soils under shrubs; by contrast, burnt soils under trees did not significantly (Permanova analysis $P > 0.05$) differ from burnt soils under shrubs (Fig. 7).

4. Discussion

The research, performed inside the Vesuvius National Park, showed that the vegetation covers more than fire occurrence had the larger and more consistent influence on soil abiotic and biotic properties. Despite the investigated trees (pines and oaks) can show differences in soil properties [38], their variability is not great enough to mask the variability between trees and shrubs. Moreover, the main evidence of the research was that the fire undid the initial differences between trees and shrubs, as highlighted by the Permanova analysis.

Fire occurrence had an effect in soils under the same vegetation cover, as some soil abiotic properties such as pH, NH_4^+ concentrations and P_{avail} were affected in burnt soils covered by trees, but not in those covered by shrubs. In fact, in these soils, fire caused a significant decrease of both pH and NH_4^+ concentrations and an increase of P_{avail}. Although many research reports increase of pH in burnt soils [48], the findings of the present research agree with those reported by Hatten et al. [6] who highlighted those late burns (after spring, same period of fire occurrence in the present research) caused a reduction of soil pH in ponderosa pine forests located in western USA. The different fire impacts on NH_4^+ concentrations and P_{avail} in soils under trees suggest that the composition of the microbial community in three-year-old burnt stands of trees was still different from the unburnt ones [27]. It is well known that different groups of soil microorganisms differently respond to fires over the time [49,50]. Particularly, the lower NH_4^+ concentrations in burnt stands of trees than in the unburnt ones could be due to the decrease of N-fixer organisms [51] that they were not still recovered after three years. This supposition is corroborated by the results reported by other researchers [9,52] who found a decrease of the N cycling bacteria abundance in burnt soils. Instead, the significantly higher P_{avail} in three-year-old burnt than unburnt stands of trees could be attributable to the combustion and mineralization processes that release the organic P in soil. The greater amount of litter under trees as compared to that under shrubs together with the burnt soil conditions seemed to stimulate the microorganisms involved in the P cycle. In fact, it is well known that, even in low-intensity prescribed fires, organic P and soil organic matter decline immediately after fire occurrence, and organic P is quickly mineralised [8].

Despite that observed for tree stands, fire did not cause significant changes in the soil abiotic properties in shrub stands. Likely, that could be attributable to the different successional stages of the two stands and their capability to respond to alteration of their stability. In fact, shrub stands, belonging to a more immature successional stage, have a major resilience and then quickly respond to the perturbations [53].

The comparison of the soil abiotic properties between the tree and shrub stands highlighted that, in the unburnt area, plant covers affected soil pH, water content, and soil C, NO_2^- and NH_4^+ concentrations. In particular, all these soil properties, with the exception of NO_2^- that was greater in soils under shrubs, showed higher values in tree stands. The higher amount of litter that accumulates under trees could be responsible for the higher water retention and carbon concentrations [54] as well as for the development of fungi and bacteria, participating in the ammonification during the decomposition of the organic matter [55]. Instead, the different values of soil pH could be linked to the chemical composition of the litter deriving by the covering plant species and by the root-soil interactions [56]. Fire would seem to reduce the differences in the soil abiotic properties between tree and shrub stands. In fact, in the burnt area, the only significant differences between the two stands regarding soil water content and NH_4^+ concentrations, suggesting that litter amount was the main driver of these soil properties.

Three-year-old burnt stands of both trees and shrubs showed an increasing trend of bacterial sequences, which was significant different

from that observed in the unburnt stands only for shrub stands. These findings suggest that, on the whole, fire stimulated the development of soil bacteria at the medium-term (after three years since fire occurrence), especially under shrubs. Barreiro and Díaz-Raviña [48] reports that fires directly affect soil organisms, causing their death, and indirectly, transforming their living environment (*i.e.*, resource availability and quantity, and environmental heterogeneity). The found results agree with those reported by Fernández-García et al. [57] who found differences in microbial biomass after fire in Mediterranean forests and those reported by Carson and Zeglin [58] who found increases of the microbial biomass already after one year since fire in some grasslands. Moreover, it can be supposed that after fire, shrub stands were more enriched in species as compared to the tree ones, belonging to a more mature successional stage, and then positively affecting the microbial diversity and biomass [59]. The lack of statistically differences in bacteria amounts between soils covered by trees and shrubs in both unburnt and burnt area suggest that different plant covers did not affect the bacterial biomass although it cannot be excluded strongly differences in taxa diversity and microbial community structure [52].

In the investigated area, the response of fungi to fire and plant cover differed from those of bacteria. In fact, within three years, the amount of fungal 18S sequences recovered the pre-fire amounts in both tree and shrub stands as no significant differences were observed between unburnt and three-old-burnt stands. According to Hernández-Rodríguez et al. [60], who observed the formation of fungal propagules stimulated by fire, it can be supposed that the soil conditions suitable for the development of the fungi already after three years since fire in the investigated area. Moreover, especially in ecosystems where fires are recurrent (such as the Mediterranean area) it has been observed the presence of numerous fungal species with heat- and smoke-activated spores [61], that may benefit from post fire ash deposits [62] and that are favoured by the reduced competition with other microbial species [61]. Moreover, some ectomycorrhizal fungi may also dominate immediately after burning as their tolerance of fire effect [63] or as they may survive in a mycelial state during the fire event [64]. Finally, the significant higher fungal 18S sequences in tree than shrub stands could be because trees favoured the fungal community, presumably triggering relationships with both symbiotic and saprophytic species [29,65].

Although the microarthropod density and taxa richness did not significantly differ between unburnt and three-year-old burnt stands in both tree and shrub stands, the investigated indices (i.e., diversity, evenness and QBS-ar indices) were significantly higher in unburnt soils only under shrubs. These findings suggest that, after three years since fire, the amount and the taxa richness of microarthropods recovered the values of the pre-fire conditions in both the stands. Moreover, conversely from that occurred in the tree stands, a meaningful difference in the structure of the microarthropod community in the shrub stands between the unburnt and the three-year-old burnt soils was observed. Particularly, the lower values of the diversity, evenness and QBS-ar indices in burnt soils suggest a negative impact on the diversity of microarthropod still evident after three years in soils under shrubs, agreeing with other studies finding that fire depressed diversity in soil organism communities [66,67]. This suggests that the effects of fire under shrubs were more severe than under trees, as soil organism communities still not recover after three years from fire occurrence. As shrub ecosystem is less dense vegetated, the impacts of fire on these soils could have directly affected soil organisms. In fact, fire events affect soil animal communities both directly, and indirectly, through physical, chemical, and biological changes to the soil environment [68]. In addition, microarthropods are strongly affected by factors at small spatial scales such as microtopography, soil temperature, soil water, soil pH [69]. Likely, in burnt stands under shrubs, more exposed to high temperature and desiccation as compared to all the other stands (*i.e.*, unburnt and burnt tree stands and unburnt shrubs stands), a shift of taxa occurred with, likely, a dominance, in terms of individuals, of the taxa more resistant to the high temperature [70]. In fact, fire induced important structural changes in soil animal communities of Mediterranean habitats [67,68]. In detail, in the investigated soils, the effects of fire caused an increase of Symphyla and Diplopoda abundances under trees and a decrease of Symphyla under shrubs. This could be due to the mineralization of litter in burnt soils under trees, that can make more accessible the food resource for these organisms [71]; by contrast, the reduction of litter layer in burnt soils under shrubs, reduce habitat and insulation from temperature and humidity [71].

5. Conclusions

An overall evaluation highlighted that vegetation cover more than fire influenced the soil abiotic and biotic properties in the investigated area. The performed research showed that the three years since fire were enough to recover the investigated soil abiotic properties (pH, water content and concentrations of C, N, C_{org}, P, NO_2^-, NH_4^+ and P_{avail}) in shrub stands, but not in tree stands. In fact, burnt tree stands became more similar to shrubs, highlighting that fire reduce the difference between the two investigated vegetation covers. In particular, at burnt tree stands, pH, and concentrations of NH_4^+ and P_{avail} were still different from the unburnt stands. Fire stimulated the development of bacteria only in shrub stands, whereas did not affect the fungi. Finally, after three years since fire, microarthropod density and taxa richness recovered the pre-fire values; whereas, the indices of diversity, evenness and QBS-ar were still negatively impacted by fire.

In the investigated area, fire modified the ecosystem development of causing a regression of soil properties from tree to shrubs ecosystem. The simultaneous investigation of fire impacts on soil under different vegetation covers could be useful to prevent and mitigate fire effects according to the vegetation in the Mediterranean environment.

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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